

BIOLOGICAL CONTROL

Reproductive Biology and Longevity of *Euplectrus ronnai* (Brèthes) (Hymenoptera: Eulophidae)

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Biologia Reprodutiva e Longevidade de *Euplectrus ronnai* (Brèthes) (Hymenoptera: Eulophidae)

RESUMO - O ectoparasitóide *Euplectrus ronnai* (Brèthes) é um dos componentes do complexo de espécies que parasitam a lagarta do trigo *Mythimna (Pseudaletia) sequax* Franclemont (Lepidoptera: Noctuidae). Não há dados na literatura sobre o potencial de parasitismo, preferência por instares do hospedeiro e longevidade desse parasitóide, o que motivou a realização do presente trabalho. O parasitismo ocorreu entre o terceiro e o quinto instares do hospedeiro; lagartas de segundo instar inviabilizaram o desenvolvimento do parasitóide, e no primeiro e no sexto instares não houve oviposição pelas fêmeas de *E. ronnai*. O número de ovos depositados pelas fêmeas aumentou com o estágio de desenvolvimento do hospedeiro, variando de 1,5 ovos/lagarta no terceiro instar a 3,6 ovos/lagarta no quinto instar. A proporção de lagartas parasitadas entre os diferentes instares mostrou que *E. ronnai* apresenta preferência pelo quarto e quinto instares de *M. sequax*. Não houve diferença no tempo de desenvolvimento, na razão sexual, no número médio de lagartas parasitadas e na porcentagem de lagartas parasitadas em testes de livre escolha entre lagartas de quarto e de quinto instares. Entretanto, o número de parasitóides/hospedeiro foi significativamente maior em lagartas de quinto instar. A fecundidade média das fêmeas de *E. ronnai* foi de 63,7 ovos e cada fêmea parasitou a média de 20,3 lagartas. A oviposição iniciou-se um dia após a emergência e manteve-se até um dia antes da morte das fêmeas. A longevidade de fêmeas em atividade de oviposição (29,7 dias) foi significativamente menor do que a de fêmeas que não receberam hospedeiros (187,5 dias).

PALAVRAS-CHAVE: Insecta, controle biológico, lagarta do trigo, *Mythimna sequax*

ABSTRACT - The larval ectoparasitoid *Euplectrus ronnai* (Brèthes) is one of the components of a species complex that attacks the armyworm *Mythimna (Pseudaletia) sequax* Franclemont (Lepidoptera: Noctuidae). No data are available on the fecundity, longevity and host instar preference of *E. ronnai*, what prompted the conduction of this research. No parasitism occurred on the first and sixth instars of the host, and a positive relationship was found between host instar and the number of parasitoid eggs/host, which ranged from 1.5 on third- instar hosts to 3.6 on fifth instars. Females of *E. ronnai* showed a preference for either the fourth or fifth instar of *M. sequax*. There were no significant differences in the developmental time of the parasitoid, sex ratio, mean number of parasitized hosts and percentage of parasitized caterpillars in free-choice tests using fourth- and fifth-instar *M. sequax*. However significantly more parasitoids/host were found on fifth than on fourth-instar caterpillars. Mean lifetime fecundity was 63.7 eggs/female and each female parasitized an average of 20.3 caterpillars. Oviposition started one day after emergence of the females, which remained reproductively active until one day before death. Females of *E. ronnai* lived significantly longer when deprived of hosts (187.5 days) in comparison to females kept in ovipositional activity throughout their lifetime (29.7 days).

KEY WORDS: Insecta, biological control, parasitoid, armyworm, *Mythimna sequax*

The armyworm *Mythimna (Pseudaletia) sequax* Franclemont is the main defoliator of winter cereals including wheat, oat, barley and ryegrass in Southern Brazil (Gassen 1984, Specht & Corseuil 2002). In the larval stage,

M. sequax is attacked by ca. 10 species of dipterous and hymenopterous parasitoids (Gassen 1986, Foerster *et al.* 2001). The biology of some of the parasitoids of *M. sequax* has been investigated; the reproductive potential and the

developmental rate in relation to temperature were determined for the gregarious endoparasitoid *Glyptapanteles muesebecki* (Blanchard) (Hymenoptera: Braconidae) (Foerster *et al.* 1999a, b) and for the solitary endoparasitoid *Peleteria robusta* Wiedman (Diptera: Tachinidae) (Foerster & Doetzer 2002) and *Microplitis mediator* Haliday (Hymenoptera: Braconidae) (Foerster & Doetzer 2003).

