

Rearing the Predator *Brontocoris tabidus* (Heteroptera: Pentatomidae) with *Tenebrio molitor* (Coleoptera: Tenebrionidae) Pupa on *Eucalyptus grandis* in the Field

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ABSTRACT

The use of natural enemies represents an important strategy in programs of Integrated Pest Management (IPM). Asopinae predators are reared with alternative prey, but supplementary plant may enhance the fecundity and longevity of their females. The objective of this research was to evaluate the development and reproduction of the predator *Brontocoris tabidus* Signoret, 1852 (Heteroptera: Pentatomidae) fed with *Tenebrio molitor* L., 1785 (Coleoptera: Tenebrionidae) pupae in a *Eucalyptus grandis* Hill ex Maiden (Myrtaceae) plant in the field. The duration and survival of the nymphal stage of *B. tabidus* were 32.07 ± 8.95 days and $77.00\% \pm 0.42\%$, respectively with 15.55 ± 8.19 egg masses, 603.00 ± 379.20 eggs and 387.45 ± 291.40 nymphs per female of this predator while longevity of its males and females was 119.80 ± 32.80 and 160.20 ± 42.13 days, respectively. Better results with *E. grandis* plant in the field and the fact that *B. tabidus* will be adapted at release time show that this predator should be reared in these conditions.

Keywords: Asopinae, Insect-Plant Interactions, Integrated Pest Management, Rearing Predators, Supplementary Food

1. Introduction

The adoption of Integrated Pest Management (IPM) reduces problems such as pest resistance and the impact of pesticides on natural enemies [1-3]. Biological control is an important tactic in IPM programs for handling pathogens and arthropods, including the Pentatomidae, a predatory bug species in reforested areas [4] where the defenses in plants can be beneficial [5], harmful or have no impact [6] on natural enemies.

Predators have a higher reproductive rate when they get plenty of food and this rate tends to decrease with less availability of food to benefit the survivors. When predatory bugs are fed more food to gain weight, there is an increase in the frequency of attacks on prey, and posture and development are faster [7].

Supputius cincticeps Stal, 1860 (Heteroptera: Pentatomidae) nymphal stage was shorter and its females heavier

when fed with pupae of *Tenebrio molitor* L., 1785 (Coleoptera: Tenebrionidae) and on seedlings of *Eucalyptus urophylla* ST Blake (Myrtaceae) [8]. *Podisus nigrispinus* Dallas, 1851 (Heteroptera: Pentatomidae) had shorter duration of nymphal stage with *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae) caterpillars, except in the fourth instar, when fed on cotton and tomato plants and prey than when they were fed only with prey [9]. Moreover, the availability of cotton or weeds reduced the impact of the shortage of prey in the partial duration, viability and nymphal stages of this predator, but it has not reached sexual maturation and not oviposited without prey [10,11].

Females of *Podisus connexivus* Bergroth, 1891 (= *P. nigrispinus*) (Heteroptera: Pentatomidae) had greater weight gain with *E. urophylla* and prey. *Podisus maculiventris* Say, 1832 (Heteroptera: Pentatomidae) showed higher survival rates and shorter pre-oviposition and nymphal periods when fed on prey and potato leaves [5].

The longevity of *P. nigrispinus* was similar for both sexes, but males showed greater longevity when fed on prey and cotton plants [9]. Females of *P. maculiventris* showed a higher survival rate when they were given potato or tomato than when they were only given water until the 35th day of life, with values of 31.9, 28.8 and 27.1 days for the potato, tomato or water diets, respectively [12]. The availability of prey with cotton plants increased longevity of *P. nigrispinus* with 30.3 ± 10.53 days for females and 43.8 ± 17.70 for males, versus 16.0 ± 11.4 and 19.93 ± 4.56 days for males and females of this predator, respectively, only, with prey [9]. The ability of predators to obtain moisture and plant nutrients can be considered an adaptation to improve survival rate at these times of scarcity of prey [13,14].

The aim of this research was to investigate the rearing of *Brontocoris tabidus* Signoret, 1852 (Heteroptera: Pentatomidae) fed with *T. molitor* pupae associated to *Eucalyptus grandis* Hill ex Maiden (Myrtaceae) plant in field conditions.

