

Olfactory response to egg odours and the effect of oviposition marker in uniform egg spacing of *Callosobruchus maculatus* (F) Brazil strain (Coleoptera; Bruchidae)

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ABSTRACT

Mechanism of egg spacing behaviour and the olfactory response towards the oviposition marker in cowpea weevil *Callosobruchus maculatus* (F) Brazil strain was investigated. An ether soluble chemical substance (oviposition marker) secreted at the time of oviposition appears to be largely responsible for uniform egg spacing. The presence of this chemical is mediated by contact chemoreception. The study demonstrated that the adult females have the ability to distinguish their own oviposition markers and appear to ignore the oviposition markers deposited by other females in further oviposition on egg-laden seeds.

Key words: *Callosobruchus maculatus*, cowpea weevil, egg-spacing behaviour, intraspecific competition, olfactory response, oviposition marker.

INTRODUCTION

When resource competition is likely, a mechanism may evolve in females to recognize and avoid conspecific egg loads or larvae on hosts (Thompson and Pellmyr 1991). In order to regulate the competition, ovipositing females tend to space eggs uniformly by using a variety of cues of which visual egg recognition (Benson *et al.* 1975; Williams and Gilbert 1981; Rothschild and Schoonhoven 1977) and chemical recognition (Corbet 1972; Prokopy 1976; Raina 1981) are the most prevalent. In majority of the known cases, chemical recognition of the presence of eggs is achieved through contact stimulus where a female reacts to some oviposition marker secreted during the course of oviposition. However, a volatile deterrent associated with their eggs is, also detected by the females of certain species (Rothschild and Schoonhoven 1977; Prokopy 1972). Among phytophagous insects, the cowpea weevil is a classic example in which the phenomenon of chemical recognition of eggs has been widely observed (Mitchell 1990). The females of cowpea weevil are known to mark the utilized

host with a pheromone (oviposition marker) which deters repeated egg laying on the seeds (Wasserman 1981; Giga and Smith 1985). However, the exact role of this pheromone in mediating egg dispersion is uncertain as similar deterrents can be obtained from egg free glass beads exposed to females (Messina and Renwick 1985a). In addition palpectomized females were able to avoid seeds with pheromone but did not distribute eggs evenly, indicating that some other cues may be used in the egg spacing mechanism (Messina and Renwick 1985b)

The experiments described below were designed to determine the following:

The role of oviposition marker and physical presence of eggs in uniform egg spacing.

The response of females to the seeds bearing eggs laid by other females, and

The olfactory response of females to the oviposition marker.

MATERIALS AND METHODS

The cultures of *C. maculatus* Brazil strain were obtained from the Natural Resources Institute (Formerly Tropical Stored Product Centre) U.K. and maintained on cowpea or black beans (*Vigna unguiculata* (L.) Walp, in the controlled temperature and humidity (CTH) room, maintained at $30 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH, in the Department of Pure and Applied Zoology, University of Reading, Reading,

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U.K. For routine cultures, about 100 unsexed adults were placed in 850 ml glass jars containing approximately 200 g of seeds. The jars were closed with filter papers sealed over the aperture with paraffin wax and were placed in oil trays to reduce the possibility of cross infestation and invasion by mites. The insects collected from routine cultures were then reared on green gram seeds (*Vigna radiata* (L.) Wilczek) for two generations by using the same experimental procedure to obtain experimental insects used in this study.

Olfactory response towards oviposition marker

The virgin males and females were obtained by isolating green gram seeds showing windows in the glass vials. The males and females were sexed using the male and female characteristics described by Southgate *et al.* (1957). The most important sexually dimorphic character in distinguishing males and females of *C. maculatus* is that in males, the pygidium possesses a median ventral curvature into the hypogidium whereas in females no such curvature is found. The virgin males and females were paired and kept for 12 h in separate petri dishes having green gram seeds. Thus the seeds with fresh eggs required for the experiment were obtained. The olfactory response of these females towards the seeds bearing their own eggs and the seeds without eggs were investigated in the two choice experiment using olfactometer. The design of the olfactometer used is illustrated in Figure 1. This olfactometer (5 cm high x 9 cm diameter) has 6 chambers on the surface at equal distance where the host seeds serving as odour sources can be housed. Each chamber corresponds to an outlet on the vertical wall to which an air pump can be connected. These chambers are superficially connected to each other and to the centre by grooves. When the seeds are housed in these chambers the air current permeated with the odour of seeds travels towards the center of the olfactometer along the grooves. Thus the test animal placed at the center is free to walk (but not fly as an inverted petri dish is placed on the top of the olfactometer to prevent escape) towards the odour source. The seeds with two eggs or without eggs were placed alternately in the chambers and covered with cotton wool to avoid visual stimulus. Thus each chamber contained four seeds with or without eggs. The female was placed in the center of the olfactometer and an inverted petridish was placed on the top of the olfactometer to prevent escape. Air current permeated with the seed odour and egg odour was directed towards the center where the insect was

