The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*

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Abstract

Uscana lariophaga Steffan (Hym.: Trichogrammatidae) is a West African egg parasitoid of *Callosobruchus maculatus* (F.) (Col.:-Bruchidae), a storage pest of cowpea. The role of semiochemicals in host location by the parasitoid was studied in airflow and diffusion olfactometers. In a four-armed airflow olfactometer, the female wasp was arrested by volatiles emanating from cowpea grains, bruchid-infested cowpea grains, virgin host females and host eggs, while volatiles from host males did not elicit a response. In a circular diffusion alfactometer, the female wasp walked more rapidly and for longer when exposed to air saturated with odour from virgin female beetles; male odour did not give such a response. Several host odours were also tested in a tube diffusion olfactometer. Male beetles and female wasps spent more time in the half of the tube containing the virgin female beetle odour or containing synthetic sex pheromone. When baited with a marble carrying bruchid eggs at one end of the tube, more wasps reached this odour source than the control marble at the other end. The parasitoid was apparently able to conduct a directional search using a host-associated odour gradient. The roles of sex pheromones and other host-associated odours (kairomones), and plant odours (synomones), are discussed.

Introduction

The stored-product insect pest *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) causes considerable losses in cowpea in West Africa. Adults of the bruchid appear soon after pod formation; and when the infested pods are harvested and stored, *C. maculatus* continues to develop in storage (Caswell 1976; Prevett 1961). Chemical control of bruchids in traditional granaries is not appropriate for resource-poor farmers and consequently biological methods of control should be encouraged (van Huis 1991).

In Niger the eggs of *C. maculatus* are attacked by *Uscana lariophaga* Steffan (Hym.: Trichogrammatidae) both in the field and in storage. Studies are focused on enhancement of the efficacy of the parasitoid by environmental manipulation. The capacity of the parasitoid to find the host eggs is one of the factors which influences the efficiency of the parasitoid. Semiochemicals could play a role in this process. For a review of the role of semiochemicals in the foraging behaviour of *Trichogramma* spp., see Noldus (1989).

Host-plant odours can have a positive effect on parasitisation rates of the egg parasitoid, as has been demonstrated for other trichogrammatids by Bar et al. (1979) and Nordlund et al. (1984 and 1985). These odours have been classified as synomones.

Adult host odours can also increase the parasitism of egg parasitoids (Lewis et al. 1982). Calling females of *Mamestra brassicae* and *Heliothis zea* and virgin females of *Pieris brassicae*, all releasing a female sex pheromone, have arrested *Trichogramma* spp. in a four-armed airflow olfactometer (Noldus and van Lenteren 1985a; Noldus 1988) and in a wind-tunnel (Noldus et al. 1991). Female sex-pheromones are involved, but host scales (Laing 1937; Lewis et al. 1971, 1975) and excretions (Lewis et al. 1982) also function as kairomones for *Trichogramma* spp.

It would be very advantageous if trichogrammatids would perceive direct signals from the egg. There is evidence of the involvement of contact chemicals (Pak and de Jong 1987; Noldus and van Lenteren 1985b), such as secretions from the accessory glands which adhere the egg to the substrate (Nordlund et al. 1987). There is some evidence of involvement of volatile kairomones emanating from host eggs (Ferreira et al. 1979; Bourarach and Hawlitzky 1984; Renou et al. 1989 and 1992). Visual perception of eggs by trichogrammatids seems to be rather poor; the wasps only reacted at a distance of a few millimetres from the egg (Laing 1937; Pak et al. 1991).

Cowpea and bruchid odours, such as sex pheromones, could also be involved in host location by *U. lariophaga*. Qi and Burkholder (1982) reported a female sex pheromone for *C. maculatus*. Virgin females displayed a calling behaviour for a period of 3–5 minutes, which was associated with pheromone release in the first two hours of the light period. A male sex pheromone or an aggregation pheromone could not be found (Rup and Sharma 1978). Cork et al. (1991) isolated some 3-methyl-heptenoic-acids isomers as components of the female sex pheromone of *C. maculatus* and *C. analis*. Another odour source could be a putative epideictic pheromone probably deposited by ovipositing female bruchids. Such a marking pheromone signals arriving conspecifics that the host seed already bears an egg. By avoiding these seeds, females reduce competition among their larval progeny for food. As a result eggs are uniformly spread over the available seeds (Giga and Smith 1985; Messina and Renwick 1985; Wasserman 1985). However, no obvious marking behaviour by ovipositing females has been reported. Credland and Wright (1990) indicated that both sexes produce compounds that deter oviposition and that there is no proof that these compounds are egg-associated. Their results suggest that deterrence only occurs when females are able to select between seeds with different egg loads.

