

Reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) with Different Densities and Parasitism Periods in *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) Pupae

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Mass production of the endoparasitoid *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) should be studied to allow its use in the biological control of Lepidoptera pests. The objective of this study was to evaluate the reproduction of *T. diatraeae* with *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) pupae with different host densities and parasitism periods in the laboratory. The experiment had 12 24/48-hour old *A. gemmatalis* pupae per replication individualized and exposed to 1:1, 3:1, 6:1, 9:1, 12:1, or 15:1 parasitoids/host densities for 12, 24, 48, or 72 hours, respectively, in a climatized chamber at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 14 hour photophase. Parasitoid emergence increased up to 10 parasitoids/host. Parasitoid life cycle (egg-adult) was shorter and parasitism percentage increased as parasitoid density and parasitism period increased. Increasing female parasitoid density reduced the sex ratio, but parasitism period did not affect this parameter. The combination of 10 parasitoids/pupa during 24 hours should be used for *T. diatraeae* reproduction.

Key words: Alternate host; biological control; mass production; parasitoids.

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Trichospilus Ferrière, 1930 (Hymenoptera: Eulophidae), mainly composed by gregarious parasitoids of Lepidoptera pupae, is a small genus of the Eulophidae family (NOYES 2003; PASTORI et al. 2012a). *Trichospilus diatraeae* Cherian & Maragabandhu, 1942 (Hymenoptera: Eulophidae) was described with individuals emerged from *Diatraea venosata* Walker, 1863 (Lepidoptera: Cram-

bidae) pupae in southern India and, in 1963, it was introduced in Trinidad & Tobago (Antillas) for lab tests with host. In 1984, *T. diatraeae* was found in *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) pupae in corn (BENNETT *et al.* 1987). In Brazil, this parasitoid was recorded in Arctiidae pupae (PARON & BERTI-FILHO 2000). Mass release in Central America – mainly Barbados – for Lepidoptera pest control, might have facilitated its migration to South America.

Trichospilus diatraeae is a promising biological control agent due to its generalist habit, ease of breeding in the laboratory and excellent biological indexes (RIBEIRO *et al.* 2013; RODRIGUES *et al.* 2013). To use the parasitoids as a promising alternative for pest control, it is necessary to multiply them on a large scale (PARRA & CÔNSOLI 2009). In mass production it is essential to guarantee the quality of the natural enemy, since properly produced parasitoids present a better capacity to locate and parasitize the host under field conditions (VREYSEN & ROBINSON 2010).

The use of parasitoids for biological control programs depends on finding adequate alternative hosts for mass production (PEREIRA *et al.* 2009; OLIVEIRA *et al.* 2016). The selection of the host is crucial in the mass creation of parasitoids (MAGRO & PARRA 2001; RAMALHO & DIAS 2003), since its physical and chemical characteristics can affect the acceptance and adaptation of the species and/or lineages, interfering in this way in the biological characteristics, impairing the quality of the parasitoid produced and its performance in field (BIGLER 1994; MONJE *et al.* 1999; VIEIRA *et al.* 2017). *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) can be easily multiplied in laboratory (GREENE *et al.* 1976) with a relatively short life cycle, around 11.0 to 16.0 days for males and females, using artificial diet (SILVA *et al.* 2012). Moreover, *T. diatraeae* develops with its pupa (PARON & BERTI-FILHO 2000), demonstrating its potential to mass produce this parasitoid.

Host density, periods of parasitism and host age affect offspring (PEREIRA *et al.* 2010; SILVA *et al.* 2014; BARBOSA *et al.* 2016), sex ratio (CHAI-SAENG *et al.* 2010), parasitism (PEREIRA *et al.* 2010), progeny (COSTA *et al.* 2014a; SILVA *et al.* 2015), life cycle duration and longevity of parasitoids (SILVA-TORRES & MATTHEWS 2003; COSTA *et al.* 2014b). Increasing host exposure time should increase parasitoid offspring production, revealing the necessity to study host density and exposure period for parasitoid production (FÁVERO *et al.* 2013).

This work aimed to evaluate the best density x parasitism time combination for producing *T. diatraeae* in *A. gemmatalis* pupae.