placed. Once the insect had moved more than 1/3 of the distance towards a particular odour source, it was recorded as orientation towards that particular odour source. The olfactory behaviour was observed in CTH room maintained at 25°C and 70% RH. Females were observed for one hour in an experiment replicated 5 times.

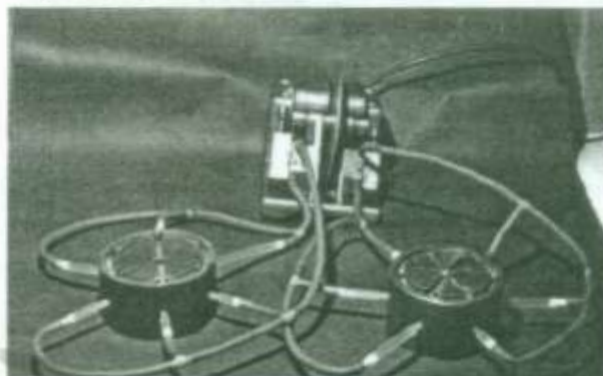


Figure 1. The olfactometer with the air pump used in the experiment

Effect of oviposition marker on uniform egg spacing

The virgin males and females were paired and kept for 12 h for mating without a substrate. To obtain egg laden seeds (one egg seed¹), few females were kept in petri dishes containing green gram seeds for 4 hours. The effect of the following treatments on the uniform egg spacing was investigated with each treatment replicated 4 times in CTH room maintained at 25 °C and 70 % RH. Each treatment consisted of 6 green gram seeds arranged in a sequence in glass petri dishes. The selection of green gram seeds for this experiment was based on two reasons. Firstly, because of small seed size, females tended to space eggs more uniformly on green gram seeds (Wijeratne 1991). Secondly, their dark green testa permitted high visibility of eggs. Individual females that were kept with males for 12 hours were introduced to each petri dish. The treatments were as follows:

Egg when laid is scraped off with a scalpel.

When an egg is laid all the seeds were ether washed.

Egg is scraped off and all the seeds were ether washed.

Egg-laden seed is replaced with a seed bearing an egg (less than 4 h old) of another female.

Control.

The experiment was terminated when the 6 seeds offered in the control treatment were saturated with

eggs (1 egg seed⁻¹).

The effect of treatments on egg spacing behaviour was analyzed using an index of uniformity (U) described by Messina and Mitchell (1989). This other index of uniformity is based on the number of mistakes committed by a female in distributing her eggs, which is defined as the minimum number of eggs that must be relocated to transform an observed distribution into the most uniform one possible. The observed number of mistakes committed by a female is then compared with the number of mistakes that would be expected if the female distributed the same number of eggs randomly among seeds. Once the observed and expected numbers of mistakes are known, $U = \text{expected number of mistakes} - \text{observed mistakes} / \text{expected mistakes}$. Thus $U=0$ if eggs are laid randomly, and reaches the maximum value of 1 if eggs are distributed in the most uniform way possible.

RESULTS

Analysis of variance of the data (Table 1) in the experiments carried out to examine the olfactory response of the female to oviposition marker indicates that orientation time towards egg-laden seeds and the seeds without eggs did not differ significantly. The mean values of the orientation time are given in Table 2.

