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Effect of Diet, Photoperiod and Host Density on Parasitism of *Anisopteromalus calandrae* on the Tobacco Beetle and Biological Parameters of the Parasitoid

Kássia C. F. Zilch[1], Simone M. Jahnke[2], Andreas Köhler[3], Eduarda Bender[1]

[1]Post-Graduation Program in Fitotecnia, Federal University of Rio Grande do Sul—UFRGS, Porto Alegre, Brazil
[2]Laboratory of Biological Control of Insects, Phytosanitary Department, Federal University of Rio Grande do Sul—UFRGS, Porto Alegre, Brazil
[3]Laboratory of Entomology, University of Santa Cruz do Sul—UNISC, Santa Cruz do Sul, Brazil
Email: kassiazilch@gmail.com, bender.ep@gmail.com, mundstock.jahnke@ufrgs.br, andreas@unisc.br

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Abstract

Lasioderma serricorne is known to be pest of tobacco, besides of attacking other products in storage. *Anisopteromalus calandrae* is an ectoparasitoid of coleopteran larvae also parasitizing the tobacco beetle. This study was aimed to evaluate the parasitism of *A. calandrae* on different densities of *L. serricorne* larvae grown in different diets and photoperiods, and to record the longevity and reproductive potential of *A. calandrae*. Individuals of *L. serricorne* were raised in three diets: wheat flour (F); wheat flour and brewer's yeast (FY) and wheat flour and dried tobacco (FT). Different amounts of host larvae (10, 20, 50 and 100) for each diet were exposed to a couple of parasitoids. The same larval densities from diet F were exposed for 24 h to a couple of adult parasitoids maintained in three photoperiods (0:24, 12:12 and 24:0 - scotophase: photophase). The highest values of apparent parasitism were in the density of 50 larvae in the FY diet (96.34%) and 100 F (92.91%). There was no significant difference in the parameters in each photoperiod in all larval densities. However, the treatment in which hosts and parasitoids always remained in scotophase, was the one that had a significantly higher sex ratio. Females had longer longevity than males surviving for up to 25 days. On the fourth day of larvae exposure occurred, the maximum number of offspring generated. It is inferred that *A. calandrae* has potential to be used as a control agent for coleopterans that attack stored products.

Keywords

Abiotic Factors, *Anisopteromalus calandrae*, Biological Control, *Lasioderma serricorne*, Reproductive Potential

1. Introduction

The species *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae), known as tobacco beetle, is found in tropical, subtropical and temperate regions of the world [1]. It is a primary pest that commonly attacks stored tobacco, but has no preference consuming products of animal origin, oilseeds, cereals, cocoa beans, flour, spices and dried fruits [1] [2].

During the tobacco storage phase, losses of 10% to 50% occur due to pest attack depending on the technological level [3]. The world losses caused by tobacco can reach 1% of the value of its production, corresponding to about 300 million dollars. The damage caused to the stored product is mainly due to insects that consume the leaves, form galleries, and contaminate the product because of their excrement and exuvia [4].

Due to damage caused by these insects, the difficulty of controlling them, and because many species, such as *L. serricorne*, have already acquired resistance to insecticides [5], new alternatives aimed at minimizing economic losses and controlling pests efficiently become necessary, as is the case of biological control with the use of parasitoids [6].

Anisopteromalus calandrae (Howard, 1881) (Hymenoptera: Pteromalidae) is an idiobiont ectoparasitoid that attacks late-instar larvae from a wide variety of hosts [7] [8], including *Sitophilus oryzae* (L. 1765) (rice weevil), *S. granarius* (L. 1758) (granary weevil), *S. zeamais* (Motschulsky, 1885) (maize weevil) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Fabricius, 1972) (lesser grain borer) (Coleoptera: Bostrichidae) and *L. serricorne* [9].

An *A. calandrae* female may oviposit about 450 eggs during life. Usually, one egg is placed per host [10] and only one parasitoid develops from each host larvae. In addition, the female feeds on the haemolymph of the parasitized larvae in order to obtain the adequate amount of proteins for the maturation of their eggs [11].

Biological interactions between parasitoid/host combined with abiotic factors such as temperature, humidity and photoperiod may limit or stimulate certain activities such as the development and reproduction of these insects [6]. The post-embryonic development of insects, in general, is directly influenced by environmental conditions. In parasitoids, the post-embryonic development is also affected by size, physical and chemical composition, as well as their host species [12].