Materials and Methods

The experiment was performed in the laboratory and had three stages:

Production of *Anticarsia gemmatalis*

Anticarsia gemmatalis caterpillars were collected in experimental soybean crops at the “Campo Experimental Diogo Alves de Melo” in “Universidade Federal de Viçosa”, Minas Gerais State, to initiate the rearing of laboratory stock. These caterpillars were put in plastic containers (1.000 ml) with perforated lids and closed with an organza-type screen. Artificial diet was supplied daily (GREENE *et al.* 1976). The obtained prepupae were transferred to similar containers with 2 cm of slightly wet sand at the bottom.

Anticarsia gemmatalis pupae were collected every two days and transferred to Petri dishes (12.0 x 1.5 cm). Emerged adults were put into screened wood cages (30.0 x 30.0 x 30.0 cm) for egg-laying on bond paper tapes. These paper tapes were removed with emerged caterpillars to continue rearing of this insect. All production stages were kept in a climatized room at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity (RH) and 12 hour photophase.

Production of parasitoids

Trichospilus diatraeae adults were kept in test tubes (14.0 x 2.2 cm) covered with cotton containing honey droplets on its interior. Five 0 to 48 hour old *A. gemmatalis* pupae (collected every two days) were exposed to parasitism by approximately 50 adult *T. diatraeae* females for 24 hours at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 14 hour photophase. After this period, the pupae were transferred to other test tubes (14.0 x 2.2 cm) covered with cotton and maintained in the same conditions described until emergence of the adult.

Experimental development

Each *A. gemmatalis* pupa up to 24 hours old was weighed (209.20 ± 4.82 mg) and transferred to a test tube (14.0 x 2.2 cm) closed with cotton. *T. diatraeae* adults were put into these tubes at densities of 1:1, 3:1, 6:1, 9:1, 12:1 or 15:1 of parasitoids/host. Parasitism was allowed for 12, 24, 48 or 72 hours per density in an acclimatized chamber at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 14 hour photophase. *T. diatraeae* females were removed at the end of the parasitism period with a fine fiber brush under a stereoscopic microscope and the tubes containing parasitized pupae were kept in the same acclimatized chambers.

Life cycle duration of parasitoids (egg to adult), parasitism percentage [(the number of *A. gemmatalis* pupae with parasitoid emergence + pupae without the emergence of *A. gemmatalis* adults) by (total number of pupa) * 100], number of parasitoids emerged per *A. gemmatalis* pupae [(number of *A. gemmatalis* pupae with adult parasitoid emergence by (number of parasitized pupae) * 100], sex ratio (SR= number of females/number of adults) and longevity of males and females were evaluated. The natural mortality of the host was determined under experimental conditions as the number of *A. gemmatalis* adults that emerged out of 50 *A. gemmatalis* pupae that were placed individually in plastic glass (100 ml) with a specific diet portion and without parasitoids. The parasitoid genus was determined based on morphological features of its antenna and abdomen.

The experiment was conducted in a completely randomized design in factorial scheme 6 x 4 (densities x parasitism times, respectively) with 12 replications, each with one *A. gemmatalis* pupa. Data of the 12 replications were used to determine life cycle duration, parasitism and emergence percentage, number of parasitoids emerged per pupae with host and sex ratio of the generation. Ten males and 20 females were randomly collected per treatment to determine male and female longevity.

Biological parameters were analyzed at 5% significance level and significant values submitted to regression analysis. The polynomial type equation from quadratic models $Y = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + e_i$ and/or the paraboloid response surface for regression analysis $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1 X_1^2 + \beta_2 X_2^2 + e_i$ were adopted, where: $i = 1$ or 2 estimated parameters; e_i = random error with one and/or two independent variables, respectively.

The equations that best fit the data were chosen from quadratic and paraboloid models with all significant parameters based on the determination coefficient (R^2), the significance of regression coefficients (β_i) and the F test (up to 5% probability), and the biological phenomenon studied. Maximum values for each equation were determined via first derivative calculation/resolution of the adjusted regression model.

Results

The life cycle duration of *T. diatraeae* with *A. gemmatalis* pupae decreased with increasing densities of this host and parasitism period, with the shortest duration using the combination of 15.0 females for 60.2 hours parasitism (Fig. 1) ($P < 0.05$).