2. Materials and Methods

This study was conducted in an area (field) of the “Departamento de Biologia Animal (DBA)” of “Universidade Federal de Viçosa (UFV)” in the city of Viçosa, Minas Gerais State, Brazil. The mean temperature during the

nymphal and adult stages of *B. tabidus* was 19.55°C , with the minimum temperature of 11.70°C recorded in July and the maximum temperature of 30°C recorded in March. The relative humidity was 77.58% (Table 1).

Nymphs of *B. tabidus* were obtained at the “Laboratório de Controle Biológico de Insetos (LCBI)” of the “Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO)” of UFV, where the referred species is reared at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $70\% \pm 5\%$ of relative humidity and photoperiod of 12 hours. Egg masses and first instar nymphs of *B. tabidus* were maintained in Petri dishes (9.0×1.2 cm) with a cotton pad soaked in distilled water. One hundred second instar nymphs of *B. tabidus* were placed in a white organza bag (20×30 cm) involving the extremity of a branch of an approximately 2-year-old *E. grandis* plant. Nymphs were fed with *T. molitor* pupae from a mass rearing facility of the UFV. Water was supplied to this predator in 2.5 mL tubes (similar to those used in dental anesthesia) fastened to *E. grandis* branches with adhesive ribbon (Figure 1).

Mortality of nymphs, change of instars and emergence of *B. tabidus* adults were daily observed [15]. Adults of this predator were weighed using a scale with 0.1 mg precision, approximately 24 hours after their emergence when they were sexed and individually placed in Petri dishes [16,17].

Table 1. Monthly mean, maximum (Max.), minimum (Min.) and mean per period of temperature (temp), relative humidity (%); mean daily rainfall (mm) and photoperiod (hours per day). Municipality of Viçosa, Minas Gerais State, Brazil. March to November 2010.

Months	Temp. Mean ($^{\circ}\text{C}$)	Temp. Max. ($^{\circ}\text{C}$)	Min. Temp. ($^{\circ}\text{C}$)	Rel. Hum. (%)	Rainfall (mm)	Photoperiod (hours)
March	23.00	30.00	19.06	80.79	1.06	11.88
April	21.86	29.86	16.95	77.65	1.12	11.52
May	19.12	26.31	14.69	78.96	1.91	11.02
June	17.92	25.61	13.30	80.71	0.01	10.76
July	16.84	25.00	11.70	77.91	0.06	10.87
August	18.33	26.53	12.41	74.81	0.21	11.03
September	19.07	25.87	14.28	74.05	2.67	11.89
October	20.49	26.72	16.19	74.29	4.91	12.49
November	22.18	27.70	18.68	81.73	7.71	13.00
Temp	19.55	26.77	14.86	77.58	2.29	11.61

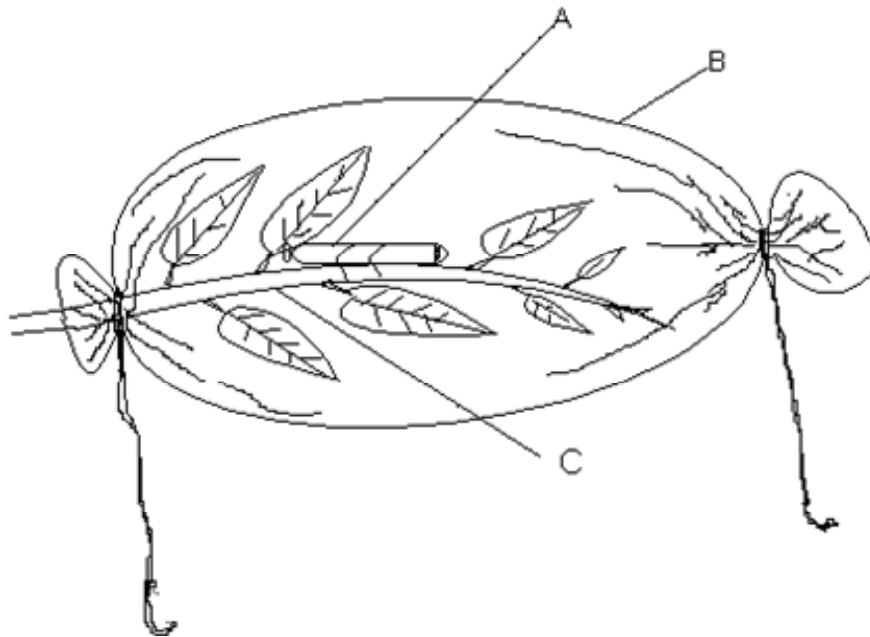


Figure 1. Organza bag used to rear *Brontocoris tabidus* on *Eucalyptus grandis* plant in the field. A—anaesthetic odonathological tube; B—organza cloth. C—*Eucalyptus grandis* branch.