Euplectrus ronnai (Brèthes) is a gregarious larval ectoparasitoid of the wheat armyworm *M. sequax* (Yamamoto *et al.* 1998). After parasitism, the hosts are unable to molt as a result of the injection of a paralyzing fluid by the parasitoid female, as observed in other *Euplectrus* species (Puttler *et al.* 1980, Coudron *et al.* 1990, Jones & Coudron 1993). Food consumption by caterpillars parasitized by *Euplectrus* species is reduced by more than 80% in relation to non-parasitized hosts (Parkman & Shepard 1981, Yamamoto *et al.* 1998). Due to these features, *E. ronnai* is a promising natural control agent of the armyworm on wheat and other winter cereals in Southern Brazil.

In view of the lack of knowledge on host instar preference, fecundity and longevity of *E. ronnai*, this paper describes its reproductive biology and longevity, as well as its host instar preference on larvae of *M. sequax*.

Materials and Methods

The experiments were conducted in climatic chambers at $21 \pm 1^\circ\text{C}$, relative humidity of $70 \pm 10\%$ and photoperiod of 12h. Laboratory cultures of *E. ronnai* and its host *M. sequax* were established from field-collected material from wheat and oat crops in Lapa County, Southern Paraná, Brazil. Voucher specimens of *E. ronnai* are deposited in the Entomological Collection "Padre Jesus Santiago Moure", at the Departamento de Zoologia, Universidade Federal do Paraná, Brazil.

Host Instar Preference of *E. ronnai*. The host instar preference of the parasitoid was evaluated in no-choice tests; 20 *M. sequax* larvae of each instar (first to sixth) were exposed to five *E. ronnai* couples in 10 cm diameter petri dishes for 24h. Each treatment was repeated twice, totaling 40 hosts exposed to 10 parasitoid females for each host instar. The number of parasitized caterpillars in each instar was recorded, as well as the number of parasitoids/host and the sex-ratio of the progeny. The caterpillars exposed to the parasitoids were reared individually in plastic vials 4 cm diameter and 7 cm high and fed with leaves of kikuyo grass (Foerster 1996). The mean number of parasitoids/host in each instar were compared by analysis of variance and classified by Tukey's test ($P < 0.05$).

A free-choice test on host instar preference was performed using fourth- and fifth-instar hosts. Ten newly emerged *E. ronnai* couples were individually confined to petri dishes 10 cm in diameter; two fourth-instar and two fifth-instar *M. sequax* larvae were exposed to each parasitoid pair for 24h. After this period the two pairs of hosts were replaced for new ones and the same procedure was followed during 10 days. The following biological parameters were

compared: percentage of parasitized hosts in each instar, developmental time of the progeny, sex-ratio, number of parasitoids/host and number of hosts parasitized in each instar. The parasitized caterpillars were reared as described for the no-choice test, and the pairs of data were compared by the 't' test ($P < 0.05$).

Fecundity and Longevity of *E. ronnai*. Newly emerged male/female pairs ($n = 25$) of *E. ronnai* were separately confined to 10 cm diameter petri dishes lined at the bottom with filter paper and fed with honey streaked to the wall of the dishes. Twelve couples received each day, from adult emergence until death, five fourth-instar *M. sequax* larvae. After the exposure period of 24h, the host larvae were removed and reared individually in plastic containers 4 cm diameter and 7 cm high and new larvae were offered to the parasitoids. The number of parasitized caterpillars and the number of parasitoids produced by each host were recorded throughout the lifetime of the female parasitoids. The longevity of these females was compared to the longevity of the other 13 couples kept in similar conditions in the absence of host larvae. Mean longevity between sexes and between females in the presence and absence of hosts were compared by the 't' test ($P < 0.05$).

Results and Discussion

Host Instar Preference of *E. ronnai*. In no choice tests, *E. ronnai* oviposited in third- to fifth-instar hosts, while no parasitism occurred on either first or sixth instar caterpillars (Table 1). Only one second-instar caterpillar was parasitized, but the parasitoid larva failed to hatch from the single egg laid on the host, a condition also reported by Parkman & Shepard (1982) for *E. plathytenae* parasitizing second-instar *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Other *Euplectrus* species also show preference for fourth- and fifth-instar caterpillars (Fonseca 1978, Puttler *et al.* 1980, Uematsu 1981).

