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Oviposition Behaviour of *Goniozus swirskiana* (Hymenoptera: Bethylidae: Bethylinae) A Parasitoid of *Batrachedra amydraula* Meyrick From The Warmest Desert of Iran

[1,2]*Elahe Sadeghi,* [2]*Valiollah Baniameri and* [2]*Aref Marouf*

[1]Department of Entomology, Islamic Azad University, Arak, Iran
[2]Department of Agricultural Entomology Research,
Iranian Research Institute of Plant Protection, Tehran, Iran

Abstract: Iran is the leading producer of dates in the world. The lesser date moth (*Batrachedra amydraula* Meyrick) is an important pest of unripe date palm *Phonix dactylifera* L., fruits in the south eastern of Iran. *Goniozus swirskiana* (Hymenoptera: Bethylidae) female attack Lepidoptera larvae which tunnel in to pulp of unripe dates. *B. amydraula* and Goniozus swirskiana were collected from Shahdad andijerd and Kerman province in the south eastern of Iran. Shahdad city is 20 km far from the Lut desert (around warmest place in the world).The most common species of wasps attacking *B. amydraula* was *Goniozus swirskiana*, attacking the larval stages of the second generation of hosts. They sting and paralyzing them and it takes less than 2 hours. They lay eggs on to hosts' surface approximately 24 h later. The egg laying process requires about 25 to 45 min. Females laid maximum of 113 eggs on *Batrachedra amydraula* at 28±1°C and 65±50 H relative humidity under a 16 L: 8 D photoperiod. The maximum numbers of eggs were laid on the 5th segment (lateral) and there were not any eggs on thorax, the first segment of abdomen and the last segment of abdomen. The results indicated that 64.20% of the female oviposited on dorsal, 29.10% on lateral and 6.70% on ventral. A comparison of theresponse of both experienced and naïve adult parasitoids to clutch size indicated that experience did not have effect on clutch size.

Key words: *Batrachedra amydraula* · Clutch size · Experienced adult · *Goniozus swirskiana* · Naïve adult · Unripe date

INTRODUCTION

Although Iran is the leading producer of dates in the world, only 10% of its product is exported. One of the factors which limit exportation is postharvest pests. Lesser date mouth (*Batrachedra amydraula*) is one of these postharvest pests [1]. It starts its activity in date palm plantations, is then transmitted into storage with infested dates and can go through multiple generations within stored dates. *Batrachedra amydraula* has existed in date palmplantation with Iran for many years [2], but it was reported as a stored date palm pest in Iran for the first time in 1998 [3]. This pest is found in all date palm plantations of Iran [4]. Insect parasitoids are free-living as adults but develop through their immature stages by feeding upon and generally killing a single host. Insect parasitoids can be ground by their pattern of brood production: 'gregarious' parasitoids can produce multiple offspring per host but 'solitary' parasitoids can produce only a single offspring per host [2, 5]. The family Bethylidae belongs to the aculeate (stinging) Hymenoptera that include the familiar bees, social wasps and ants [6]. It is placed in the superfamily Chrysidoidae, members of which are almost axclusively parasitoids [7]. They belonging to Chrysidoidae and known as a group of primitive aculeate Hymenoptera, is widely distributed from the tropics to the subarctic regions of the world. They are aculeate parasitoids of Lepidopteran or Coleopteran larvae [8]. They are a species-rich and globally distributed family of aculeate wasps [9]. Bethylids are closely related to the cuckoowasps (Chrysididae) [10]. Most species lead a concealed life and are hard to find; therefore little is

Corresponding Author: Elahe Sadeghi, Department of Entomology, Islamic Azad University, Arak, Iran.
Tel: +989123257139.