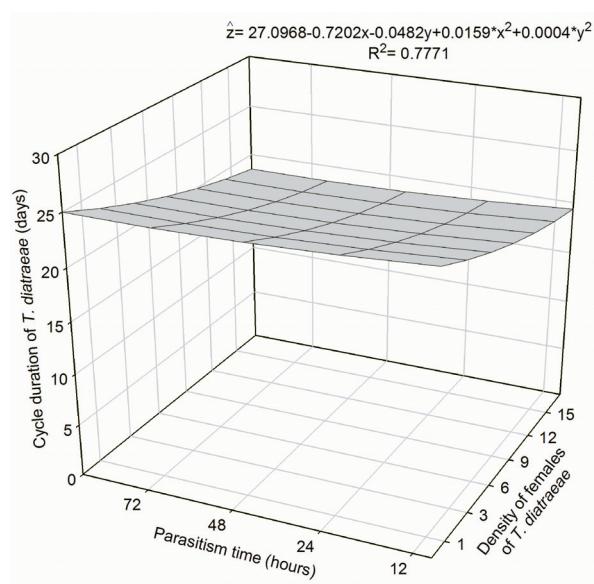


Fig. 1. Life cycle (days) by *Trichospilus diatraeae* (Hymenoptera: Eulophidae) on *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) pupae as a function of female density and parasitism periods ($y_0 = P < 0.0001$; $x = P < 0.0001$; $y = P < 0.0070$; $x^2 = P < 0.0002$; $y^2 = P < 0.0469$).

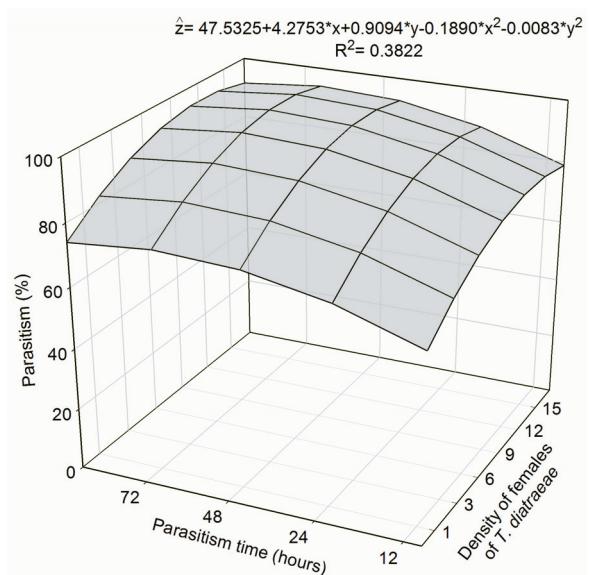


Fig. 2. Parasitism by *Trichospilus diatraeae* (Hymenoptera: Eulophidae) on *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) pupae as a function of female density and parasitism periods ($y_0 = P < 0.0001$; $x = P < 0.0001$; $y = P < 0.0019$; $x^2 = P < 0.0039$; $y^2 = P < 0.0141$).

Parasitism by *T. diatraeae* on *A. gemmatalis* pupae increased with host exposure period and parasitoid density, with a maximum value for the combination of 11.3 parasitoid females for 54.8 hours of parasitism (Fig. 2).

The parasitism period did not affect the emergence of *A. gemmatalis* adults and the sex ratio of

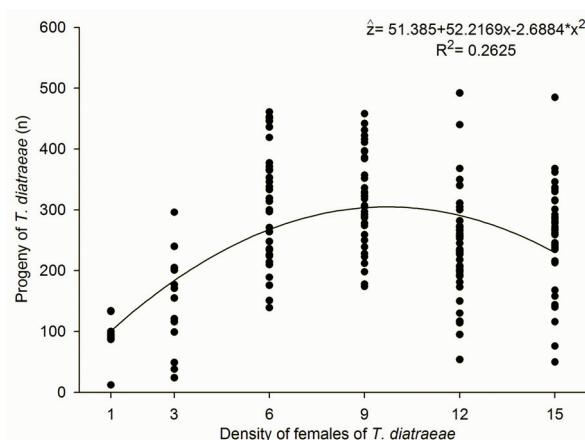


Fig. 3. Number of emerged adults of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) from *Anticarsia gemmatalis* pupae as a function of parasitoid density and host exposure period ($y_0 = P < 0.0579$; $x = P < 0.0001$; $x^2 = P < 0.0001$).