For the production of natural enemies in laboratory, studies on the influence of biotic and abiotic factors in the development of both organisms (host and parasitoid) are needed [13]. Some morphological, nutritional and biometric criteria should also be used in the evaluation of diets, making it possible to verify if it is adequate for the development and creation of these arthropods [14].

Although the occurrence of parasitism of *A. calandrae* in *L. serricorne* is known [15], there is a gap in knowledge about the biology of the parasitoid in this host. The understandings of reproductive attributes such as reproductive rate and longevity of natural enemies, biotic and abiotic requirements and parasitism capacity are essential for successful parasitoid growth and commercial use as a biological control agent [16].

The aims of this study was to: a) evaluate the influence of different diets and photoperiods on post-embryonic development, emergence, offspring size, sex ratio and parasitism rate of *A. calandrae* in *L. serricorne* larvae of 4th instar and b) record the longevity and reproductive potential of *A. calandrae* parasitizing *L. serricorne* in order to evaluate the potential of this parasitoid as a control agent of coleopteran pests of stored products.

2. Material and Methods

2.1. Insects Rearing

The rearings of *L. serricorne* and *A. calandrae* were kept in the Entomology Laboratory at University of Santa Cruz do Sul—UNISC, in Santa Cruz do Sul municipality, inside of plastic containers adapted with openings covered by white organza crystal cloth for aeration and kept in controlled conditions chamber (28˚C ± 2˚C, 60% ± 10% RH and 12-hour photophase).

Lasioderma serricorne individuals were caught in dry tobacco from producers in Santa Cruz do Sul municipality and surrounding region and also from traps installed at Japan Tobacco International (JTI) in Santa Cruz do Sul, RS, Brazil (29˚45'01.3"S 52˚25'38.3"W). The traps consisted of a 10-liter glass jar with a plastic funnel attached, filled with wheat flour-based diet, brewer's yeast, a dry tobacco leaf of variety Virginia, and two Bio Serrico® sex pheromone lozenges.

The parasitoids (*A. calandrae*) were obtained from the host *S. zeamais* (maize weevil)that was infesting dried and stored corn grain from farmers in Candelaria municipality, RS, Brazil (29˚31'31.62"S 52˚45'28.70"W), which were taken to the laboratory for beginning of the rearing.

Adults of *A. calandrae* collected were transferred to pots (120 ml) containing fourth instar larvae (with cocoon formation) of the host *L. serricorne* and fed with honey droplets. Subsequently, these pots were packed in larger pots of 11 liters (40.8 × 29 × 12.8 cm in size) with paper lined bottom moistened daily and lid fitted with organza crystal cloth for ventilation. The parasitoids were reared for at least three generations in the host *L. serricorne* before being submitted to the bioassays.

2.2. Influence of Diet and Host Density on Parasitism of *A. calandrae*

The individuals of *L. serricorne* were raised in three different diets a) 1000 g of wheat flour (F), b) 950 g of wheat flour and 50 g of brewer's yeast (FY) and c) 950 g of wheat flour and 50 g of triturated dried tobacco (FT). At each adult emergency, these were transferred to new breeding pots identified with date and type of diet.

Fourth instar larvae (with cocoon formation) from one of the diets were exposed to newly emerged adult couples (24 h) of *A. calandrae*. Different densities of *L. serricorne* larvae (10, 20, 50 e 100) of each diet (F, FY e FT) were placed inside plastic pots (120 ml) for exposure to parasitism, totaling 12 treatments.

The parasitoids remained in the assay pots until their death, around 20 days. Soon after, they were removed, and the pots with the host larvae kept in incubator for daily observation of emergence of either *L. serricorne* or *A. calandrae*.

As control treatment, two pots with the same densities of host larvae used in each treatment (10, 20, 50 and 100) were kept without exposure to the parasitoids, aiming to verify the natural mortality rate of the host.

The bioassays were performed with 10 replicates per treatment, with one parasitoid couple per replicate and kept in an incubator under the same environmental conditions of the creations.

2.3. Influence of Photoperiod on the Parasitism of *A. calandrae*

Couples of parasitoids with 24 to 48 hours of age, reared in host larvae exposed to 12 hours of light were submitted to the following photoperiods: a) 0:24, b) 12:12 and c) 24:0 (scotophase: photophase). Four densities of the fourth instar larvae of *L. serricorne* (10, 20, 50 and 100), raised on a wheat flour diet, were exposed to *A. calandrae* in a total of 12 treatments. The bioassays were performed with 10 replicates per treatment and kept in an incubator with the same temperature and humidity conditions described above, varying only in the photoperiod.