known about their biology or distribution and many taxonomic problems still have to be resolved. The species of the subfamily Bethylinae appear to be external parasites on the larvae of small moths and in order to reach even the narrowest hiding places of borers and leafrollers, the adult females possess remarkably flat bodies [11]. *Goniozus swirskiana* is the most species of the subfamily Bethylinae, represent by 50 described species from the oriental regions of which only 36 species are known from the Indian subcontinent [12], they listed 140 nominal species of this genus worldwide and all most of them are presumed to be primary external parasitoids of Lepidoptera larvae [13]. *Goniozus swirskiana* is a cosmopolitan genus with the potential of being a biological control agent various Lepidoptera pests [14]. In the present study, we investigated the detail observation on the ovipositional behavior of *Goniozus swirskiana* and the position of *Goniozus swirskiana* eggs on *Batrachedra amydraula* larvae. We also examine the influence of parasitoid experience on clutch size.

MATERIALS AND METHODS

Insects: *B. amydraula* larvae were collected from palm grove infested fruits during 2008-2011 from Shahdad andijerd and Kerman province. Additional larvae were acquired from eggs laid by adult moth in the Iranian Research Institute of Plant Protection. This culture had been intermittently reared on a semi-artificial diet of yeast, middling, date powder, honey and glycerin [15]. We reared *Goniozus swirskiana* wasps which were obtained from a territorial storage room and field. Adult wasps were provided with dried honey on the wall of the glass vial as food for aminimum of three days. The cultures were kept at 28°C (±1) and 65% (±5) H relative humidity maintained by evaporation from a water bath in the Iranian Research Institute of Plant Protection under a 16L:8D photoperiod. Identification the parasitoid wasps were sent to University of Calicut, India (Dr. Santhosh Sheervihar) the University of Calicut reported *Goniozus swirskiana* in his trial.

Host Feeding, Host Attack and Oviposition: A single mated female parasitoid was isolated with a single host larva, which the female paralyzed in to a gelatin capsule (diameter = 9mm) resting on the squash surface and allowed it to oviposit. The squash was placed under a stereomicroscope and observed parasitoid behavior. Parasitoid behavior, including host attack, host feeding and oviposition wererecorded.

Table 1: Position of *Goniozus swirskiana* eggs on *Batrechedra amydraula* larva

Segment	Dorsal	Latral	Ventral	Total
T1	0	0	0	0
T2	0	0	2	2
T3	0	0	1	1
A1	0	0	0	0
A2	0	0	0	0
A3	0	18	0	18
A4	8	20	2	30
A5	10	18	3	31
A6	9	10	2	21
A7	5	5	0	10
A8	0	0	0	0
Total	32	71	10	113

T= Thoracic Segment; A= Abdominal Segment

Position of Eggs on Hosts: One hundred larvae of *Batrachedra amydraula* were collected picked from main culture. An adult female wasp and 2 larvae of the host were placed in a plastic dishes (diam 85 mm) plugged by gauze and cotton. Larvae were fed by honey streaks on surface of each Petri and a formed napkin as a shelter for decreasing exost. One of the larvae was as a food resource of wasp. They were observed 2 days. 113 eggs were studied and position of eggs on larvae was recorded in Table 1.