The reaction of *U. lariophaga* female wasps to volatiles emanating from cowpea grains and *C. maculatus* adults and eggs was tested in the current study.

Materials and Methods

The experiments were carried out by using one airflow and two diffusion olfactometers.

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Four-armed airflow olfactometer (Fig. 1)

Because the olfactometer used by Vet et al. (1983) was too large for *U. lariophaga*, the modifications introduced by Noldus and van Lenteren (1985a) were applied. Four odour fields were created in the exposition chamber by sucking air through a hole in the centre of the chamber floor. Clear odour fields were established by adjusting the airflow to 27 mL/min in each of the four arms of the chamber. Each arm was connected to a set of two in series 50 mL glass vials. The vial closest to the chamber contained the odour source and the outer vial distilled water. The incoming air was passed over the water to create a high and uniform humidity. The wasps were introduced into the olfactometer via the hole in the centre of the chamber. The vacuum was disconnected during introduction of a wasp. The top of the introduction tube was made level with the bottom of the exposition chamber so that there were no disturbing edges for the wasps walking into the olfactometer. The olfactometer was cleaned with ethanol (70%).

A circular fluorescent tube (35 cm diameter), placed 15 cm above the olfactometer, provided an even light intensity of 5300 lux. The temperature in the climate room was 30°C and the relative humidity (r.h.) 45–65%. To record the behaviour of the wasps, a video camera was positioned centrally above the chamber. Because the wasps could be disturbed by movement in the surroundings the whole device was covered by white cloth. Observations were made exclusively from the outside via the video monitor. One airflow was tested against three others for a period of 10 minutes for cowpea/bruchid odours and 5 minutes for beetle and egg odours. The time spent by the wasp in each sector was recorded. After 12 observations, odour vials were interchanged so that the odour came from a different arm in the exposition chamber.

The following odours were tested: cowpea grains, bruchid-infested cowpea grains, unmated male bruchids, virgin female beetles, and bruchid eggs. To obtain cowpea odour, 35 g of beans were kept for 1 day in a sealed odour vial of the olfactometer. Odour of bruchid-infested cowpea was obtained by placing 150 male and female bruchids (< 24 hours old) on 35 g of cowpea grains in the sealed odour vial for a day. Odour of unmated males or virgin females was obtained from 15 beetles (all < 24 hours old) confined in darkness for at least 16 hours in a closed vial. These unmated males and females of *C. maculatus* were obtained by isolating adults emerging from individually placed cowpea beans which received only one to three eggs per bean. Beetle eggs of 4–7 hours old were obtained by allowing oviposition to occur on plastic vials (Eppendorffs), and then scraping them off (about 100) into the odour vial of the olfactometer. Mated wasps without oviposition experience and <2 days old were tested in the olfactometer. Only in the treatments using cowpea odour, and male and female bruchid odour, were the wasps younger, viz. < 9 hours old. Wasps were either reared from eggs laid on Eppendorffs or on cowpea (without and with cowpea experience).

Circular diffusion olfactometer (Fig. 2)

The effect of host odour on the walking behaviour of the parasitoid was studied in a circular diffusion olfactometer. This olfactometer consisted of two identical round glass vessels which fit together and are sealed with a rubber ring to ensure an airtight closure. A filter paper was inserted between

