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EGG-LYING BEHAVIOR OF *Anisopteromalus calandrae* (Howard), AN ECTOPARASITOID OF *Lasioderma serricorne* (Fabricius)

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ABSTRACT: This paper describes the egg-laying behavior and reproduction capacity of *Anisopteromalus calandrae* in laboratory conditions. *Anisopteromalus calandrae* lay eggs only on the *Lasioderma serricorne* larvae hiden inside of grains or nests. The maximum lifespan of *A. calandrae* females was 32 days when they were kept with of aqua feed flour plus honey (30%) and infested with larvae of *Lasioderma serricorne*. One female laid 71.13 ± 4.24 eggs. The eggs were laid mostly (81.95%) during the first half of the lifespan, with the peak of 7.40 eggs/day on the 9th day. The females usually lay one egg/host larva, but sometimes they lay 2-5 eggs/host larva. They prefer to lay eggs on the 4th larval instar.

Keywords: Anisopteromalus calandrae, *Lasioderma serricorne*, ectoparasitoid, egg-laying rhythm, mass rearing, insect pests, stored products.

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INTRODUCTION

Anisopteromalus calandrae (Howard), a widely distributed ectoparasitoid, is recorded as an important agent for biological control to protect stored agricultural products from several insect pests, such as *Lasioderma serricorne*, *Callosobruchus maculatus*, *Sitophilus zeamais*, *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica* (Hayashi et al., 2004; Kraaz, 2008).

Ahmed (1996) investigated the reproduction of *A. calandrae* growing on *R. dominica* larvae. Schmale et al. (2001) reported that egg laying duration and reproduction of parasitoids *A. calandrae, Dinarmus basalis* and *Heterospilus posopidis* was prolonged when they were fed on honey compared with those fed on sugarcane or without supplementary nutrition. Visarathanonth et al. (2010) investigated the egg laying duration of *A. calandrae* parasitizing *S. zeamais* fed with milled rice. Several authors determined the ovipositional preference of the parasitoid on different sizes of hosts (Choi et al., 2001; Smith, 1993).

There are few studies on *A. calandrae* in Vietnam. The presence of *A. calandrae* was recorded from stored maize grains in Son La Province (Nguyen Van Duong & Khuat Dang Long, 2017). We reported previously the effects of food supplements on the longevity of *A. calandrae* (Nguyen Thi Oanh et al., 2017). *Lasioderma serricorne* is one of the common insect pests damaging aqua feed as well as bean grains in Viet Nam (Nguyen Thi Oanh et al., 2016). The investigation on egg-laying behavior of *A. calandrae* can provide a scientific basis for reproduction process and encourage its use for biocontrol of common insect pests, such as *L. serricorne* for stored agricultural products.

For these reasons, we investigated the egg-lying behavior of *A. calandrae* fed on *L. serricorne* larvae.

MATERIALS AND METHODS

Insect hosts and parasitoids

Anisopteromalus calandrae, *Lasioderma. serricorne,* *Sitophilus zeamais* and *Callosobruchus maculatus* used in this study were obtained from the stock cultures in stored agricultural products and stored aqua feed grains in several places in the Mekong Delta: Ben Tre, Dong Thap, Tien Giang and Tra Vinh provinces, Vietnam. The pests were maintained in the laboratory fed with stored aqua feed grains, *Zea mays* and *Vigna unguiculata*. The grains of stored aqua feed (Ø = 8 mm) were made from rice bran, broken rice, wheat, maize, soy-bean and several other components, which were used for feeding catfish. The stored aqua feed were made by several companies (Hung Vuong, Cargill, New Hope, Songfish) in Dong Thap Province, southern Vietnam.

For the observation of egg-laying behavior of *A. calandrae*, the experiments were conducted in clear round plastic containers of 7 cm height with the bottom and top diameters of 9 cm and 12 cm, respectively. When flours are used as nutrient source for pests, 15 individuals of the third and fourth instar larvae were placed in a box containing 50 g of the corresponding grain flour. *S. zeamais* was fed with the grain flour of *Zea mays*, *C. maculatus* with the grain flour of *Vigna unguiculata* and *L. serricorne* with the grain flour of aqua feed.

For experiments carried out in grains, parasites were released into boxes containing 100 g of *Zea mays* and *Vigna unguiculata* grains infested with the third and fourth instar larvae of *Sitophilus zeamais* and *Callosobruchus maculatus*, respectively. Each experiment consisted of 6 replications. The parasitoid's eggs on hosts were determined every 24 hr-period, for 2 weeks. To maintain the constant number of host larvae, the dead larvae were removed and the equal number of larvae were added to the culture every day.

