

## A Study of Ovipositional Behaviour of *Anagyrus pseudococci* a Parasitoid of Mealybugs

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### ABSTRACT

The ovipositional behaviour and success of *Anagyrus pseudococci* (Girault), an encyrtid endoparasitoid of mealybugs, *Planococcus citri* (Risso) and *Pseudococcus affinis* (Mask.) were studied in the laboratory. Behavioural sequences during oviposition starting from the searching for the host to the post-oviposition activities were described. Once encountered, the mealybug host was examined and probed by the ovipositor of the parasitoid. Oviposition success was influenced by the defence activity of the host. *P. citri* was less able to defend itself and was more susceptible to parasitism than *P. affinis*. Oviposition experience of the parasitoid played an important role in increasing the efficacy of subsequent oviposition.

**Keywords:** *Anagyrus pseudococci*, *Planococcus citri*, *Pseudococcus affinis*, Parasitoid, Oviposition.

### INTRODUCTION

Mealybugs, *P. cirri* and *P. ajjinis* (Homoptera: Pseudococcidae) are serious pests of many crops and ornamental plants and attack the barks, twigs, leaves, flower buds, and fruit [2,5]. (*Pseudococcus ajjinis* has been recently renamed to: *P. viburni sig.*). Control of mealybugs by pesticides is not easy due to their waxy body covering and also because they shelter in crevices [9]. The use of broadspectrum insecticides to control mealybugs often eliminates natural control agents [8] and induces rapid increases in mealybug population that damage the crop severely. Spraying also causes problems, with public access to displays of ornamentals in botanical gardens, amenity areas, and offices. Because of the difficulties associated with chemical

control the use of chemicals is not recommended also [6]. Attention is now being turned to ways of biologically controlling mealybugs. The encyrtid parasitoid *Anagyrus pseudococci* is a promising agent for controlling mealybugs biologically.

Successful parasitism requires that the following successive steps be carried out: a) habitat location, b) host location, c) host acceptance, and d) host suitability, [3]. A fifth step, referred to as host regulation, has since been added by Vinson [12]. The first three steps make up the host selection process. The first step, habitat location, is further divided into two steps, and host acceptance has been divided into three steps by Vinson [13]. Thus, altogether, seven steps are involved in successful parasitism by parasitoids: i) habitat preference, ii) potential

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host community location, iii) host location, iv) host examination, v) ovipositor probing, vi) ovipositor drilling and vii) oviposition.

Through these successive processes, the host list becomes limited, though many potential species are available in nature [4]. With the advancement of biological control programmes, the factors involved in successful parasitism have been more frequently studied.

Ovipositional success is one of the important factors that results in effective parasitism. Oviposition behaviour does not always imply oviposition [7]. Females of *Cardiochiles nigriceps* with ovipositional experience are more likely to search for and attack hosts than an inexperienced female [11].

The objectives of this study were to observe: a) the behavioural sequence exhibited by female *Anagyrus pseudococci* at the time of oviposition on two different mealybug hosts, and b) the influence of previous ovipositional experience on egg laying and host selection.

## MATERIALS AND METHODS

The adult mealybugs *Planococcus citri* and *Pseudococcus affinis*, which were used as hosts, were reared on potato sprouts in the laboratory at a temperature of  $27\pm1^{\circ}\text{C}$ , 12 hL: 12 hD photoperiod and  $60\pm10\%$  r.h. The parasitoid was reared on *P. citri* larvae in the same environmental conditions. Adult parasitoids were fed on 50% honey solution.

Female wasps, 4-7 days old, were selected for this study. A small Perspex dish (15 mm diameter and 5 mm height) with a lid was used as the experimental arena. An experienced (with previous oviposition experience on mealybug hosts of both species) female was introduced into the arena containing an adult female mealybug. The behavioural sequence of *A. pseudococci* during oviposition on mealybug hosts was observed under the microscope. Those parasitoids which did not

respond to mealybugs up to 10 minutes after being transferred into the arena were discarded and replaced by new ones. Successful egg deposition by the parasitoid in two mealybug species was observed.