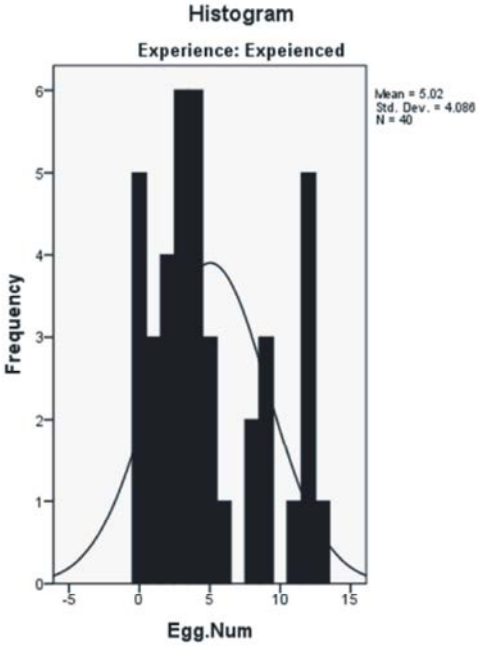
Influence of Parasitoid Experience on Clutch Size: To test the influence of experience on the ability of parasitoids to adjust their experience on clutch size, two sets of 40 individual standardized female parasitoids were exposed to two consecutive host larvae. A 'naïve' set of female parasitoids were first exposed to a host larva. Conversely, an 'experienced' set of female parasitoids were first exposed to a host larva of standard large size, to gain experience, followed by a second host larva to determine the clutch size response. For both groups of females a second vial containing the second host was connected to the first vial, after 48 h, by a small copper tube (approx. 1•5 mm diameter and 40 mm length) to allow the parasitoids to 'choose' when to leave the first host and attack the second. This avoided any disruption of the oviposition and 'brood guarding' behaviour of *Goniozus swirskiana* which can last for more than 48 h [16]. The copper tubing did not seem to affect parasitoid movement from one vial to the next, but in some cases the second host moved into the first vial and those replicates were discarded. Clutch size was again determined by physical removal of individual eggs from parasitized host larvae.

RESALTS

Host Attack, Host Feeding and Oviposition: The female examines the host for about 9 to 12 seconds. It moves its antenna and searches around larva. The female immediately moves to the dorsum of the hosts thorax and attempts to attaching its mandibles to it. The parasitoid attempts to move to the head of the host larva and sting between the head and thorax which is in the vicinity of the sub esophageal ganglion. The larva remains passive at first and then attempts to keep away the parasitoid, but it is useless. The female leaves the larva for a short time and returns to it for walking upon its back, moving its antenna endlessly for assessing size of the host and cleaning the external surface of the segment which was selected after examining with its mandibles. The female usually feeds haemolymph by cutting legs of the host and greedily licks it from wound which cuts by mandibles. Sometimes the larva was moved by the female to find a suitable position. Feeding takes about 4 minutes. They deposit both single and multiple-egg clutches on their hosts, which are stinged stung and safe. The time take from paralysis to laying an egg is 20 to 30 min and during this time some paralyzed larvae are moved to a better position. They usually are moved to the corner of the dishes. The time for depositing a single egg varies from 45 to70s. The paralyzed of the larva lasts for about 2h, after which it beings to change its position. After the egg laying process is completed the female shows different protecting behavior, which studying on it is continuing.

Position of Eggs on Hosts: The eggs are usually laid parallel to the longitudinal axis of the larva and their anterior ends concave between the rings of the host. Eggs on the host were usually laid with the same orientation on 3 different positions. An obvious preference of egg deposition is detailed in Table 1.

Influence of Parasitoid Experience on Clutch Size: Observations are not from a normal distribution so we use Equality of the means test for large samples (80>30). The observed Z value is -1.04 and the observed P-value of the two-tailed alternative is 0.3(>0.05) so we don't have enough evidence to reject the null. With confidence level of 95% or 99% using these data we can't conclude there is significant difference between numbers of egg on two levels experienced and non-experienced. This indicates that experience does not have effect on clutch size.



DISCUSSION

Our observation show that Goniozus swirskiana usually attacks some paralyzed larvae just for feeding. The wasps attack them and feed haemolymph by cutting their legs and make wounds on their segments. The wasps use their mandibles for it. Host feeding of *parasierola swirskiana* were frequently observed feeding upon *Batrachedra amydraula* larvae that they had paralyzed, often before and occasionally after eggs. Some hosts were

used only for feeding and not for oviposition. Females provided with hosts apparently utilized them as their sole food source and were never observed feeding on the honey streaks [17]. Most of the *Goniozus swirskiana* move the hosts by their mandibles for finding a suitable position and protect them. They usually put them beside the dishes or into the formed napkin for better protection. Some species have observed transporting hosts from one location to another [18]. possibly in an attempt to conceal them in a protective position in number conspecifics [19, 20, 21, 22, 23].