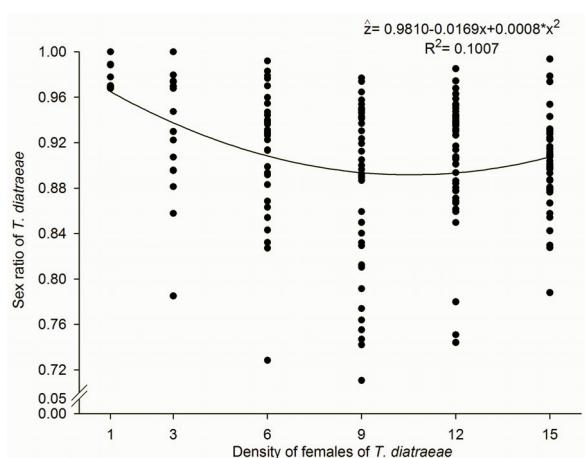


Fig. 4. Sex ratio of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) from *Anticarsia gemmatalis* pupae as a function of parasitoid density and host exposure period ($y_0 = P < 0.0001$; $x = P < 0.0001$; $x^2 = P < 0.0007$).

T. diatraeae, fitting the quadratic functions according to parasitoid density per *A. gemmatalis* pupa (Fig. 3 and 4), with maximum and minimum sex ratio with 9.7 and 10.6 parasitoids/pupa, respectively.

Parasitism period and mostly parasitoid density did not affect *T. diatraeae* female longevity (Fig. 5), with a maximum point at 24.0 days with the combination 8.8 females for 40.2 hours of parasitism. Parasitism period did not affect male longevity with a maximum value of 23 days at the density 8.6 females/host pupa (Fig. 6).

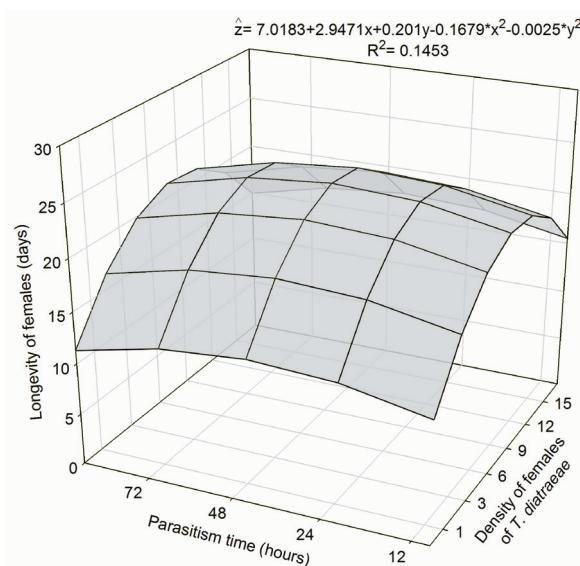


Fig. 5. Longevity of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) females from *Anticarsia gemmatalis* pupae as a function of parasitoid density and host exposure period ($y_0 = P < 0.0007$; $x = P < 0.0001$; $y = P < 0.0313$; $x^2 = P < 0.0001$; $y^2 = P < 0.0180$).

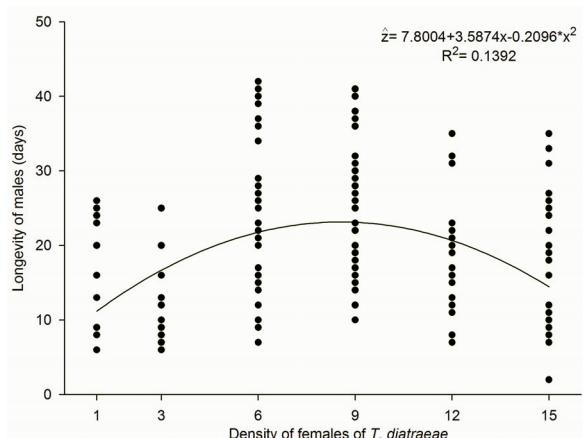


Fig. 6. Longevity of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) males from *Anticarsia gemmatalis* pupae as a function of parasitoid density and host exposure period ($y_0 = P < 0.0011$; $x = P < 0.0001$; $x^2 = P < 0.0001$).

Discussion

The reduced duration of parasitoid life cycle with increasing female density and parasitism periods is due to super-parasitism by the attack of more than one *T. diatraeae* female per *A. gemmatalis* pupa. This reduction was also observed in *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) pupae, showing that food availability can influence their life cycle (VARGAS *et al.*

2014). The number of parasitoid females used per host is one of the factors that can affect the lifespan of the progeny (CHEN *et al.* 2017). A reduction of egg-adult development time of the parasitoid by increasing the density of females of the natural enemy per host, may be related to factors such as immature competition (PEREIRA *et al.* 2010), and competition between immature, physical combat or physiological suppression (ANDRADE *et al.* 2010; PEREIRA *et al.* 2017). After parasitism, the host becomes the source of food and shelter of the natural enemy (CÔNSOLI & VINSON 2002). Thus, if the number of immatures is higher than the number supported by the host, there may be a change in development time.