Twenty *B. tabidus* females weighing more than 120 mg were selected to reduce the influence of this trait on female productivity and mated four days after their emergence [18]. Pairs of this predator were involved in organza bags similar to those used for their nymphs and maintained on *E. grandis* plant in the field until the death of males and females. The males that died before their respective females were replaced by other ones reared under similar conditions. Pairs of *B. tabidus* were given water daily and fed on *T. molitor* pupae, when the duration of pre-oviposition, oviposition and post-oviposition periods, as well as the number of egg masses, eggs and nymphs per female, egg viability and longevity of females of this predator were evaluated. Egg masses of *B. tabidus* were daily collected and placed in Petri dishes (9.0 × 1.2 cm) with a cotton pad soaked in distilled water and its nymphs were counted 24 hours post-eclosion.

3. Results

Brontocoris tabidus did not prey during the first instar that lasted 3.52 ± 0.50 days, whereas the second, third, fourth and fifth instars lasted 5.80 ± 1.38 , 4.93 ± 1.50 , 6.10 ± 3.04 and 11.72 ± 2.68 days (**Figure 2(a)**). The duration of the nymphal stage of this predator was 32.07 ± 8.95 days on *E. grandis* plant (**Figure 2(a)**).

The survival rate of *B. tabidus* was $91.00\% \pm 0.28\%$, $92.30\% \pm 0.26\%$, $94.04\% \pm 0.23\%$ and $97.46\% \pm 0.15\%$ during the second, third, fourth and fifth instars, whereas adult emergence of this predator reached $77.00\% \pm 0.42\%$

on the *E. grandis* plant with *T. molitor* pupae (**Figure 2(b)**).

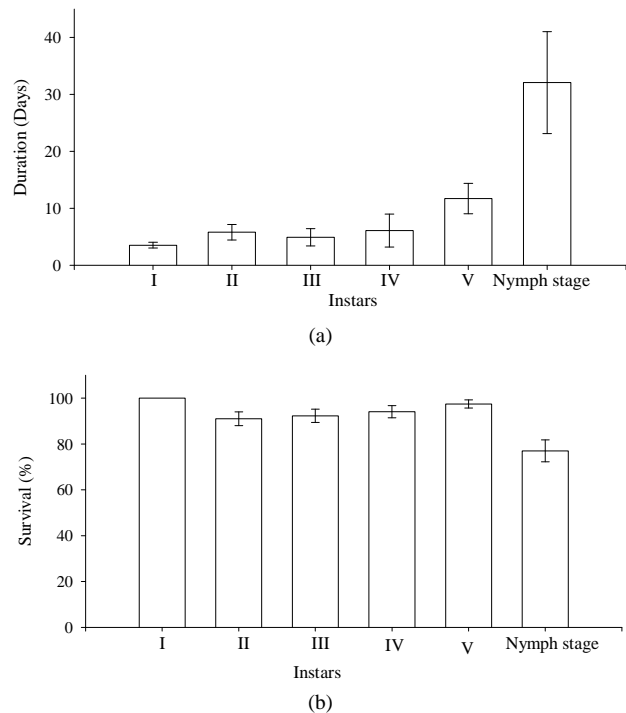


Figure 2. Duration (a) and survival (b) (mean ± standard deviation) during each instar and of the nymphal stage of *Brontocoris tabidus* fed with *Tenebrio molitor* pupae on a *Eucalyptus grandis* plant in the field. Municipality of Viçosa, Minas Gerais State, Brazil.

The pre-oviposition, oviposition and post-oviposition periods of *B. tabidus* females were 50.16 ± 35.12 , 108.79 ± 38.76 and 7.84 ± 6.85 days, respectively with 603.00 ± 311.20 eggs and 15.55 ± 8.19 egg masses per female (**Table 2**). The nymphal stage of this predator was as follows: 387.45 ± 291.40 nymphs per female and 58.83%

$\pm 24.23\%$ nymph hatching (**Table 2**).

The survival rate of *B. tabidus* females was over 50% until they were 170 days old and egg production was greater between days 60 and 160 of their life (**Figure 3**). This predator had 0.0 to 5.49 eggs and 0.0 to 4.72 nymphs per day per fertile female (**Figure 4**).