There was a relationship between host instar and the mean number of parasitoids/host, which ranged from 1.5 parasitoids/host on third-instar caterpillars to 3.6 parasitoids/host on fifth-instar caterpillars (Table 1). A

Table 1. Percentage of parasitized caterpillars and mean number (\pm s.e.) of eggs/host by *E. ronnai* on different instars of *M. sequax*. ($n = 20$). Temperature: $21 \pm 1^\circ\text{C}$, R.H.: $70 \pm 10\%$, photoperiod: 12h.

Host instar	Parasitism rate (%)	Eggs/host $\bar{x} \pm$ s.e.
First	0	0.0 ± 0.00
Second	5	1.0 ± 0.00 b
Third	30	1.5 ± 0.22 b
Fourth	85	3.2 ± 0.26 a
Fifth	40	3.6 ± 0.65 a
Sixth	0	0.0 ± 0.00

Means followed by different letters are significantly different by Tukey's test ($P < 0.05$).

similar relationship was found for other *Euplectrus* species (Gerling & Limon 1976, Fonseca 1978, Puttler *et al.* 1980, Uematsu 1981).

In free-choice tests using fourth- and fifth-instar hosts, no differences were recorded regarding percentage of parasitized hosts, developmental time from oviposition to adult emergence, number of parasitized caterpillars/female and sex-ratio of the progeny (Table 2). However, fifth-instar hosts yielded a significantly higher number of parasitoids than fourth-instar ones, a trend that was also observed in the no-choice experiment, although in that case without statistical significance.

Table 2. Biological characteristics (means \pm s.e.) of *E. ronnai* parasitizing fourth- and fifth-instar caterpillars of *M. sequax*. Temperature: $21 \pm 1^\circ\text{C}$, R.H.: $70 \pm 10\%$, photoperiod: 12h.

Biological parameter	4 th instar	5 th instar
% parasitism	12.5 ± 2.26 a ¹	17.5 ± 1.11 a
Number of eggs/host	6.0 ± 0.42 a	16.3 ± 0.90 b
Parasitized hosts/female	2.5 ± 0.45 a	3.5 ± 0.22 a
Sex-ratio	0.6 ± 0.06 a	0.6 ± 0.05 a
Developmental time	26.0 ± 0.20 a	26.4 ± 0.16 a

Means followed by the same letter in the rows are not significantly different by the 't' test ($P < 0.05$).

The difference in the mean number of eggs/host between the two experiments indicates that in the free-choice experiment the hosts were superparasitized, yielding twice more eggs in the fourth instar and four times more eggs in the fifth instar (Table 2) in comparison to the number of eggs/host obtained in the no-choice experiment (Table 1). The competition among the five females exposed to 20 hosts in the no-choice experiment probably increased competition and regulated oviposition, while the lack of competition in the free-choice experiment induced superparasitism.

Fecundity and Longevity of *E. ronnai*. The biological parameters of the adult stage of *E. ronnai* are shown in Table 3. An average of 20.3 caterpillars were parasitized during the female lifetime, resulting in the production of 63.7 descendants by each female. The number of parasitized hosts during the female lifetime is similar to

Table 3. Longevity and reproduction of *E. ronnai*, on fourth-instar larvae of *M. sequax*. Temperature: $21 \pm 1^\circ\text{C}$; R.H.: $70 \pm 10\%$; photofase: 12h.

Biological parameter	Mean \pm s.e.	Range
Parasitized hosts/female (total)	20.3 ± 2.80	8 – 41
Fecundity (total)	63.7 ± 9.07	28 – 111
Fecundity/day	2.1 ± 0.12	1.4 – 2.8
Parasitized hosts/day	0.7 ± 0.05	0.5 – 1.0
Female longevity with hosts (days)	29.7 ± 3.52 c	13 – 46
Female longevity without hosts (days)	187.5 ± 13.84 a	86 – 242
Male longevity (days)	145.6 ± 8.57 b	87 – 184

Means followed by different letters in the longevity of males and females are significantly different ('t' test, $P < 0.05$).