Following oviposition, parasitized mealybugs were kept separately in small glass vials for 72 hours at  $27^{\circ}\text{C}$  and then preserved for 24 hours in 80% ethanol, which removes the wax covering of the mealybugs. Each mealybug was then dissected under the microscope in insect saline solution (9% NaCl diluted with water) to determine the presence of eggs.

In another experiment, similar-aged, inexperienced females (which were not previously exposed to the host for oviposition) were selected for parasitizing adult female *P. citri* only. The parasitoid was transferred to another mealybug after each oviposition. If a parasitoid showed no response to the host up to 30 minutes after being transferred, the observation was discontinued. Three successive ovipositions were observed between 1400 and 1600 at  $26\pm0.5^{\circ}\text{C}$  in the laboratory. The time spent in different activities during successive oviposition was recorded by stopwatch. Data of the experiments were analysed by one way ANOVA.

## RESULTS AND DISCUSSION

The parasitoid *A. pseudococci* showed the following behavioural responses during oviposition:

- 1) Searching: The parasitoid moved randomly while moving its antennae upward and downward successively.
- 2) Approach to host: When she approached the host, she usually made a sharp turn towards it.
- 3) Host encounter: After reaching the host, she examined it for  $9.7\pm0.37$  to  $13.56\pm0.43$  seconds, depending on her experience, by drumming the antennae. After antennation, the parasitoid accepted or rejected the host.

4) Ovipositor tapping: After antennation, if a suitable place for drilling was found, she turned her body clockwise or counterclockwise and flexed the tip of her abdomen to place the ovipositor in position. The body of the parasitoid was arched and the ovipositor was sheathed.

5) Drilling: After searching for a suitable region for ovipositor insertion, she began drilling for a few seconds. Drilling usually began from the edge of the host body. If the ovipositor was deflected, it was again placed into position.

6) Ovipositor insertion: The ovipositor was inserted into the host fast and rhythmically. During penetration of the ovipositor and insertion of the egg into the host body, the wings of the parasitoid were raised vertically above her body. At the moment of penetration, the host showed a sort of defence activity by bending her body and rapidly moving the abdomen up and down. Sometimes a drop of fluid exudate came out alongside the ovipositor. This defence activity was more frequent in *P. affinis*.

7) Oviposition: A single egg was inserted into the host through its ovipositor, and a stalk of the egg protruded out of the host body surface after a successful oviposition. In almost all cases, when insertion of the ovipositor was followed by a pumping movement of the abdomen, an egg was found upon dissection and the encounter was classified as oviposition. The process took  $30.5 \pm 1.6$  to  $37.1 \pm 2$  seconds depending on experience.

8) Withdrawal: The ovipositor was withdrawn from the host and sheathed.

9) Post-antennal drumming: This occurred for a few seconds immediately after the ovipositor was removed.

10) Departure: The parasitoid left the host and moved away.

II) Preening: After a successful attack, the wasp cleaned the different parts of its body

using its antennae, legs, mouthparts and wings. Preening took  $97.7 \pm 3.3$  to  $101.2 \pm 4$  seconds. 12) Resting: At this stage, she remained motionless.

The percentage of successful oviposition by *A. pseudococci* in its two mealybug host species is presented in Table 1, and indicates that the parasitoid significantly prefers *P. citri* to *P. affinis* ( $P < 0.01$ ). This may be due to more defence activity on the part of the latter species and demands further research work.

Table 1. Successful oviposition by *A. pseudococci* in *P. citri* and *P. affinis*

Host	% Mean oviposition	s.e
<i>P. citri</i>	91.07	1.09
<i>P. affinis</i>	76.79	1.54

Means in the same column are significantly different from each other ( $F = 7.59$ ,  $P < 0.001$ )

No. of replications 7. Each replicate consisted of 24 mealybugs.

The amount of time spent on each activity by the parasitoid on its host (Table 2) indicated that those females having previous ovipositional experience required significantly less time in antennation than inexperienced females ( $P < 0.01$ ), but no significant difference was observed in ovipositor drilling-withdrawal and preening between experienced and inexperienced parasitoids (Table 2).