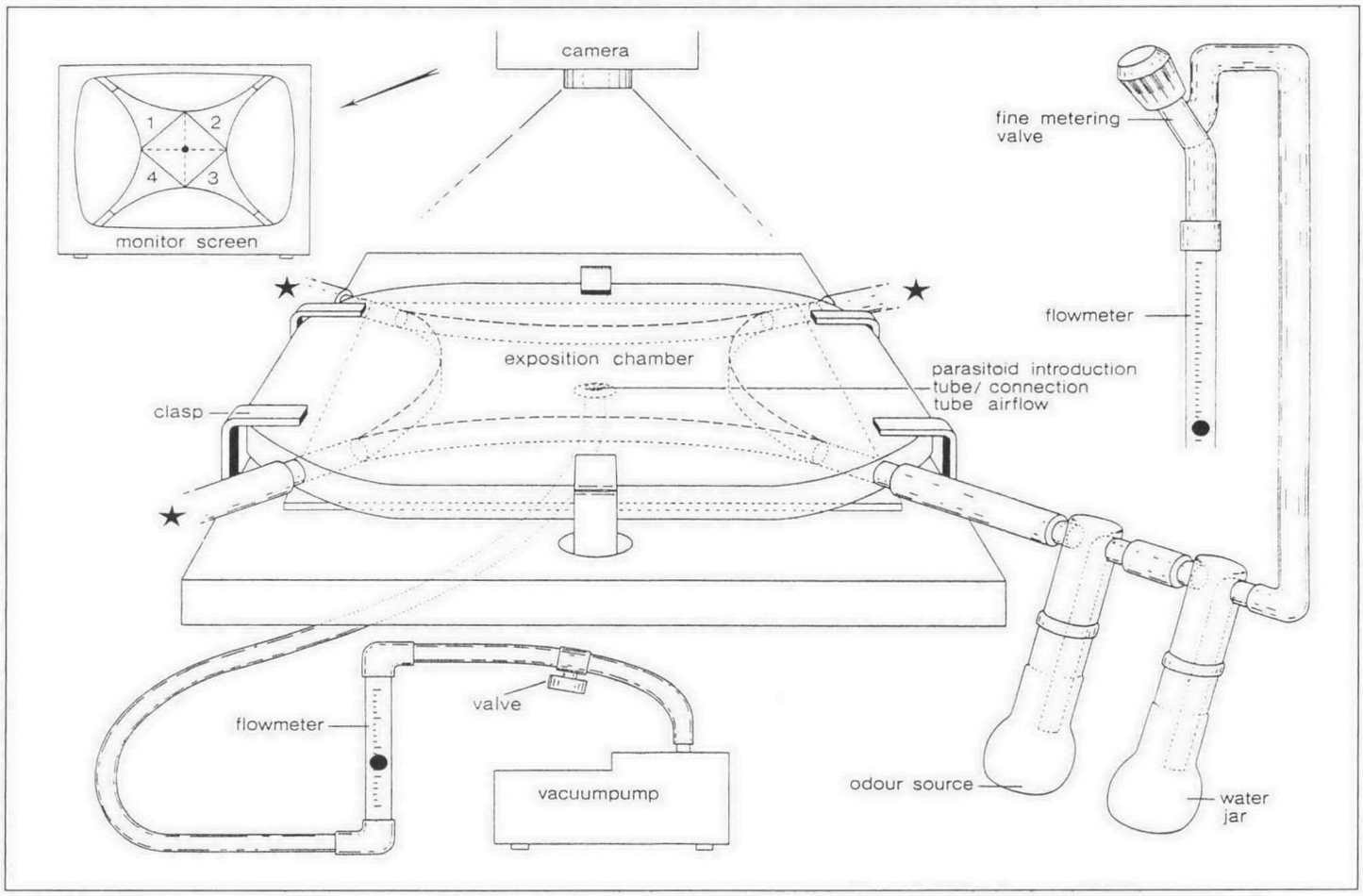

Fig. 1. Perspective view of the four-armed airflow olfactometer.

the two glass vessels in order to create two chambers. The lower chamber contained the odour source and the upper one was used as walking arena for the wasp. Visibility of the wasp was improved by introducing a plastic ring in the upper chamber to create a walking arena of 6.3 cm diameter. The ring was smeared with vaseline to prevent wasps from mounting the wall. Into the lower chamber *C. maculatus* females or males were placed in a plastic netting to prevent them walking onto the filter paper. The female parasitoid was introduced in the middle of the walking arena in the upper chamber via a small metal tube inserted in a small hole (diameter 0.5 cm and normally closed by a gum stopper) in the wall of the upper chamber. The wasp performed a short flight ('jump') to leave the tube. The arena was placed on a platform of plexiglass illuminated underneath to prevent wasps from flying upwards. The behaviour of the wasp was recorded by a video camera. To prevent disturbance the whole experimental set-up was covered by white cloth. The experiments were executed in a climate room at 30°C and 40% r.h.