Table 2. Reproductive characteristics, longevity and weight (mean \pm standard deviation) of males and females *Brontocoris tabidus* fed with *Tenebrio molitor* pupae on a *Eucalyptus grandis* plant in the field. Municipality of Viçosa, Minas Gerais State, Brazil.

Characteristics evaluated	Results
Incubation period (days)	5.16 ± 0.50
Pre-oviposition period (days)	50.16 ± 35.12
Oviposition period (days)	108.79 ± 38.76
Post-oviposition period (days)	7.84 ± 6.85
Number of eggs/female	603.00 ± 311.20
Number of egg masses/female	15.55 ± 8.19
Number of eggs per egg mass	37.01 ± 7.44
Number of eggs per day	3.5 ± 1.60
Percentage of nymph hatching (%)	58.83 ± 24.23
Number of nymphs per female	387.45 ± 291.40
Number of nymphs per egg mass	21.52 ± 8.02
Longevity of males (days)	119.80 ± 32.50
Longevity of females (days)	160.20 ± 42.13
Weight of males (mg)	102.70 ± 5.91
Weight of females (mg)	138.80 ± 11.24

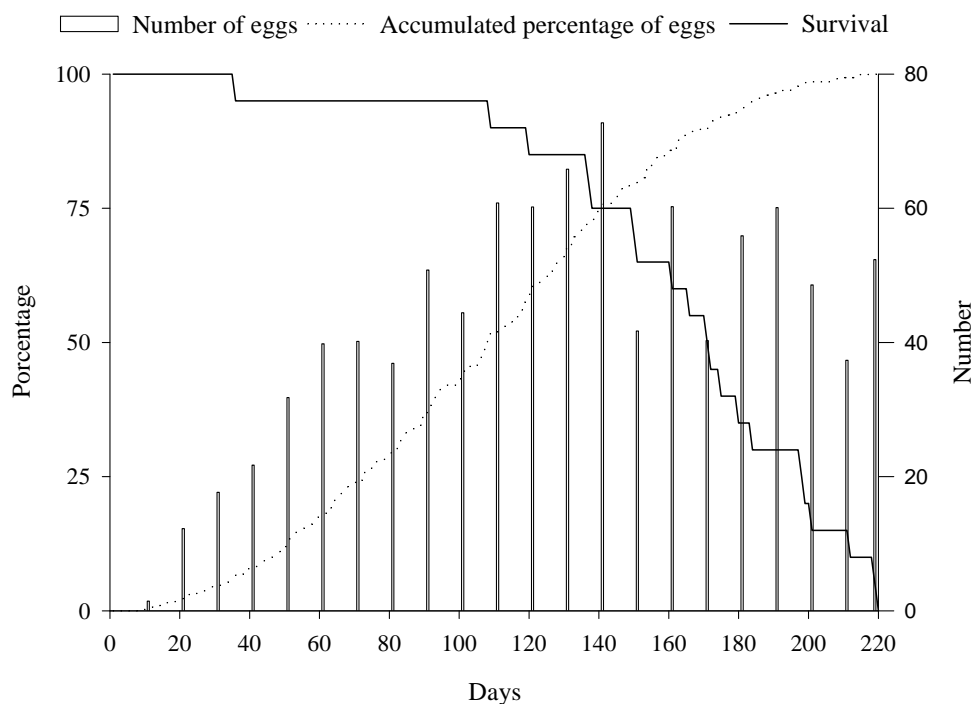


Figure 3. Number of eggs per period of 10 days, accumulated percentage of eggs produced and survival of *Brontocoris tabidus* females fed with *Tenebrio molitor* pupae on a *Eucalyptus grandis* plant in the field. Municipality of Viçosa, Minas Gerais State, Brazil.

