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RESEARCH REPORT

Prey scarcity at the beginning of fifth instar: effect of *Eucalyptus urophylla* (Myrtaceae) plants on reproduction, longevity, and weight of the predator *Brontocoris tabidus* (Heteroptera: Pentatomidae: Asopinae)

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Abstract

Intervals without prey during the fifth instar and nutrient quality may affect reproduction, longevity, and weight of the zoophytophagous predator *Brontocoris tabidus* (Heteroptera: Pentatomidae: Asopinae). This asopine was reared on *Eucalyptus urophylla* (Myrtaceae) trees under field conditions at 23 ± 6 °C, 76 ± 9 % RH and 13:11 (dark:light) h photoperiod. The experiment was developed with 60 *B. tabidus* nymphs individualized in organza bags (31 cm long × 21 cm diameter). One group of nymphs received only *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae and water without *E. urophylla* trees (T1). Other nymphs were reared on *E. urophylla* trees and fed with *T. molitor* pupae and water during the second, third, and fourth instars and twenty of them per group after zero, five, and 10 days from the beginning of the fifth instar making up the treatments T2, T3, and T4, respectively. The period without prey increased duration of the fifth instar for males in the T3 and T4 while female weight was lower in the T4. The oviposition period was shorter and the number of egg masses of *B. tabidus* was lower in the T1 than in the T2, T3, and T4. The highest egg numbers were found in the T2 and T3 with about 4 times more eggs than in the T1. The number of nymphs was low and the percentage of nymph hatching higher in the T1. The interval of five and 10 days without prey from the beginning of the fifth instar did not affect the duration and survival of this instar and supplementation with *E. urophylla* increased the reproductive capacity of the predator *B. tabidus*.

Key Words: insect predator; plant feeding; starvation; supplementation; survival; zoophytophagy

Introduction

Asopine predators can be used in the biological control of defoliator caterpillars (Lepidoptera) in eucalyptus (*Eucalyptus* spp., Myrtales: Myrtaceae) plantations (Tavares *et al*., 2013, 2014, 2015). The most common asopine predators are *Brontocoris tabidus*, *Podisus maculiventris*, *Podisus nigrispinus*, and *Supputius cincticeps* (Heteroptera: Pentatomidae) feeding on coleopteran and lepidopteran specimens (Mohaghegh *et al*., 2001; Pereira *et al*., 2008; Zanuncio *et al*., 2014).

Asopine predators have diversified feeding behavior, including plant material in their diet

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Table 1 Duration (days) and survival (%) of fifth instar *Brontocoris tabidus* nymphs (mean ± SE, standard error, and variation interval) fed on *Tenebrio molitor* pupae after zero (T2), five (T3), and 10 (T4) days from the beginning of the fifth instar on *Eucalyptus urophylla* or without plants (T1) (Tr. = Treatments) at 23 ± 6 ºC, 76 ± 9 % RH, and 13:11 (dark:light) h photoperiod.

Tr.	Duration (days)		Survival (%)
	Females	Males	
T1	9.3 ± 0.20 a (7.0-10.0)	9.3 ± 0.39 b (7.0-17.0)	88.3 ± 0.05 a
T2	11.0 ± 0.52 a (8.0-15.0)	10.6 ± 0.36 ab (7.0-14.0)	90.0 ± 0.04 a
T3	12.1 ± 0.65 a (8.0-18.0)	11.1 ± 0.47 a (8.0-18.0)	91.6 ± 0.04 a
T4	11.7 ± 0.82 a (8.0-23.0)	11.5 ± 0.58 a (8.0-18.0)	93.3 ± 0.03 a

Means followed by the same letter, per column, do not differ between them by the Tukey's honest significant difference (HSD) test ($p > 0.05$).

(Zanuncio *et al*., 2000, 2004, 2011a). In the laboratory, *B. tabidus* females fed on mealworm pupae, *Tenebrio molitor*, (Coleoptera: Tenebrionidae), *Eucalyptus urophylla* seedlings, and water were heavier and had higher oviposition rates than those fed only on this prey and water (Zanuncio *et al*., 2000). *Supputius cincticeps* had higher egg production fed on mealworm pupae, *E. urophylla* trees, and water in the field (Zanuncio *et al*., 2004) and mealworm larvae, common bean pods, *Phaseolus vulgaris* (Fabales: Fabaceae), and water in the laboratory (Mourão *et al*., 2003). The importance of plant material for asopine predators suggests that these insects might obtain water and nutrients from these plants (Coundron *et al*., 2002; Holtz *et al*., 2011; Castro *et al*., 2015).

Food quality and quantity affect the life cycle, reproduction, and longevity of asopine predators (Wittmeyer *et al*., 2001; Holtz *et al*., 2009; Zanuncio *et al*., 2011b). Intervals without prey and protein levels in nutrients affect body weight, and total number and viability of eggs (Mourão *et al*., 2003; Ramalho *et al*., 2008; Holtz *et al*., 2009). Prey shortages and plant material may affect the life cycle of asopine predators and, consequently, their efficiency to control insect-pests (Molina-Rugama *et al*., 1998; Lemos *et al*., 2001; Zanuncio *et al*., 2012).

The objective of this work was to evaluate duration and survival during the fifth instar and the reproduction and longevity of *B. tabidus* adults after zero, five, and 10 days without prey from the beginning of the fifth instar.