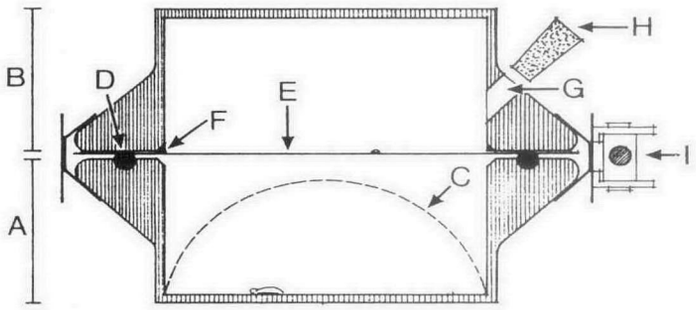

Fig. 2. Cross section of the circular diffusion olfactometer: A. lower glass vessel; B. upper glass vessel; C. plastic netting to prevent beetles to contact filter paper; D. rubber ring; E. filter paper; F. plastic ring covered with vaseline to prevent beetles from climbing the wall; G. introduction channel; H. gum stopper; I. clasp.

Unmated male and virgin female beetles were obtained in the same way as described earlier. Mated females of *U. lariophaga*, which had an oviposition, were obtained as follows. Females enclosed in tubes for a period up to 16 hours were sexed, isolated in capsules and moved to the climate room (30°C; 40% r.h.), where they were offered a one-day old host egg on a cowpea bean. The wasps were used in the experiment 45 to 90 minutes after oviposition. The time lapse was necessary because wasps immediately after oviposition showed a reduced walking speed and an increased turning rate, as experienced for *Trichogramma evanescens* by Gardner and van Lenteren (1986).

There were three treatments repeated with 20 wasps: odour of virgin female beetles, unmated male beetles, and no odour (control). Under rearing conditions unmated males and virgin females were left for 24 hours in the lower chamber to ensure accumulation of host odours in the olfactometer. An introduced wasp was observed for 4 minutes. Wasps flying up were discarded from the analysis. Each treatment was repeated no more than three times per day because a wasp could leave an odour trace on the surface, which could influence the behaviour of subsequent wasps.

The recorded walking patterns were drawn with a marker on a plastic sheet fixed to the screen of the video recorder. The pattern was divided into intervals of 5 seconds and the middle point of the arena marked on the sheet. The pattern was transferred to the Leescal program by reading the coordinates with the help of a mouse in a X-Y tablet (Pijnacker-Hardijk 1985; van Rijsoort and Sutterlin 1991). A new coordinate was registered if the coordinate was 2 mm vertically or horizontally from the previous coordinate. As the walking pattern on the sheet had been enlarged four times, a coordinate was taken every 0.5 mm. The Leescal program calculated walked distance by totalling distances between coordinates. The average speed was defined as the total distance walked divided by the time spent walking. Time not walked was only registered when the wasp stood still for the entire time interval of 5 seconds. The exactness of this parameter thus depends on the length of the time interval selected. A higher speed does not necessarily make searching more intense. There is evidence that walking speed decreases with a more tortuous walking path (Casas 1988).

Tube diffusion olfactometer

A tube diffusion olfactometer was used to test the reaction of *C. maculatus* males and *U. lariophaga* females to female beetle odour, synthetic sex pheromone and beetle egg odour. The olfactometer consisted of an open glass tube of 100 mm (diameter 8 mm) with a hole in the middle (at 50 mm) through which the insect from a capsule was introduced. To test the reaction of bruchid males, plastic caps were inserted in the open ends, one with an odour source and the other with the control. For the wasps, caps could not be used because the edges appeared to form a physical barrier. The odour source, therefore, was put at one end of the tube and the control at the other end. The ends were covered with parafilm.

Three odour sources were tested: female beetles; synthetic sex pheromone; and eggs. Filter paper could absorb for 2–3 days natural odours from 15 *C. maculatus* females in the circular diffusion olfactometer (Fig. 2). There was no contact between the beetles and the filter paper.