The behavioural sequence of oviposition of the parasitoid was more or less similar to both host species. The behavioural sequences which have been presented in this study, usually occurs in almost all successful oviposition but may be interrupted at any point. Once interrupted, the parasitoid would begin again with "searching".

The antennae appear to play an important role, not only in host finding but also in helping to locate the place of oviposition. Witsack [15] has described the role of the antennae in host finding by the parasitoids.

Table 2. Time spent in various activities by female *A. pseudococchi* in successive oviposition in *P. citri*

Activity	Mean time(s) $\pm$ s.e in successive oviposition			LSD at 5% level for oviposition	
	1st	2nd	3rd	1st & 2nd	2nd & 3rd
Anlennalioiv	13.56 $\pm$ 0.43 a	10.83 $\pm$ 0.58 b	9.70 $\pm$ 0.37 b	1.31	1.47
F2, 35=18.34					
Drilling-ovipositor withdrawal	37.06 $\pm$ 2.01	33.67 $\pm$ 2.02	30.50 $\pm$ 1.60	---	---
I <sup>2</sup> 2, 35=2.74 NS					
Preening	101.19 $\pm$ 3.97	106.75 $\pm$ 4.57	97.70 $\pm$ 3.28	---	---
F2, 35 = 1.09 NS					
Replication	16	12	10		

Mc;ms followed by the same letter in the same row are not significantly different. NS= Nonsignificant at 5% level.

The cleaning of different parts of the body and resting may be considered auxiliary phases because they do not contribute directly to the host acceptance process [10]. Sometimes the wasp does not show any grooming activity at all.

There are some host insects which are defenceless during parasitization [1], but mealybugs defend themselves by walking away, releasing a drop of fluid to force the parasitoid away and frequently move the rear half of their bodies up and down. These defence activities during oviposition were more frequent in *P. affinis* than *P. citri*, probably because of the larger body size of the former one.

The handling time of the mealybug by an experienced parasitoid was less than that of an inexperienced parasitoid (Table 2). The mealybug began defence activity in response to ovipositor penetration which hampered the inexperienced parasitoids more than the experienced individuals. As a result, the parasitoid would walk away but come back several times, trying more oviposition attempts. In contrast, the experienced parasitoid would thrust its ovipositor sharply into the mealybug's body in spite of all defence activities. Waage

[14] showed that for parasitoid *Nemeritis canescens*, oviposition experience resulted in a marked increase in the duration of a patch visit, also this increase did not carry across to subsequent visits.

Prior experience of oviposition increases the efficiency of subsequent oviposition by *A. pseudococchi*. Experienced parasitoids would, therefore, parasitize more hosts than inexperienced parasitoids per unit of time.

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## بررسی نحوه تخم‌گذاری و رفتار پارازیت‌کردن

### *Anagrus pseudococci* (Girault) پارازیتوئید

#### شپشکهای آردآلود

#### چکیده

نحوه تخم‌گذاری و موفقیت پارازیت‌کردن زنبور *Anagrus pseudococci* (Girault) که یک پارازیتوئید داخلی انسرتید (Encyrtid) شپشکهای آردآلود *Planococcus citri* (Risso) *Pseudococcus affinis* (Mask) می‌باشد در آزمایشگاه مورد مطالعه قرار گرفت. ترتیب دوره تخم‌گذاری که شامل جستجو برای یافتن میزبان تا رفتارهای پس از تخم‌گذاری است در این بررسی توصیف شده است. در این آزمایش نشان داده شد که پدیده تخم‌گذاری تحت تأثیر فعالیتهای دفاعی میزبان می‌باشد، همچنین مشاهده گردید که *P. citri* در مقایسه با *P. affinis* قابلیت دفاعی کمتر و حساسیت بیشتری برای پارازیت‌شدن دارد. علاوه بر این تجربه قبلی زنبور پارازیتوئید نقش مهمی در افزایش عملکرد تخم‌گذاریهای بعدی ایفا می‌کند.