Hosts and parasitoids remained in the same pot during the adult life of the parasitoid couple and subsequent emergence of either *L. serricorne* or *A. calandrae*.

As a control, two pots with the same amount of host larvae were kept under the same conditions and in the same period of each treatment, but without exposure to the parasitoids.

The following factors were analyzed in the bioassays of the diet (2.2) and photoperiod influence:

- Mortality of host: $\frac{n^{\circ}\ \text{hosts emerged in controls}}{\text{host density}} \times 100$
- Mean emergence of offspring: $\frac{n^{\circ}\ \text{of emerged parasitoids}}{\text{host density}}$
- Apparent parasitism:

$$\frac{n^{\circ}\ \text{of emerged parasitoids}}{\left(n^{\circ}\ \text{of emerged parasitoids} + n^{\circ}\ \text{of emerged host}\right)} \times 100$$

- Sex ratio of parasitoids: $\dfrac{n^{\circ}\ \text{of females}}{\left(n^{\circ}\ \text{of females} + n^{\circ}\ \text{of males}\right)}$

2.4. Longevity of *A. calandrae* and Fertility of Females

Fifteen couples of *A. calandrae* with 24 to 48 hours of age were evaluated. Ten fourth instar larvae of *L. serricorne* were offered to each couple, exposed to parasitism for 24 hours. Daily, the couples were transferred to a new pot with 10 larvae, allowing the female to parasite new larvae every day. The bioassays were carried out in the same environmental conditions described for the maintenance of the rearing.

The emergence of offspring and the mortality of couples were recorded daily. The number of emerged parasitoids and sex of the offspring were recorded. In the bioassays that couples remained in the pot, a male and a female of the total value were subtracted. The remaining cocoons were opened to check for possible trapped parasitoids, which were also registered.

2.5. Statistical Analysis

The longevity data were used to construct survival curves by using the Kaplan-Meier estimator in the statistical software SPSS version 22.

The emergency data and apparent parasitism were tested for normality by Lilliefors and as they did not meet the assumptions for parametric data, they were submitted to the Kruskal-Wallis test and the means compared by the Dunn test Differences in the proportion of emerged females (reflecting the sex ratio of the group) in each treatment were tested by Binomial with two proportions. The analyses were performed using the software Bioestat 5.0 [17].

3. Results and Discussion

3.1. Influence of Diet and Host Density on Parasitism

The mean emergence of *L. serricorne* in the control (without the presence of parasitoids) was 78%, 80%, 95% and 88% at the densities 10, 20, 50 and 100, respectively, in F diet; 100 (10), 90 (20), 88 (50) and (100) at FY diet and 100 (10), 85 (20), 77 (50) and 91% (100) at FT diet. These values differed significantly of the treatments, which was 1%, 7%, 16% and 4% in the F diet ($H = 40.73$; $p < 0.001$); ($H = 35.44$, $p < 0.001$), at the densities of 10, 10, 10, 10, 20, 50 and 100, respectively.

Considering the means of emergency of the tobacco beetle in the control, we considered the natural mortality of the species at approximately 16%.

Comparing the diets at the same density, the emergence mean of parasitoids was higher in FL diet compared with the FT in densities of 20 and 50. At the density of 100 larvae, in the FT diet, the emergence was lower. In apparent parasitism, there were also significant variations between the diets at the densities of 20, 50 and 100. Only the density of 10 larvae did not differ of the others (**Table 1**).

Table 1. Mean emergence (±SD) (mean/treatment) and mean apparent parasitism (%) of *Anisopteromalus calandrae* according to the larval density of *Lasioderma serricorne* and diet offered to the host.

	DIET					
	Wheat flour		Wheat flour + Brewer's yeast		Wheat flour + Tobacco	
Larvae quantity	Emergence	Apparent parasitism (%)	Emergence	Apparent parasitism (%)	Emergence	Apparent parasitism (%)
10	0.02 ± 0.042 cA	20 bA	0.06 ± 0.126 cA	23 bA	0.08 ± 0.131 bA	17.93 bA
20	0.04 ± 0.043 bcAB	54 abAB	0.11 ± 0.099 bcA	73 aA	0.02 ± 0.035 bB	19.16 bB
50	0.33 ± 0.102 abAB	68,1 abB	0.41 ± 0.071 abA	96.34 aA	0.22 ± 0.105 aB	89.09 aA
100	0.51 ± 0.142 aA	92.91 aA	0.44 ± 0.156 aA	89.2 aAB	0.25 ± 0.069 aB	84.98 aB

*Different lowercase letters in column and capital in the row for the same parameter differ significantly ($p < 0.05$) by the Kruskal-Wallis test followed by Dunn test.