The National Resources Institute (NRI), Chatham, U.K. provided the pheromone, which was an identified component of the sex pheromone of *C. analis*, viz, (Z)-3-Methylhept-2-enoic acid (Cork et al. 1991). A mixture of this component with its isomer (Z)-3-Methylhept-3-enoic acid in a ratio 10:90 gave the highest response of *C. maculatus* males in a pitfall trap (A. Cork, pers. comm.) and was therefore used in our experiments. The synthetic sex pheromone of *C. maculatus* was solved in n-hexane and 1 mL of the solution was absorbed to filter paper. The control filter paper absorbed 1 mL of n-hexane. To study whether *U. lariophaga* was attracted by egg odours, 5–10 eggs on a marble were placed at one end of the tube, and a clean glass marble at the other end as a control.

Experiments were carried out in a climate room (30°C; 30% r.h.). The experimental setup was placed under a white cloth on a white surface. The light intensity was 4100 lux. Before use, the olfactometers were cleaned with n-hexane, rinsed with hot water and dried in a stove (50°C). Caps, capsules and parafilms were used only once and then disposed off.

Unmated male beetles of < 22 hours old and naive female wasps of < 19 hours old were used. Thirty minutes was found to be the optimal period to establish an odour gradient for the wasps and 15 minutes for the beetles. The location of the beetle or the wasp in the tube was continuously recorded. The attraction of wasps to egg odours was established by recording the time of arrival of the wasp at one end of the tube. Recording was then stopped because behavioural changes were expected to occur once the wasp encountered an egg.

Results

Four-armed olfactometer

The time spent in the four sectors of the olfactometer was considered. Wasps without previous experience to cowpea and without oviposition experience spent about 5 times longer in the cowpea odour sector than in the clean airflow sectors (Fig. 3). Of the 48 wasps observed, 34 spent most time in the odour sector. Female wasps reared on cowpea and without oviposition experience were also able to detect whether cowpea was infested with *C. maculatus* as they spent 2 to 3 times longer in the sector with odour of infested cowpea than in the sectors with cowpea odour only (Fig. 3). Of the 35 wasps observed, 26 spent most time in the sector with infested cowpea odour.

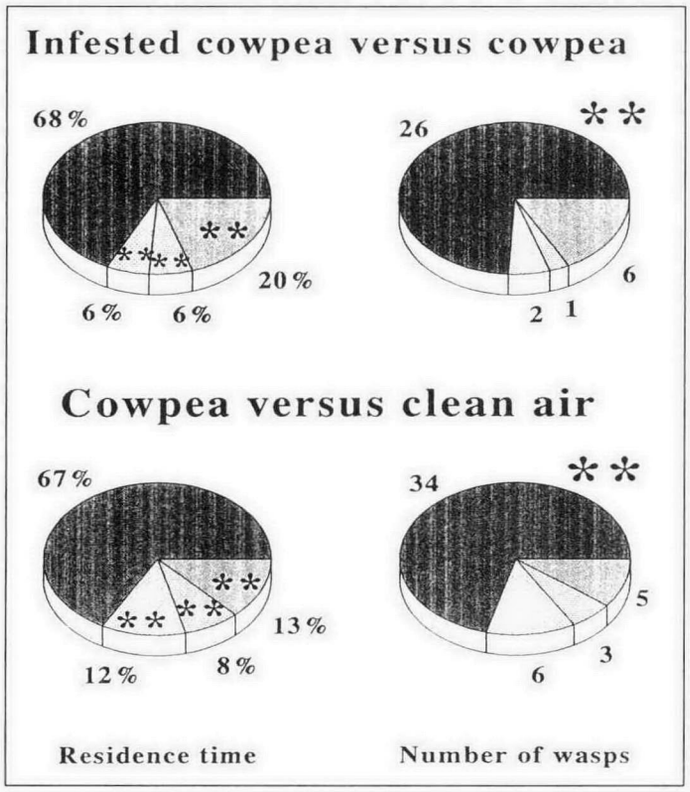

Fig. 3. Response of *U. lariophaga* female wasps to odours of cowpea and cowpea infested with *C. maculatus* in a four-armed airflow olfactometer: average time per sector (%) and the number of wasps longest in that sector. Significant differences between sectors for pies with number of wasps (χ^2-test) and between the treatment sector (dark shaded) and the control sectors (light shaded) for pies with residence time (Friedman 2-way Anova test): **= P≤ 0.01.