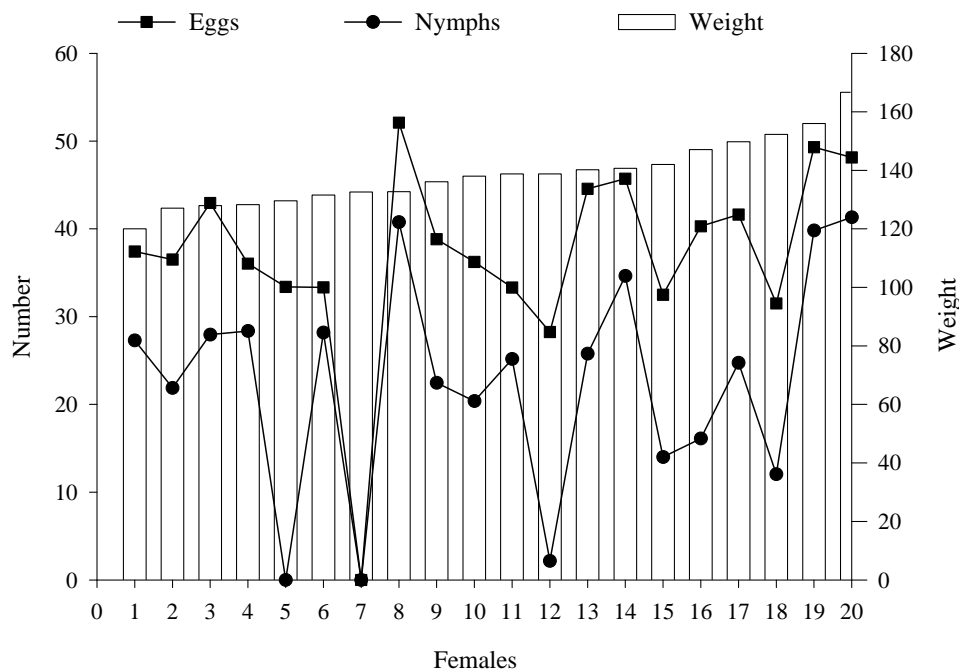


Figure 4. Number of eggs and nymphs per day per *Brontocoris tabidus* female (related to its weight) fed with *Tenebrio molitor* pupae on a *Eucalyptus grandis* plant in the field. Municipality of Viçosa, Minas Gerais State, Brazil.

Some *B. tabidus* findings regarding oviposition are: 15.67 to 51.21 eggs and 5.00 to 37.50 nymphs per egg mass (**Figure 5**), whereas the mean number of eggs and nymphs for all egg masses of each female ranged from 0.00 to 52.11 and 0.00 to 40.78, respectively (**Figure 6**).

The mean longevity and weight of *B. tabidus* males were 119.80 ± 32.50 days and 102.70 ± 5.91 mg, respectively, whereas these values were 160.20 ± 42.13 days and 138.40 ± 11.24 mg for females of this predator with *T. molitor* on the *E. grandis* plant in the field (**Table 2**).

4. Discussion

The *B. tabidus* had five instars on *E. grandis* plant, with similar results for this predator fed with an artificial diet based on beef meat and liver and/or *T. molitor* pupae [19]. This suggests that the number of instars of *B. tabidus* is similar for this predator fed with prey and/or on a diet with or without plants.

The fourth instar of *B. tabidus* had a similar duration on the *E. grandis* plant with *T. molitor* pupae compared to this predator fed only with *T. molitor* pupae [19]. However, the duration of the fifth instar of *B. tabidus* with *T. molitor* on *E. grandis* was longer than that observed for this predator with pupae of *T. molitor* [19]. This is consistent with the longer duration of the fourth and fifth instars of *P. maculiventris* with prey [third instar of *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae)] and plants [*Solanum tuberosum* L. and *Lycopersi-*

con esculentum Mill. (Solanaceae)] compared to that of *B. tabidus* fed only on prey [12].

The nymphal stage of *B. tabidus* was longer on eucalyptus plant in the field than this predator in the same stage fed only with *T. molitor* pupae [19]. The longer duration of the nymphal stage of *B. tabidus* fed with plant is similar to that found for *S. cincticeps* fed with *T. molitor* and Myrtaceae plants [20]. Although this may delay the emergence of *B. tabidus* adults, the predation rate is increased, since the *Podisus rostralis* Stal, 1860 (Heteroptera: Pentatomidae) killed a greater number of *B. mori* caterpillars when these had a longer nymphal period [17].

The survival rate during the nymphal stage of *B. tabidus* was longer than during the stage where the predator was fed with pupae of *T. molitor* [19]. The higher survival of *B. tabidus* in the field can be due to a change in temperature, relative humidity and photoperiod [21]. It may affect biological traits of insects because their activities can be regulated by duration of the day [22].