Considering the same diet, there were also differences in these parameters in relation to the density of hosts. The mean parasitoid emergence at the densities of 50 and 100 was significantly higher than the lower densities (10 and 20).

In relation to the apparent parasitism in the F diet, the density of 10 had a significantly lower parasitism than the density of 100. In FY diet, only the density of 10 larvae was lower than the others. In the FT diet, the apparent parasitism was lower in the densities of 10 and 20 compared to the ones of 50 and 100 (**Table 1**).

Although there are studies such as Meneses *et al.* [18] stating that host diet can influence the performance of the natural enemies, this was not observed in this study in relation to parasitism and emergence index of the parasitoid. A similar result to the present study was found by Pratissoli *et al.* [19], who evaluated the influence of host diet of *Anagasta kuehniella* (Zeller, 1879) on *Trichogramma pretiosum* (Riley, 1879) parasitism. They observed that use of *A. kuehniella* eggs, raised in different proportions of wheat and maize flour (0:100, 25:75, 50:50, 75:25 e 100:0), also did not have a significant effect on the parasitism and emergence of *T. pretiosum* adults.

Mean sex ratio, on the other hand, showed a significant difference between the F diet (0.66) compared to FY (0.46) or FT (0.40) ($p < 0.05$). We observed a significant difference of the sexual ratio between the densities in all diets (**Figure 1**), however, not presenting a pattern between them.

Despite finding a better production of females in the diet with only flour,some studies such as Panizzi and Parra [20] consider brewer's yeast a sexual maturer, and therefore should be added in some diets. However, this may be determinant only for host species maturation, since to *A. calandre* the increment of this compost in the FL diet did not influence significantly in sex ratio.

The combination of dietary influence and sex ratio density of the tested parasitoids was not clearly identified in this study. Pratissoli *et al.* [19], on the other hand, reported that a diet composed only of corn meal affected negatively the sex ratio of *T. pretiosum*, which was not noticed for *A. calandrae*.

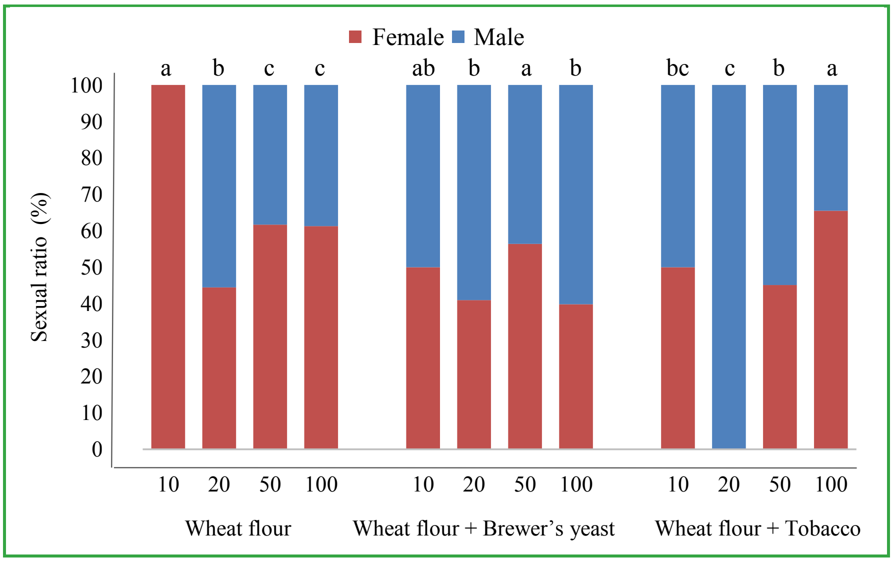

Figure 1. Proportion of males and females of *Anisopteromalus calandrae* emerged at different densities of the host (larvae of *Lasioderma serricorne*) and in the evaluated diets. Different lower case letters on the bars differ significantly ($p < 0.05$) in each treatment (Binomial test two proportions).

Sitthichaiyakul and Amornsak [21] showed that the proportion of males and females emerged from *Theocolax elegans* (Westwood, 1874) differed between host diets. More females were generated in the diet with brown rice than in the other evaluated diets (Maize, Sorghum, Wheat). Similar to the present study, where the diet with only flour, offered to *L. serricorne*, stood out from the others, generating more females.