In *G. swirskiana* the number of egg was laid per host varied from 1 to 20. The maximum numbers of eggs were laid on the 2nd and 3nd day after which the rate decreases gradually. The numbers of eggs were host by other *Goniozus swirskiana* varied greatly. Some species were capable of laying up to 40 eggs per host [16], but most species do not deposit more than 20 eggs on a host. *Goniozus emigrates* lays 9-15 eggs per host [24]. *Goniozus aethiops* average 16.27 eggs per host [25, 6]. All *Goniozus* sp. deposit eggs on the middle segments of the host. Relatively few eggs are deposited on the segments towards the head or anus [26]. The maximum haemolymph flow (volumetrically) is present along the middle segments of the host body; these segments are preferred for oviposition [25]. Adult *Goniozus nephantidis* females sting and permanently paralyze host larvae and lay a clutch of up to 18 eggs 24 h later [27].

Our data show the eggs are laid parallel to the longiudinal axis of the larvae. Third five percent of eggs are deposit the anterior end of larvae and 55% deposit the posterior end. In *Parasierola swirskiana* Argman (Hymenoptera: Bethylidae) egg is usually laid parallel to the longitudinal axis of the host. In general, the female's oviposition stance is random: 25% of the ecolosing larvae faced the anterior end of the host, while 48% faced the posterior end. However, eggs on a particulate host are usually laid with the same orientation: on 31 of the hosts all eclosing larvae faced the anterior end, on 31% all larvae faced the posterior end and on 38% of the hosts the parasitoid larvae faced either direction (n=144) [17]. Eggs are laid on to the host approximately 24 hours later, with ranging about 10 eggs. The paralyzed condition of the host lasts for about 2 h and then the larva begins to move for finding a shelter. The time required for the actual deposition of a single egg varies from 40 to 80 s. After depositing about 4 eggs on one lateral surface of the segment the parasite begin to oviposit on the other lateral area of the same segment. Finally a few eggs are placed on the dorsal region of the same segment. These form acontinuous row of egg from the lateral region of a segment to the dorsal area and down the lateral area of the same segment. After the egg laying process is completed the female of *Goniozus sensorius* moves away from the host. The oviposition process lasts 30 to 60 min. The paralyzed condition of the host lasts for about 2 h, after which the larva begins to move [25].

Position of Eggs on Hosts: In *Goniozus swirskiana* the distribution of eggs on segments and the number of the host segments required for oviposition were recorded for 113 eggs and the data was recorded in the Table 1.

The maximum numbers of eggs were laid on the 5^{th} segment (lateral) and there were not any eggs on the first segment of abdomen and thorax and the last segment of abdomen. The results indicated that 64.20% of the female oviposit on dorsal, 29.10% on lateral and 6.70% on ventral.

In *Goniozus sensorius* eggs are glued firmly to the host integument and do not drop off with the movement or feeding activities of the host larva. The eggs are laid diagonally on the host larval segment. Eggs are laid side-by-side, in contact with each other, only one of the intrasegmental region and more toward the posterior end of the body. The maximum numbers of eggs were laid on the 7^{th} segment (36.46%) followed by the 6^{th} segment (34.45%), 5^{th} segment (14.54%), 8^{th} segment (9.17%), 4^{th} segment (5.15%) and 9^{th} segment (0.22%). Four other segments 1, 2, 3 and 10 did not have any eggs at all. The distribution pattern of the parasite eggs on the segments of the host larva was recorded. The numbers of host required for oviposition by a parasite was recorded for 209 parazited larvae and the data were segments tabulated in the form of frequency distribution. The results indicated that 85.61% of the female oviposited only on 1 segment, 13.35% on 2 segments and 1.02% on 3 segments [25]. Position of eggs on the host of *Parasierola swirskiana* is detailed in Table 2. There is a clear preference for the host □s dorsum (55%) and sides (43%), with only 2% of eggs laid ventrally. Egg deposition upon the host ?s dorsum is typical of Parasierola species [28]. Sixty- six percent of the eggs were deposited on abdominal segment 3-5 (n=966) [17].

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