Adequate parasitoid density per host provides a balance of numbers and quality of the natural enemies produced, which is attributed to the nutritional resources for their development (PEREIRA *et al.* 2010). The use of parasitoid numbers higher than those borne by the host can lead to the rapid reduction of nutritional resources as a consequence of superparasitism and low benefit of host defense (ANDRADE *et al.* 2010). Superparasitism is a peculiar characteristic of parasitoids, defined as the deposition of one or more eggs in a host already parasitized by an insect of the same species (PEREIRA *et al.* 2017) and directly affects the duration of the insect cycle, usually slowing it down (BARBOSA *et al.* 2008).

Increasing the density of *Melittobia digitata* Dahms, 1984 (Hymenoptera: Encyrtidae) by pupa of *Neobellieria bullata* (Parker, 1916) (Diptera: Sarcophagidae) reduced parasitoid development time (SILVA-TORRES & MATTHEWS 2003). The duration of the life-cycle of *Palmistichus elaeensis* Delvare & LaSalle, 1993 (Hymenoptera: Encyrtidae) decreased with increasing density of females by pupae of *A. gemmatalis* (PASTORI *et al.* 2012b). In *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Encyrtidae), variation of the density of females per pupae of *D. saccharalis* did not influence the duration of the cycle (days) of the parasitoid (COSTA *et al.* 2014b). The period in which the host is exposed to the parasitoid is also one of the crucial factors for a parasitoid performance check (FÁVERO *et al.* 2013). It may influence the nutritional status of parasitoid females due to the higher ingestion of fluids from the host pupae (WYLIE 1966) causing superparasitism (MELLO *et al.* 2010).

The exposure of pupae of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) for 72 h to the parasitoid wasp *Nasonia vitripennis* Walker, 1836 (Hymenoptera: Pteromalidae) caused an increase in immature developmental time (MELLO *et al.* 2010). The use of 11.3 female parasitoids for 54.8 hours of exposure time increased the parasit-

ism rate such that density/time of exposure was necessary for the parasitoid to perform successfully. This result also suggests that *T. diatraeae* shows population regulation potential of *A. gemmatalis* at the density and time reported. Some species of parasitoids have a higher rate of parasitism with increasing host density and time exposed to the host, stabilizing when reaching the maximum parasitism capacity of the female (CHEN *et al.* 2018).

The parasitism rate of *T. diatraeae* ranged from 33.3 to 100.0%, when the proportion was 1:1 and 35:1 females of the parasitoid per pupa of *Thrinaxia arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) (ZACHÉ *et al.* 2011). A higher rate of parasitism occurs in the proportion of three to five females of *N. vitripennis* per pupa of *C. megacephala* (MELLO *et al.* 2009). For females of *T. howardi* and *T. diatraeae*, 24 hours are sufficient to reach 100% parasitism in *Helicoverpa armigera* (Hubner, 1808) (Lepidoptera: Noctuidae) pupae (OLIVEIRA *et al.* 2016). The density of six *A. gemmatalis* pupae with ten *T. diatraeae* females shows that these characteristics are the most appropriate to mass production of this parasitoid, as well as for obtaining more females (sex ratio). Using density of parasitoid females above the host-supported density may result in a decrease in the percentage of emergence (BARBOSA *et al.* 2008; ZACHÉ *et al.* 2011); this is correlated to the capacity limit of the host (available food) in supporting the eggs of the parasitoid. Parasitized larvae need to ingest sufficient amounts of nutrients to improve the physical condition and the perpetuation of the species (GATHALKAR *et al.* 2017).

For gregarious parasitoids, an increase in the number of postures in a host is proportional to the number of parasitoids that emerge from it with a maximum host load, that is, it limits the reduction of the emergence of parasitoids (RASOOL *et al.* 2017). The emergence rate of *T. diatraeae* at proportions above 28 females of the parasitoid per pupa of *T. arnobia* is significantly reduced. This proportion includes the maximum number of descendants (ZACHÉ *et al.* 2011). The exposure time of the parasitoid / host is also an important factor in the acceptance by the parasitoid (CARNEIRO *et al.* 2009; CHEN *et al.* 2018), i. e., it influences the percentage of emergence (PEREIRA *et al.* 2010). The emergence of *N. vitripennis* did not vary between 24, 48, 72 and 96 h of exposure to pupae of *C. megacephala* (MELLO *et al.* 2010).