Comparing larval densities in the same diet, it was observed that the higher densities, mainly of 100 larvae, had a positive effect on the parasitism index and on the average emergence of offspring.

These differences can be related to the occurrence of superparasitism at low densities, such as 10 and 20 larvae, in this study. As the couple of parasitoids remained throughout the entire life in contact with the host at a low density of available larvae, it may have led the female to oviposit more than once in the same host. This superparasitism probably generated intraspecific competition and, due to a lack of food resources, either prevented or impaired the development of the parasitoid.

The occurrence of superparasitism in host shortage conditions is pointed out by studies such as Wu and Noordlund [22], which evaluated the parasitism of *Anaphes iole* Girault, 1911 (Hymenoptera: Mymaridae) in relation to the density of the host *Lygus hesperus* Knight, 1917 (Hemimptera: Miridae). According to the authors, in the ratio of 1:40 (parasitoid: host eggs), only 10% of the eggs were superparasitized after 24 h. In a ratio of 1:9, the proportion of superparasitism was around 33%, 67% and 82% after exposure of 2, 6 and 24 h, respectively.

So, we emphasize the importance of correct density and the exposure time of hosts in rearing of natural enemies in laboratory. This fact is also highlighted by

Parra *et al.* [23], who state the necessity of having an optimal host/parasitoid relationship that, for a certain period, does not allow superparasitism. Thus, it was expected that, at higher densities, there would be a lower superparasitism, which actually occurred, since the proportional supply of larvae was higher.

Hanan *et al.* [24] who also evaluated the density of the host, *Trialeurodes vaporariorum* (Westwood, 1856) (Hem. Aleyrodidae) under parasitism of *Eretmocerus warrae* Naumann & Schimidt, 2000 (Hym. Aphelinidae), similarly observed that increased density of host nymphs from 20 to 140, made the rate of superparasitism decrease significantly.

Furthermore, the increase in the percentage of parasitism with increasing density can indicate a functional and/or numerical response of the parasitoid in relation to its host. The terms "functional response" and "numerical response" were proposed by Solomon [25] to denominate, respectively, the changes in the behavior of predators and the population increase of these, in function of the abundance of hosts.

According to Godfray [26], a natural enemy will be more effective if its functional response is dependent on the density of the host. Although in this study the type of functional response has not been evaluated, increased parasitism at higher densities indicates that *A. calandrae* has a good potential to be used as a biological control agent in environments with high host density.

In nature, *A. calandrae* mainly uses the beetle larvae of the family Curculionidae, which generally occur in high density in stored products [27]. Females lay many eggs over a long period and because of this, *A. calandrae*is considered a r-strategist [27]. Such fact is exemplified in the study of Gokhman, Fedina and Timokhov [28], in which *A. calandrae* deposited an average of 271 eggs for 40 days.

Those authors emphasize, however, that the values can vary depending on the environmental conditions of the creation system. We do not count the number of eggs laid per female, but we observed that in none of the treatments did the parasitoids emerge in the same amount of larvae offered. The emergence values of *L. serricorne*, on the other hand, were low, demonstrating that the paralization of the host larvae before oviposition is sufficient to carry out the control.

Considering such aspects, the highest larvae densities treatments (50 and 100), especially in the wheat flour diet (F), were those that presented the best rearing conditions. However, it should be considered that other factors can influence these results, such as photoperiod.

3.2. Influence of Photoperiod on the Parasitism of *A. calandrae*

In the control treatment, the average emergence of *L. serricorne* in scotophase was 85%, 85%, 92% and 91% at densities 10, 20, 50 and 100, respectively; in photoperiod of 12:12, of 85 (10), 75 (20), 83 (50) and 95% (100) and in photoperiod of 0:24, 75 (10), 82 (20) and 88% (100), respectively. This percentage differed significantly from that observed in the treatments of three photoperiods evaluated, which presented emergency values of 0%, 11%, 11% and 11% in treatment with scotophase (H = 34.39; $p < 0.001$); 8%, 0%, 8% and 3% in photoperiod of 12:12 (H = 38.12, $p < 0.001$) and 10%, 1%, 4% and 6% at 24:0, at densities 10, 20, 50 and 100, respectively (H = 31.35, $p < 0.001$).

There was no difference in the average parasitoid emergence and in the apparent parasitism between the different photoperiods tested, except for the apparent parasitism at the density of 100 larvae, which was lower in scotophase than in 12:12 photoperiod (Table 2). It was observed that emergence of offspring and apparent parasitism in most treatments (photoperiods and diets) had significantly lower values at the lowest densities (10 and 20 larvae) compared to larger ones, at 50 and 100 larvae.