The pre-oviposition and oviposition periods of *B. tabidus* were longer than that with *Musca domestica* L., 1758 (Diptera: Muscidae) larvae [23], which can be explained by the low temperature before the reproductive stage of this predator and to the decreasing photoperiod in the field. The longer interval between egg mass laying and preoviposition and oviposition periods, and the longevity of *P. nigrispinus* and *S. cincticeps*, were also reported as possibly due to the reduction on their metabolic activities at

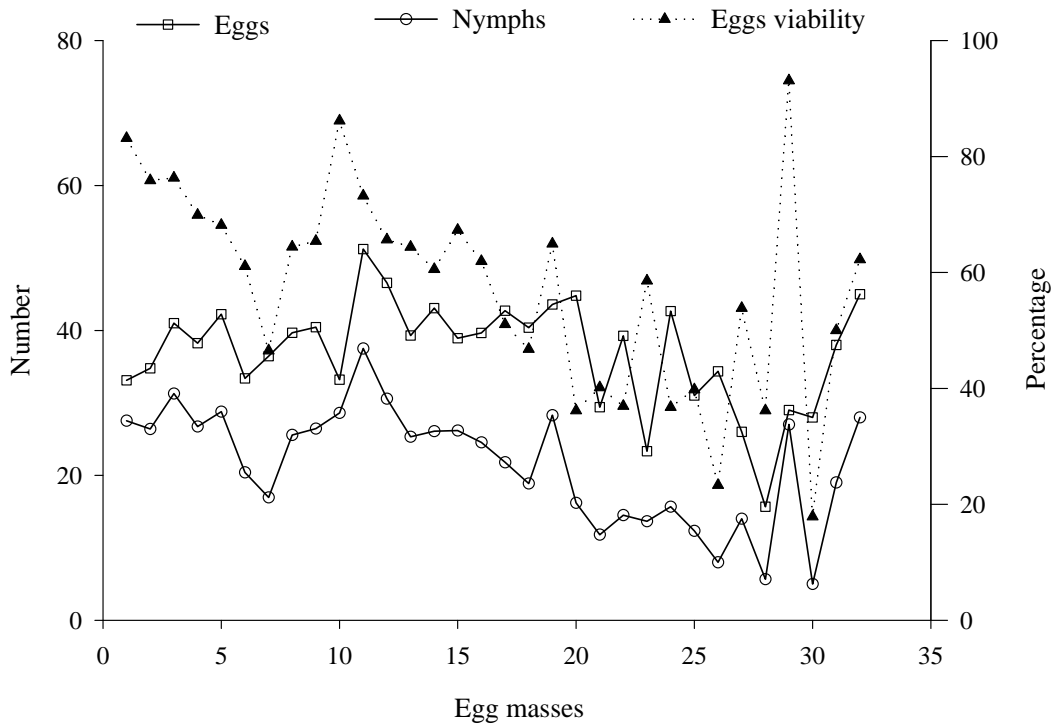


Figure 5. Number of eggs and nymphs per egg mass of *Brontocoris tabidus* fed with *Tenebrio molitor* pupae on a *Eucalyptus grandis* plant in the field. Municipality of Viçosa, Minas Gerais State, Brazil.