In the present work the densities and time provided more than 85% of females in all treatments. Densities above those recommended may cause superparasitism, and consequently host contact through superparasitism is necessary for the symbiont's spread in populations of its primary host

N. vitripennis, such that when superparasitism rates are high, the symbiont causes highly female biased population sex ratios and consequently causes local host extinction (PARRATT *et al.* 2016).

Sex ratio variations may be beneficial or detrimental to biological control programs (BARBOSA *et al.* 2016), but larger female numbers must be sought, since males are not parasitic (PANDEY & TRIPATHI 2008). Moreover, the number of females produced is important to maintain and increase the population of natural enemies (HEIMPEL & LUNDGREN 2000). Female parasitoids can change their progeny sex ratio when submitted to certain conditions, such as the presence of several females per host pupa, but the parasitoid female tends to produce a sufficient number of males to fertilize the offspring (CARNEIRO *et al.* 2009). The strategy to invest in a larger number of males in the progeny can assure what is known as “local mate competition” (HAMILTON 1967). The number of females of Eulophidae always exceeds 90% (PEREIRA *et al.* 2009, PASTORI *et al.* 2012a,c), suggesting not enough time to promote size reduction and “fitness” of males as in females.

The parasitism period and female density affected *T. diatraeae* sex ratio confirming that parasitoid fitness depends on its ability to manipulate reproductive strategies when in competition. For example, *Diaeretiella rapae* (McIntosh, 1855) (Hymenoptera: Braconidae) females adjust their progeny production and progeny sex ratio with changing competition; the number of female progeny decreased when multiple females competed for the same host (KANT & MINOR 2017). There was an increase in the number of females compared to males of *N. vitripennis* for the 24, 48, 72 and 96 h of exposure to pupae of *C. megacephala* (MELLO *et al.* 2010).

The longevity of adults (males and females) of *T. diatraeae* was similar between treatments. The densities were sufficient for the survival of the adults and provided the females, in laboratory or field conditions, the time necessary to seek their hosts (CARNEIRO *et al.* 2009). Females permanently exposed to parasitism tend to have lower longevity when compared to females deprived of hosts, it was observed that even after nine days of host absence there was no significant reduction in *Telenomus remus* (Nixon, 1937) (Hymenoptera: Platygastridae) parasitism rates (CARNEIRO *et al.* 2009). In mass production, the survival capacity of natural enemies is one of the requirements for quality control (QUEIROZ *et al.* 2017). Establishing the best exposure time is essential, as well as the ideal amount of parasitoid/host to guarantee greater longevity of the progeny, because, at high densities and longer contact time there will be less nutritional utilization by high competition, leading

to less longevity (COSTA *et al.* 2014b; BARBOSA *et al.* 2016; CHEN *et al.* 2018). In general, female parasitoids tend to present greater longevity in relation to males, since the females have the ability to feed on the host’s fluid through the ovipositor.

The longevity of males and females did not show differences when different densities of *T. diatraeae* were used per pupa of *D. saccharalis* (VARGAS *et al.* 2014). *Melittobia australica* Girault, 1912 (Hymenoptera: Eulophidae) and *M. digitata* present higher adult longevity at a density of up to three parasitoids per pupa of *N. bullata* (SILVA-TORRES & MATTHEWS 2003).

Conclusion

Ten *T. diatraeae* female should be used per *A. gemmatalis* pupa during 24 hours as the best density to mass produce this parasitoid.

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Author Contributions

Research concept and design: G.S.A., F.F.P, P.L.P.; Collection and/or assembly of data: F.A.L.D.O., R.O.S.; Data analysis and interpretation: F.A.L.D.O., R.O.S., C.R.C., P.L.P.; Writing the article: F.A.L.D.O., R.O.S., N.R.X.D.O., G.S.A., F.F.P, C.R.C., P.L.P.; Critical revision of the article: N.R.X.D.O., G.S.A., F.F.P, J.C.Z. C.R.C., P.L.P.; Final approval of article: P.L.P.

Conflict of Interest

The authors declare no conflict of interest.

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