Table 2. Mean emergence of parasitoid (±SD) (mean/treatment) and mean percentage (%) of parasitism according to the photoperiod during adult phase of the parasitoid *Anisopteromalus calandrae* and the density of the host *Lasioderma serricorne*.

	PHOTOPERIOD					
	0:24		12:12		24:0	
Larvae quantity	Emergence	Apparent parasitism (%)	Emergence	Apparent parasitism (%)	Emergence	Apparent parasitism (%)
10	0.09 ± 0.223 bA	20 bA	0.09 ± 0.099 cA	43.2 bA	0.03 ± 0.048 cA	25 bA
20	0.13 ± 0.105 bA	90 aA	0.11 ± 0.062 bcA	90 aA	0.11 ± 0.183 bcA	68 abA
50	0.50 ± 0.216 aA	85.3 aA	0.38 ± 0.155 abA	90.8 abA	0.29 ± 0.162 abA	92.1 aA
100	0.35 ± 0.143 aA	73.6 abB	0.48 ± 0.136 aA	93.6 abA	0.40 ± 0.233 aA	74.2 abAB

*Different lower case letters in column and capital in the row for the same parameter differ significantly ($p < 0.05$) by the Kruskal-Wallis test followed by Dunn test.

Although the total number of offspring differed between photoperiods, except for 12:12 h, the treatment in which hosts and parasitoids always remained in scothophase was the one that presented a sex ratio significantly higher than the total photophase. The density of host larvae influenced the sex ratio of the offspring only in the full scotophase and in the full photophase, being significantly higher the number of females in the higher densities. In the photoperiod of 12:12 there was no difference in sex ratio between the tested densities (Figure 2).

It was observed that *A. calandrae* is able to develop in different light regimes, however there was an advantage of the scotophase, both in the densities and in the sexual ratio of offspring.

On the other hand, Zart *et al.* [29], evaluating the influence of different photoperiods on the parasitism of *T. pretiosum* in *A. kuenhiella*, observed that in photoperiods of 12:12 and 0:24 the sex ratio remained high, indicating that the obtaining of females is favored when the number of light hours is equal to the number of darkness hours. The result found here is interesting, since stored products generally remain stored in closed and dark places. Thus, we believe that *A. calandrae* may carry out the parasitism even with variations in the substrate and in the photoperiod of the storage environment.

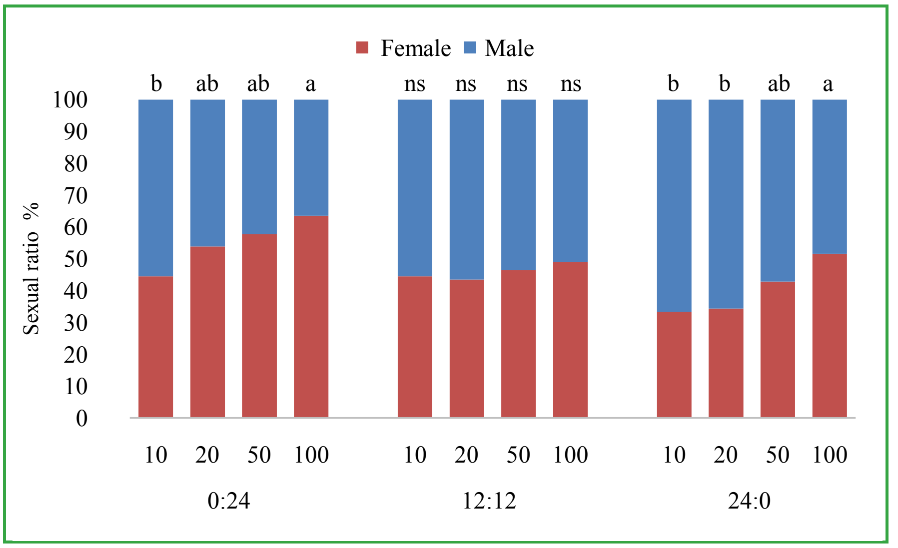

Figure 2. Proportion of males and females of *Anisopteromalus calandrae* emerged at different densities of the host (larvae of *Lasioderma serricorne*) and in the photophases evaluated. Different lower case letters on the bars differ significantly ($p < 0.05$) in each treatment (Binomial test two proportions); ns indicate no significant difference.