that described by Puttler *et al.* (1980) for *E. puttleri* (23 caterpillars), however total egg production by *T. puttleri* (100.5 eggs) is higher than that of *E. ronnai*. This difference is due to the lower mean number of parasitized hosts/day (0.7) and lower mean number of eggs/host (3.0) in comparison to *E. puttleri* which laid a mean of 7.2 eggs/day and parasitized an average of 1.6 caterpillars/day. The number of parasitoids/host is also higher in *E. plathypenae* (12.1), however a smaller number of hosts (8.3) are parasitized during the female lifetime in comparison to *E. ronnai* (Parkman *et al.* 1981). Females of *E. ronnai* started oviposition one day after emergence and remained in activity until one day before death (Fig. 1). All females remained alive up to 13 days after emergence, a pattern similar to the one observed for *E. puttleri* (Puttler *et al.* 1980). However, mean longevity of *E. ronnai* females (29.7 days) was ca. twice as long as *E. puttleri* (14.2 days). Unlike other parasitoid species that deposit most of their eggs in the first five days, *E. ronnai* showed ovipositional peaks until 41 days after emergence (Fig. 1). Like other *Euplectrus* species, *E. ronnai* feed on host fluids after puncturing the host with their ovipositor (Puttler *et al.* 1980). The ingestion of the proteinaceous fluid of the host enables the females to continue oogenesis (Clausen 1962) and oviposition continues for long periods after emergence, as observed for *E. ronnai*.

Female longevity was significantly affected by ovipositional activity; when deprived of hosts, females lived on average for 187.5 days, compared to 29.7 days when the females were continuously exposed to *M. sequax* larvae (Table 3). Males of *E. ronnai* showed a mean longevity of 145.6 days when kept in the presence of females which did not receive host eggs during the adult stage. Other species of *Euplectrus* are shorter lived than *E. ronnai*; *E. laphygmae* females showed a mean longevity of 45 days in the absence of hosts and 34 days when exposed to caterpillars (Gerling & Limon 1976), however the experiment was conducted at a higher temperature (26°C), suggesting that at lower temperatures the longevity of *E. laphygmae* may also be extended. For *E. puttleri*, Puttler *et al.* (1980) obtained a mean longevity of 14.2 days for females in reproductive activity and 39.1 days in the absence of hosts at $22\text{--}24^\circ\text{C}$; maximum longevity however reached 90 days. The longevity recorded for *E. ronnai* at 21°C provide the species with a high searching capacity, which is a desirable feature in the field, under conditions of low host availability.

The preference of *E. ronnai* for the fourth- and fifth instars of *M. sequax* associated to the sharp reduction in food consumption by parasitized hosts (Yamamoto *et al.* 1998) provide a significant reduction in plant damage, since leaf consumption by *M. sequax* increases from the fourth instar onwards (Doetzer & Foerster 1998). Moreover, the extended longevity of the females in the absence of hosts suggests that *E. ronnai* possesses a high searching capacity under conditions of scarcity of hosts. For these reasons and by the fact that *E. ronnai*, like other eulophids, inhibits host molting, this species may have a significant impact on the biological control of the armyworm in winter cereals in Southern Brazil.

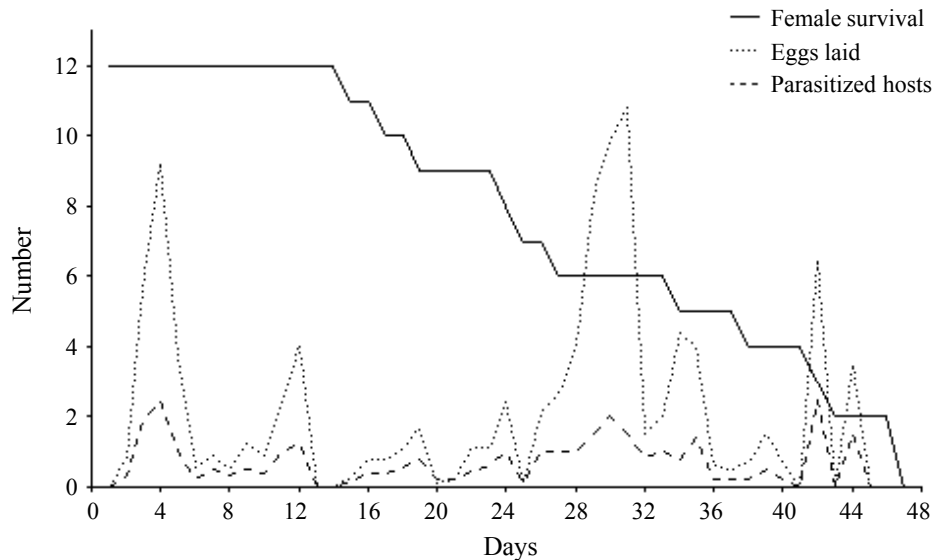


Figure 1. Female survival and fecundity of *E. ronnai* parasitizing fourth-instar larvae of *M. sequax*.

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