The egg spacing differed significantly between the treatments (Table 3). Females of *C. maculatus* were able to disperse eggs extremely uniformly in t

Table 1. Analysis of variance of the olfactory response of the females of *C. maculatus* Brazil strain towards the oviposition marker

Source	DF	SS	MS	F	P
Treatment	1	40.0	40.0	1.09	0.357
Replicate	4	484.6	121.15	3.30	0.137
Residual	4	147.0	36.75		
Total	9	671.6			

Table 2. Mean olfactory response of females of *C. maculatus* Brazil strain towards egg-laden seeds and pristine seeds (values indicate mean time in minutes during one hour)

	Pristine seeds	Egg-laden seeds
Olfactory response (min.)	18.8	14.8

Standard error of the difference of means = 3.83

the control treatment and in the treatment where the egg was scraped off without ether washing (Table 4). Random egg distribution was observed in all the other treatments. When the seeds were ether washed after the egg was scraped off, the egg distribution appeared to be slightly clumped ($U=0.19$). However, the U value was not significantly different from the U values of the treatments where all the seeds were ether washed ($U=0.03$) and where the egg-bearing seeds were replaced with a seed bearing an egg laid by another female.

DISCUSSION

The results of the experiments clearly demonstrated that an ether soluble substance deposited at the time of oviposition is largely responsible for uniform egg spacing.

Table 3. Analysis of variance of the effect of oviposition marker in egg spacing behavior of *C. maculatus* Brazil Strain

Source	DF	SS	MS	F	P
Treatment	4	5.2236	1.3059	5.95	0.007
Replicate	3	0.9075	0.3025	1.38	0.297
Residual	12	2.6346	0.2195		
Total	19	8.7657			

Table 4. Mean U values in different treatments

Treatment	U value	Distribution
Scraped off	1.00a	Uniform
Ether washed	0.03b	Random
Scraped off & ether washed	0.19b	Random
Replaced with another female's egg	0.09b	Random
Control	1.00a	Uniform

Means followed by the same letter are not significantly

These findings agree with Wasserman (1981) and Giga and Smith (1985) who also demonstrated the same phenomenon in *C. maculatus*. It also appears that the existence of this substance is mediated by contact chemoreception as females failed to show any olfactory response to egg odours. Detection of the chemical is thought to be mediated by the receptors located in the labial palpi and maxillary palpi of this insect (Messina and Renwick 1985b). Generally both males and females are known to produce these chemicals. However, females produce much larger quantities than males which are several

times more active (Yamamoto 1990).

The results further demonstrated that the presence of eggs with ether soluble marker removed, had little effect in uniform egg spacing. This is contradictory to the findings of Messina and Renwick (1985a) and Yamamoto (1990) who reported that the presence of eggs acts as a deterrent for oviposition. The most important of all is that a female did not respond to the oviposition marker of other females. This indicates that the females have the ability to distinguish their own eggs from the eggs laid by other females. The ability of a female of one species to distinguish hosts bearing her own eggs from those bearing eggs of another female of the same species may be an evolutionary solution to intraspecific competition enabling her to place eggs where offspring have the best chance of survival (Wijeratne 1994). This ability is specially important for the insects that complete the entire life cycle within the seeds chosen by their parents. A female faced with seeds bearing eggs laid by other females will generally benefit more if she places an egg on them rather than on a seed with her own eggs. Although the chances of survival under such conditions are vague, any survivors will increase the female fitness above zero level which would apply if she did not lay an egg at all (Smith and Lessells 1985).

A proximate mechanism for the discriminatory pattern of oviposition observed in this study may be individual variation in oviposition marking pheromones. The intraspecific differences in the pheromones composition has been shown in a wide range of insect pests (Birch and Haynes 1982).

Oviposition deterrent chemicals can form an important component of a sound pest management system. The potential use of these chemicals in the management of cherry fruitflies and other fruitfly species has been highlighted (Boller 1981). Use of these chemicals in combination with other effective trapping systems might become a useful component of future integrated pest control programmes for stored-grain insects where chemical pesticides have restricted use. However, identification of the chemical nature of these compounds and understanding the exact behavioural mechanism towards them will be a primary prerequisite before such control programmes are implemented.

CONCLUSION

Females of *C. maculatus* tend to distribute eggs uniformly among host seeds and thereby reduce

competition among larvae within seeds. This study reveals that a chemical deposited at the time of oviposition largely governs the uniform egg distribution. The detection of this chemical is mediated by the contact chemoreception; the physical presence of eggs did not discourage females from laying eggs on seeds that already bear conspecific eggs. The females have the ability to distinguish the seeds bearing their own eggs from those bearing the eggs of other females.

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