3.3. Longevity of Adults and Fertility of *A. calandrae* Females

Females had an average of 11.3 ± 4.74 days of life, while males lived an average of 9.2 ± 5.45 days (**Figure 3**), although there was no significant difference between the means ($H = 5.664$, $p > 0.05$).

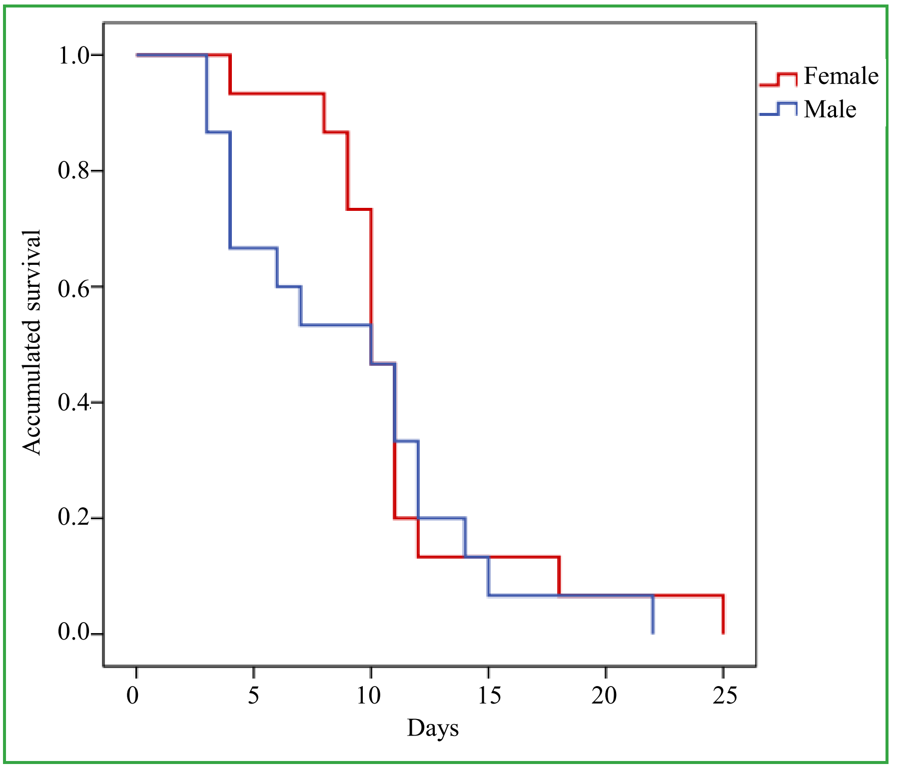

Figure 3. Survival curve of *Anisopteromalus calandrae* males and females.

The mortality pattern of the population, however, is different for males and females. There was a marked mortality of females between 7 and 12 days, with only 20% of females surviving for more than 20 days. To males, the mortality pattern was more homogeneously distributed during the exposure period, gradually increasing over the days (**Figure 3**).

The increase in longevity can potentially improve parasitoid performance, considering that the longer the parasitoid survival time in environment, the greater the chance of finding larvae suitable for oviposition (Aung *et al.*) [16]. Thus, *A. calandrae* can be considered an effective control agent of coleopteran pests because it can survive up to 25 days in the same environmental conditions where *L. serricorne* completes its cycle around 35 days (28˚C) [30].

Berger *et al.* [31] highlights that the timing of the releases of natural enemies is crucial and needs to be determined specifically for every single system of pest, showing the importance of knowing the period of survival of the parasitoid, to assist in control measures.

Belda and Riudavets [32], evaluating the potential of *A. calandrae* and *Lariophagus distinguendus* (Foster, 1841) (Hymenoptera: Pteromalidae) on the hosts *S. oryzae* and *R. dominica*, observed that, after one week of exposure to parasitism, 73% of *A. calandrae* and 90% of *L. distinguendus* were still alive, with males longer-lived than *A. calandrae* females. In this study, we also observed that after a week, about of 80% of the exposed couples to parasitism were alive, although, in this case, the females demonstrated longer-lived, suggesting that in different hosts the same parasitoid can show distinctive behaviors.