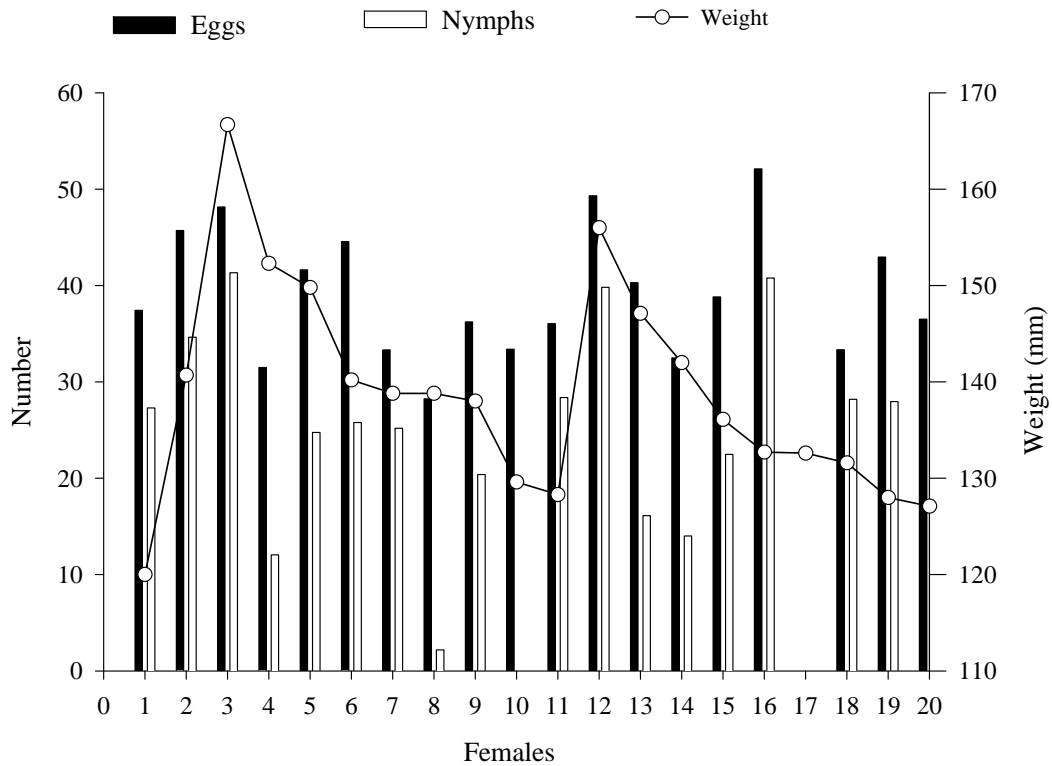


Figure 6. Mean number of eggs and nymphs per egg mass of each female of *Brontocoris tabidus* fed with *Tenebrio molitor* pupae on a *Eucalyptus grandis* plant in the field. Municipality of Viçosa, Minas Gerais State, Brazil.

temperatures below 25°C [15,20]. The longer pre-oviposition period with *Eucalyptus* spp. plants may also result from the higher nitrogen content in their leaves [14,24]. However, these parameters can be affected by other factors, because the females of this predator that weigh 95 to 150 mg and 160 to 220 mg had a greater pre-oviposition period (16.53 ± 3.33 and 16.09 ± 2.57 days, respectively) [25] than that observed in the present work. The oviposition period of this predator was 5.7 to 10.4 days when fed with pupae of *T. molitor*, *M. domestica* and the combination of both, respectively [23]. Again, the high values for this period demonstrate that the *B. mori* has a higher nutritional quality as a prey for the predator species [1,17].

The number of eggs (603.00 ± 311.20), nymphs (387.45 ± 291.40) and longevity (160.20 ± 42.13 days) of *B. tabidus* females showed higher values than that with *T. molitor* pupae alone [9]. The females live longer when predators oviposit more often and stay on the field during a longer period of time, but may be affected by the type of food ingested, since females of *P. maculiventris* and *Podisus sagitta* F., 1794 (Heteroptera: Pentatomidae) did not lay eggs when fed only with green beans and potato leaves [13]. Other predators such as *P. sagitta* [13], *P. maculiventris* [5] and *S. cincticeps* [8] have also shown greater longevity and higher reproduction rates when fed on plant and prey.

The number of eggs per egg mass was not influenced by the age of *B. tabidus* females, which is not consistent with the findings obtained for *P. nigrispinus*, since the older females of this species laid a lower number of eggs per egg mass [26-28]. Females of *B. tabidus* had similar weight, but a variable number of eggs and egg masses, indicating that these traits may be related to genetic characteristics of this predator besides its weight [18,25].

The greater longevity and the number of eggs of *B. tabidus* fed with *T. molitor* and seedlings of *E. grandis* are important factors for the improvement of the potential of this predator as a biological control. However, the type of prey alone cannot affect the longevity of stinkbug predators, because males and females mated *S. cincticeps* showed similar longevity, 19.2 ± 3.3 and 21.2 ± 2.7 days and 29.2 ± 6.1 and 30.7 ± 4.1 days when fed with *M. domestica* or *T. molitor*, respectively [8].

5. Conclusion and Future Prospects

The nymphal stage of *B. tabidus* was longer when the predator was fed with *E. grandis* and, thus, this must be considered in mass rearing and in the implementation of programs involving this predator. The *B. tabidus* had better reproductive parameters such as longer longevity and shorter period to double its population when fed with

plant and prey. Therefore, this predator should be reared on eucalyptus plants in the field where its adults would be better adapted at the moment of their release in biological control programs.

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