To investigate the effects of instar-age on the preference for feeding and oviposition, experiments were carried out using 8 cm diameter Petri dishes. Each dish contains 50 g of aqua feed flour and *L. serricorne* larval instars from the first to fifth instars, prepupae and pupae (10 each). Larval instar stages of *L. serricorne* were determined based on their width of head and postembryonic moult (Nguyen Thi Oanh, 2017). A pair of the parasite immediately after developed to adult was released to search for hosts in the box. The box was covered with a plastic lid. After 24 hr, eggs on the hosts were examined. Parasitized hosts were transferred into another Petri dish and placed in plastic boxes. Every day, the dead larvae were removed and the equal number of host larvae were added until the parasite female died. The experiments were replicated 10 times.

The third and fourth instar larvae were released into 8 cm diameter Petri dishes. Each dish contains 50 g of aqua feed flour. The dishes were then placed in plastic boxes. Mated *A. calandrae* was released into the boxes and fed with honey solution (30%) streaked to cotton stuck on the box wall. The box was covered with a plastic lid. The presence of eggs on the hosts was examined every 24 hrs. Parasitized host larvae were individually removed to other Petri dishes, and placed in plastic boxes. Every day, host larvae were added into the box up to 30 larvae. The process was repeated till the parasitoid female died. The experiments were replicated 15 times.

A stereomicroscope Meiji Techno DK3000 (Japan) installed with Lumenera INFINITY1-3C camera (Canada) was used to observe parasitoid's eggs and the development of larval parasites. It was also used to measure the width of the head capsule of the host's larval instars.

All experiments were conducted in laboratory conditions, at a temperature of 28-33^{o}C, 69 - 83% relative humidity (RH) and natural light condition.

RESULTS AND DISCUSSION

Egg laying behavior of *A. calandrae*

When *L. serricorne* larvae are released into aqua food flour, most of them fixed food on their body to made a nest within 24 hr (fig. 1-a1). *Anisopteromalus calandrae* laid eggs using its genitalia on the host in the nest, but never lay eggs on free larvae. Similarly, *A. calandrae* did not lay eggs on *S. zeamais* and *C. maculatus* larvae released into flour of *Zea mays* and *Vigna unguiculata*, respectively. The larvae of those two host species live freely in those flours and do not make nests. However, *A. calandrae* laid eggs on hosts hiding in the grains. This behavior probably account for the survival of its progeny.

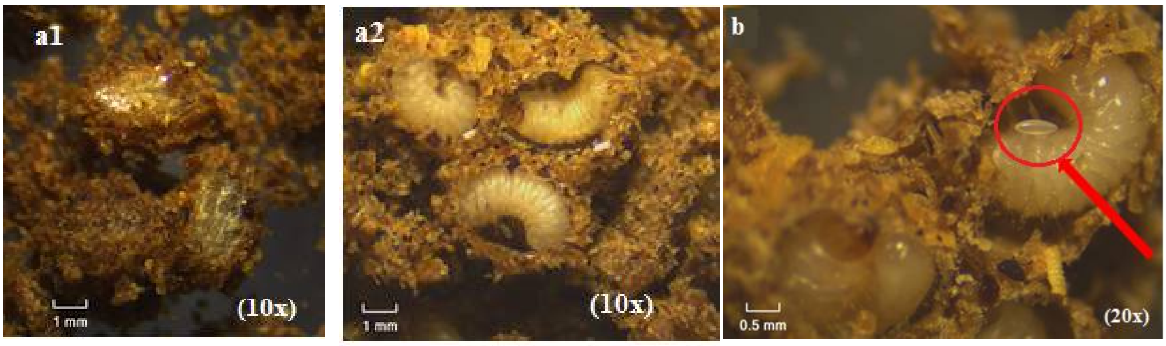

Figure 1. Nests made of aqua feed flour (a1) and larvae of *L. serricorne* inside the nests (a2), and an *A. calandrae* egg laid on *L. serricorne* larvae (b).

In the present study, *A. calandrae* lay eggs on the 2nd, 3rd, 4th larval instars, prepupa and pupae, but not on the 1st and 5th larval instars. *Anisopteromalus calandrae* preferentially lay eggs on the 4th larval instar (76.03%), followed by on the 3rd (18.90%), 2nd (1.90%) larval instars. The infection rate of prepupae was 2.01% and that of pupae 1.16%. The 1st and 5th larval instars were probably not suitable nutrient for the ectoparasitoid.

The infection rate was variable among each developmental stage of larval instars of hosts. Smith (1993) described the host-size preference of *A. calandrae*: 87% of them laid eggs on larvae with tunnel diameters of 0.9 to 1.8 mm and 6% of them on prepupae and pupae. The highest number of eggs was laid on the larvae with tunnel diameter of 1.6 mm and the lowest was seen in pupae and small larvae. Choi et al. (2001) also reported that *A. calandrae* preferred to lay eggs on relatively large *S. oryzae* larvae.