Females of *A. calandrae* produced an offspring average of 35.5 ± 1.68 individuals parasitizing *L. serricorne* throughout life. The maximum reproductive potential of females was reached in the first 10 days of exposure, with a marked and progressive decrease afterwards (**Figure 4**). Considering that females were

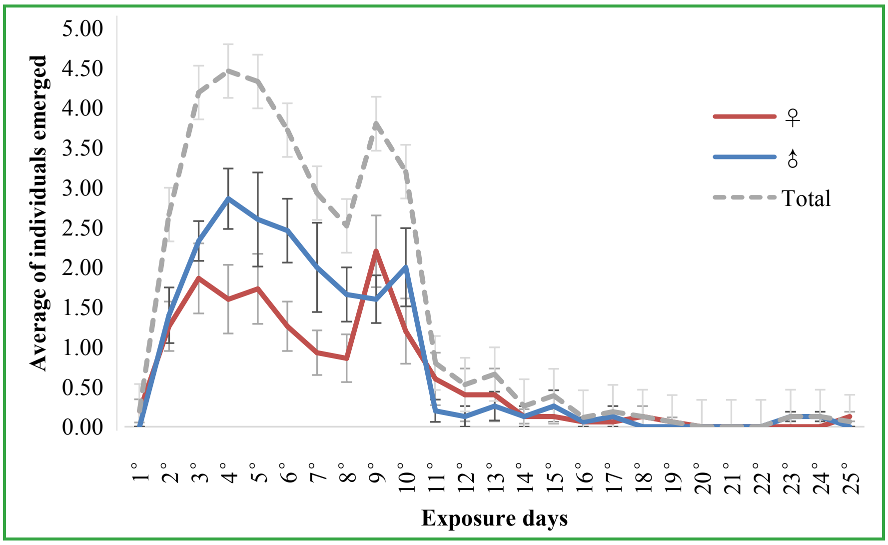

Figure 4. Mean emergency of males, females and total offspring (±SE) relative to the day of larvae exposure to the couples.

inserted in bioassays with a maximum of 48 h of life, it is inferred that they express their maximum reproductive potential when they are 4 - 12 days old.

The total daily apparent parasitism was 7.11% in average, the sex ratio of the total offspring was 0.95:1 (female to male) and most of the offspring emergency occurred between the third and the 10^{th} day of exposure (**Figure 4**).

Anisopteromalus calandrae females oviposited in the first 24 hours of exposure to the host larvae. However, emergencies related to the first day indicated that only females were generated at a mean of 0.2 ± 0.56. From the second day onwards, the number of offspring generated increased by 13.3 times. On the fourth day of larval exposure, the number of offspring occurred with an apparent parasitism of 21.13%. Visarathanonth *et al.* [33] observed, when evaluating the parasitism of *A. calandrae* on the host *S. zeamais*, that females had a reproductive period of 11 days with peak number of offspring on the fifth day, similar to this study, although in a different host.

In the same way as observed for *L. serricorne*, the sex ratio of *A. calandare* parasitizing *S. zeamais* was diverted to males, 0.44 (females) [33]. Evaluating the reproduction of *A. calandrae* and *L. distinguendus*, Belda and Riudavets [32] observed that sex ratio was also deviated for males (0.45 females) in *A. calandrae* and for females (0.65 females) in *L. distinguendus*.

Differences in sex ratio can be explained because, after detection of a host, the female is able to decide whether the larva is suitable for oviposition of a male or female, or either it will be used only for feeding [34]. This choice determines the offspring proportion and is based on several factors. Large larvae are commonly used for oviposition of females, while smaller ones are used to either generate males or for feeding [9]. In addition, the nutritional quality of the host, abiotic conditions and also genetic characteristics of the species, can act on offspring sexual ratio [34].

4. Conclusions

The factors that provided the best development conditions for *A. calandrae* were diet with only wheat flour and photoperiod 12:12 hours. In relation to densities, it is more appropriate to provide larger amounts (from 50 larvae per couple), promoting a single oviposition in each host larvae, especially when the exposure occurs for a longer period, thus decreasing the pressure of superparasitism and maximizing the fitness of the parasitoid.

The longevity of adults of *A. calandrae* was similar for males and females. The mortality pattern, however, is different being more pronounced for females between seven and 12 days. For males, the mortality was distributed more homogeneously during the exposure period, gradually increasing over the course of days.

We concluded that for a better result in control of *L. serricorne* in storage environments, the parasitoids should be released being 4 to 12 days old, since it is in this period that females present their maximum reproductive potential.

The data presented in this study provide important information about the biology of *A. calandrae*. Further studies evaluating the role of the agents involved in this interaction may be performed to make applicable the use of this biological control agent. Being a cosmopolitan parasitoid that attacks a large variety of beetles considered agricultural pests, it can be used in several storage environments to control these insects, reducing the application of chemicals, benefiting the product and the environment.

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