It was also studied whether *U. lariophaga* females without oviposition experience and without previous exposure to cowpea remained longer in the sector with either unmated male or virgin female beetle odour than in the clean air sectors. Although in these treatments the wasps spent most time in the sector with bruchid odour, this was only significant for females (Fig. 4). Female wasps spent almost twice as long in the female bruchid odour sector than in the control sectors. Of the 57 wasps observed, 29 spent most time in the odour sector.

Female wasps without an oviposition experience and without previous experience to cowpea were arrested by odours emanating from bruchid eggs as the residence time in this sector was at least twice as long as in the control sectors (Fig. 4). Of the 47 wasps observed, 26 spent most time in the egg odour section.

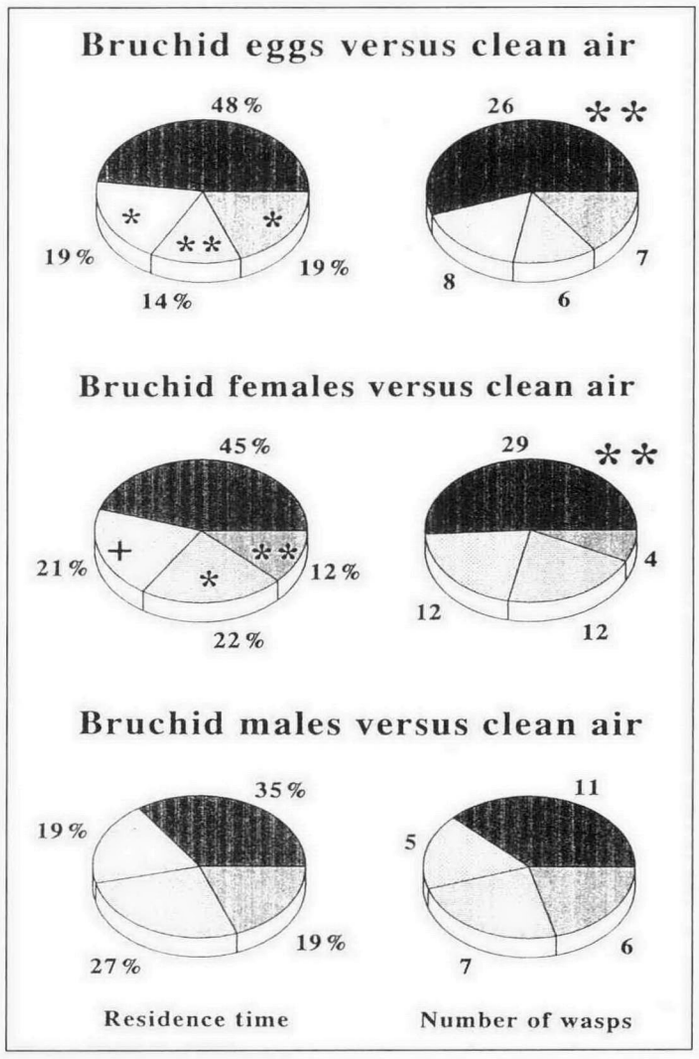

Fig. 4. Response of *U. lariophaga* female wasps to odours of female and male adult bruchids and their eggs in a four-armed airflow olfactometer: average time per sector (%) and the number of wasps longest in that sector. Significant differences between sectors for pies with number of wasps (χ^2 -test) and between the treatment sector (dark shaded) and the control sectors (light shaded) for pies with residence time (Friedman 2-way Anova test): ** = P ≤ 0.01; *= P≤ 0.05; += P≤ 0.10.

Diffusion circular olfactometer

About one-third of the introduced wasps flew up shortly after they were introduced in the olfactometer. This response occurred in all treatments (χ^2-test). Of the remaining wasps, those exposed to the odour of virgin females walked more, faster and a longer distance than those exposed to male odour or no odour (control; see Table 1).