In this study, *A. calandrae* laid 1-5 eggs on a host larva, predominantly 1 egg per larva (82.47%) followed by 2 (10.31%), 3 (5.62%), 4 (1.12%) and 5 (0.47%) eggs per host. Our results are in agreement with the previous report of Chaisaeng (2007). When *A. calandrae* lay multiple eggs on a host, all eggs might hatch on the host, but only one larva will develop to a pupa. This might be due to one host can provide enough nutrient for only one parasitoid to develop into an adult, as the results of evolution of host selection-behavior of ectoparasitoid wasps (De Bach, 1964).

In the laboratory condition, 825 out of 1067 (77.32%) *A. calandrae* developed from pupae. However, this ratio was variable depending on the numbers of eggs per host. From the host with a single egg, 85.45% of *A. calandrae* emerged could complete their life cycle. On the other hand, the mortalities of *A. calandrae* before they developed to pupa were 52.73, 71.67, 75.00 and 80.00% from the hosts with 2, 3, 4 and 5 eggs, respectively. These results indicate that the more eggs *A. calandrae* laid on a host, the higher the premature mortality ratio of *A. calandrae*. Similar phenomenon was reported in other endoparasitoid and ectoparasitoid wasps of Hymenoptera (De Bach, 1964; Vu Quang Con & Khuat Dang Long, 1989). When parasitoid wasps lay more than one eggs on a larval host, only one will develop to an adult. In this study the mortality of the larval instars and pupae was 19.59% and 3.09%, respectively. Similarly, the mortality of *Euplectrus laphygmae* parasitizing on *Spodoptera littoralis* increased sharply with the number of eggs laid on each host, being 30% at 1 egg per host, and 46% at 7 eggs per host (Gerling & Limon, 1976).

Egg-lying rhythm of *A. calandrae* on *L. serricorne*

Female *A. calandrae* fed on honey (30%) lived up to 32 days with the mean survival time of 27.07 ± 2.89 days at 28-33°C and 69-83% RH (fig. 2). When they were fed on pure water, the mean survival time was 16.10 ± 1.52 days. The females started to lay eggs 24 hrs after mating. The number of eggs per day ranged from 0.00 to 7.40, and the mean total number of eggs produced by one female was 71.13 ± 4.24. Schmale et al. (2001) reported that the mean longevity of *A. calandrae* fed on honey was 49.8 days. However, the time was 10.4 days when they was not supplemented with any food or fed with sugarcane. Ahmed (1996) reported that at 30°C and 60 ± 5% RH, the daily and total numbers of eggs laid per *A. calandrae* female on full grown larvae of *R. dominica* were 8.3 and 132.6, respectively.

Female *A. calandrae* laid eggs on *L. serricorne* as a function of time in which the parabola $Y = -0.15X^2 + 2.61X - 2.71$, fixed well with the first stage of their life from the 1st to the 15th day, and $Y = -0.16X + 4.86$ for the second stage starting from the day 16th and ended up at 32th day (fig. 2).

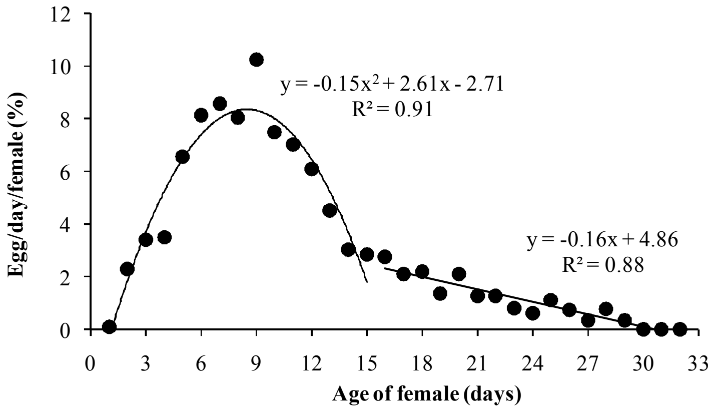

Figure 2. Egg-lying rhythm of *A. calandrae* on *L. serricorne*

The large part of the eggs were laid during the first stage (81.95%, fig. 2). The peak egg production per day per female was observed on the 9th day with 7.4 eggs/female in average (10.25% of total eggs/female). Visarathanonth et al. (2010) reported that *A. calandrae* laid eggs for 11 days with the peak on the 5th day with 12 ± 5 eggs/female. In common, peak egg production of *A. calandrae* is in the middle of their life. Obviously, the time to lay eggs and the number of eggs/female are affected by environmental conditions such as hosts, supplemented foods etc.

Conclusion

After developing from pupa, the survival time of *A. calandrae* fed on honey (32 days) was significantly longer than that fed on pure water (16.10 days). Female *A. calandrae* started to lay eggs 1 day after mating. Most eggs were laid on from the 7th day until the 11th day (41.65% of eggs laid). The present results provide the basic data for the mass rearing of *A. calandrae* for biological control of stored insect pests.

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