Diffusion tube olfactometer

When bruchid female odour was placed in one end of the tube, *C. maculatus* males spent 80% in that end and 64% of the time when synthetic sex pheromone was used. From the longer residence time, it seems that bruchid males are more arrested by the female odour than synthetic sex pheromone (Fig. 5).

Table 1. Effect of bruchid adult volatiles in a circular diffusion olfactometer on the behaviour of *Uscana lariophaga* females (20 wasps per treatment).

Odour source	Clean air	Bruchid males	Bruchid females
Path length (mm)	163 ± 92a	202 ± 106a	336 ± 112b
Time not walked (s)	117 ± 51a	98 ± 57a	54 ± 40b
Walking speed (0.01 mm/s)	120 ± 40a	135 ± 34a	177 ± 31b

Means with a common letter in the same row are not significantly different at the 5% level of significance (Mann-Whitney U test).

Similarly, *U. lariophaga* females were also arrested by female bruchid odour and the synthetic sex pheromone: 64 and 61% of the observation time respectively was spent by the wasps in the halves of the tube containing the odour (Fig. 5).

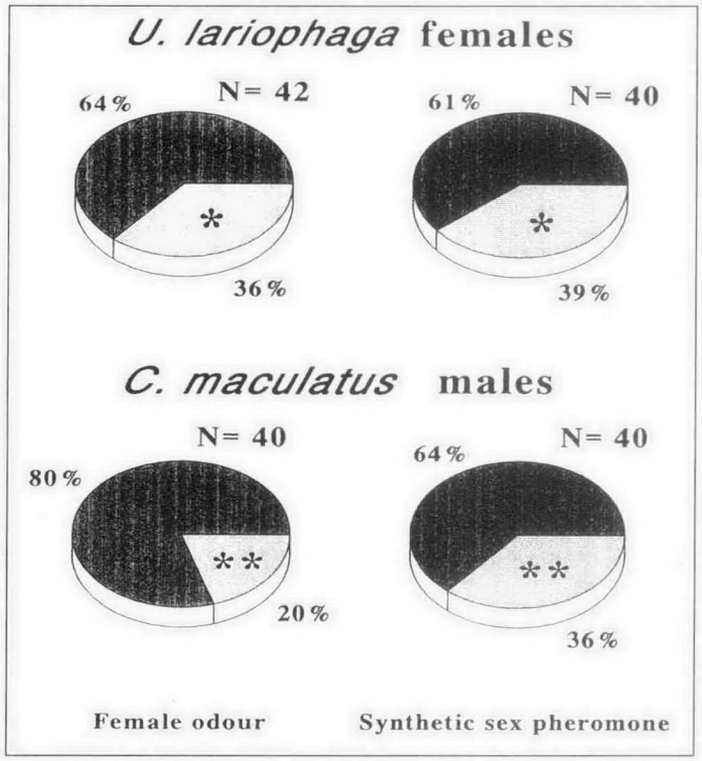

Fig. 5. The effect of odours of female bruchids and synthetic sex pheromone of *C. maculatus* in a tube diffusion olfactometer on percent time spent by male bruchids and *U. lariophaga* in the treatment half (dark shaded) and the control half (light shaded) of the tube (total observation time 10 minutes). Wilcoxon signed-ranks test: **= P≤ 0.01; *= P≤ 0.05; N= number of replicates.

The attraction of eggs on the *U. lariophaga* female wasp was checked by scoring which end of the tube a wasp would reach first, the marble with eggs or the marble without eggs. Of the 40 wasps tested, 33 reached the baited end first indicating attraction by egg odour. The egg bait was reached almost twice as quickly by the female wasp (average 166 seconds) compared to the baits of female odour (281 seconds) and synthetic sex pheromone (301 seconds).

Discussion

Experiments in the four-armed airflow olfactometer showed an arrestment effect of volatile chemicals emanating from dry cowpea grains. In this experimental set-up it could not be ascertained whether attraction was also involved. During the dry season, the wasp develops in granaries where the air is saturated with cowpea odour. Arrestment or attraction by cowpea odour would be beneficial if different harvested products are stored in the same granary or if wasp migration into the granary occurs. But no data on migration are available. Long range attraction may occur in the field when the female wasps are searching for *C. maculatus* eggs laid on maturing cowpea pods.

In the four-armed airflow olfactometer *U. lariophaga* was arrested by odour of bruchid-infested cowpeas compared to cowpea odour. This effect was analysed further by checking whether the arrestment was caused by odour from unmated male or virgin female beetles, or from host eggs. Although the residence time in the treatment sector was longest for all these host odours it was only significant for virgin females and eggs. This suggests that a female host component, such as a sex pheromone, is involved.

Initially *U. lariophaga* reacted satisfactorily in the four-armed airflow olfactometer. However, during subsequent studies the locomotory activity of the wasps ceased. Checking and changing experimental conditions such as underpressure, electrostaticity, light, humidity and age of the wasps did not produce improvements. Odour field boundaries were also checked but appeared clearly separated when visualised with introduced smoke. The experimental set-up was abandoned.

The investigation continued using a circular diffusion olfactometer in which the locomotory reaction of the wasp was studied in air saturated with odours of bruchid males and females. The volatile chemicals emanating from virgin bruchid females did have an effect on the walking behaviour of experienced female wasps, but again no effect was obtained from male bruchid odour. The activity and mobility of the wasps increased in the presence of virgin female odour. They walked faster, longer and consequently a longer distance in a given time. In an area where virgin female odours are present, the parasitoid is more likely to find new cues leading to the host egg than in an area without odours. Therefore, prolonged searching will increase the chance of meeting new cues. But, is it logical to expect a higher mobility of *U. lariophaga* wasps in an area where more cues can be expected. Moving more slowly in orthokinetic and klinokinetic behaviour in the odour laden air may also increase the chance of encountering new cues (Li et al. 1987; Kennedy 1978; Shu and Jones 1989). However, tortuosity of walking was not checked because the walking arena was too small and the result would be too much influenced by turning at the edges. The higher mobility of the wasp would be adaptive if the odour of virgin female bruchids acts as an attractant. In this experimental set-up it could not be studied whether arrestment occurs in turning behaviour when leaving the odour field because there were no odour field boundaries (Bell and Tobin 1982; Gardner and van Lenteren 1986; Casas 1988; Waage 1978).

The walking arena of the circular diffusion olfactometer was considered to be too small because of the high mobility of the wasp. At a speed of up to 2 mm per second, a wasp quickly reached the edge, and repeated encounters with the edge may have affected the wasp's behaviour. Thus a plastic ring was covered with vaseline to prevent the wasps mounting the wall and consequently has restricted their movements. However, a larger arena could not be covered by the video camera which was used to register the wasps. Therefore, other diffusion olfactometers were used taking into account that in cowpea granaries little airflow can be expected and that female wasps may search their hosts by using odour gradients brought about by diffusion.

The tubular diffusion olfactometer was designed by trial and error because there were no data on the rate of diffusion of bruchid odours in stagnant air. The tube was divided into a treatment and a control section and 15 and 30 minutes (for beetles and wasps respectively) was allowed to lapse after introducing the odour source to obtain a detectable odour gradient in the tube. The apparatus has the advantage that tube and caps were cheap and therefore disposable. Consequently, there were no problems about traces remaining and cleaning the device.

In this diffusion olfactometer, bruchid males were arrested by filter paper that had absorbed bruchid female odours in the circular olfactometer, while clean filter paper did not elicit such a response. The synthetic sex pheromone, a blend of two components (as described in material and methods) was also attractive to bruchid males. When the same components were tried out on the *U. lariophaga*, they elicited a similar reaction indicating that at least sex pheromones act as a kairomone. More female wasps reached the marble with bruchid eggs than the marble without eggs at the other end of the tube.

The test with the tube diffusion olfactometer indicates that sex pheromones are involved in host searching by *U. lariophaga*. However, it is not certain that this is the only component involved. Egg volatiles have been shown to cause an arrestment in the four-armed airflow olfactometer and an attraction in the tube diffusion olfactometer. This could have been explained if the eggs had adsorbed sex pheromones. However, other female components, such as the putative epideictic pheromone, specific egg odours or mixtures of components may also be involved. Further investigations should be carried out to clarify which chemical components act as sex and epideictic pheromones in *C. maculatus* and as kairomones in *U. lariophaga* and to establish their origin.

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