Material and Methods

Experimental site

This research was carried out in the Laboratory of Biological Control of Insects (LCBI) and in the Insectarium of the Department of Entomology (DEN) at the "Universidade Federal de Viçosa (UFV)" in Viçosa, Minas Gerais state, Brazil. *Brontocoris tabidus* was mass reared in the vicinity of the Insectarium (20° 45' S × 42° 52' W and 648 m altitude) in organza bags (31 cm length × 21 cm diamater) on *E. urophylla* branches (approx. three-day-old trees) at 23 ± 6 °C, 76 ± 9 % RH, and 13:11 (dark:light) h photoperiod.

Fifth instar Brontocoris tabidus *nymphs*

A total of 900 first instar *B. tabidus* nymphs reared in the Insectarium was divided in three groups of 300 each, into organza bags on *E. urophylla* branches from the second to the beginning of fifth instar. Fifth instar nymphs were reared in groups of 20 individuals per bag on *E. urophylla* branches in the field. Nymphs were supplied *ad libitum* with mealworm pupae and water in 5 mL plastic containers. These nymphs were weighed at the beginning of the fifth instar before feeding to obtain a similar number of males and females per treatment. Although it is not possible to determine sex during the nymph stage, heavier asopine nymphs usually become females (Mohaghegh *et al*., 1998). First instar *B. tabidus* nymphs are not predators, feeding only on their eggshell and water (Pires *et al*., 2011).

Description of treatments

Eighty fifth instar *B. tabidus* nymphs were separated from each group of 300 individuals and divided into four treatments with eight of them individualized into the bags on *E. urophylla* tree branches with mealworm pupae supplied daily and water *ad libitum.* A group of nymphs was maintained without *E. urophylla* plants, feeding only on mealworm pupae and receiving water (T1). At the beginning of fifth instar, the nymph groups had periods of zero, five, and 10 days without prey constituting the treatments T2, T3, and T4, respectively.

Assessment of nymphs

The duration (days) and survival (%) of fifth instar nymphs were verified daily.

Assessments of adults

Brontocoris tabidus adults obtained from nymphs of the treatments T1, T2, T3, and T4 were weighed just after their emergence and sexed, based on their genitalia appearance (Lemos *et al*., 2005; Guedes *et al*., 2007). These adults were mated on the fourth day of adult age (Zanuncio *et al*., 2006) and those from T2, T3, and T4 placed into the bags on branches of *E. urophylla* plants (Zanuncio *et al*., 2004). Adults received a mealworm pupa and water daily. Those of T1 received only pupae of this prey and water in similar bags without *E. urophylla* branches. The number of *B. tabidus* pairs, per treatment, varied according to males and females obtained, with 23, 25, 20, and 22 for treatments T1, T2, T3, and T4, respectively.

Assessment of eggs

Brontocoris tabidus egg masses were collected daily and the number of eggs was counted. These egg masses were maintained in plastic Petri dishes (9.0 cm diameter × 1.2 cm height) with a moistened cotton ball at 24 ± 2 °C, 70 ± 6 % RH, and 12:12 (dark:light) h photoperiod in the laboratory.

General assessments

The male and female weight (mg) and the pre-oviposition, oviposition, and post-oviposition periods (days), besides the number of eggs, nymphs, egg masses, eggs per egg mass, nymphs per egg mass, percentage of nymph hatching, and longevity of males and females (day) were recorded.

Statistical analysis

The results were analyzed using the Cochran's C test (Cochran, 1941), Bartlett's test (Bartlett, 1937), and Lilliefors test (Lilliefors, 1967) to determine the homogeneity of variance and normality, respectively. The duration of the fifth instar, adult weight, and reproductive data were transformed to $\sqrt{x}$, except for the percentage of nymph hatching which was transformed to arcsen x. The data was subsequently analyzed using a one-way ANOVA and Tukey's honest significant difference (HSD) test at 5 % probability (Tukey, 1949).

Results

Survival of *B. tabidus* fifth instar nymphs varied from 88.3 to 93.3 % without difference between treatments (Table 1). The duration of this instar was longer for males in the T3 and T4 than in the T1 (Table 1). The duration of the fifth instar for females was similar between treatments (Table 1).

The weight of *B. tabidus* females was lower only for those in the T4, while male weight was similar between treatments (Table 2).

The pre-ovipostion and post-oviposition periods of *B. tabidus* were similar between treatments (Table 3). However, the oviposition period was about 4 times longer with mealworm pupae and *E. urophylla* trees than with only pupae (11.8 days), regardless of the period of prey shortage (Table 3).

Brontocoris tabidus females fed only mealworm pupae presented a lower number of egg masses (3.6) than in the other treatments (Table 3). The number of eggs per female was similar in the treatments where nymphs received pupae after zero, five, and 10 days from the beginning of fifth instar and about 4 times higher (410.5, 452.8, and 332.2, respectively) than for those fed only mealworm pupae (Table 3). The number of eggs per egg mass was similar between treatments (Table 3).

The number of nymphs was higher for *B. tabidus* females on *E. urophylla* and fed on mealworm pupae after zero, five, and 10 days from the beginning of the fifth instar (259.9, 299.2, and 201.2 nymphs, respectively) than only with pupae (86.5 nymphs) (Table 3). The number of nymphs per egg mass was similar between treatments, but the percentage of nymph hatching was higher with only mealworm pupae (Table 3).

The longevity of *B. tabidus* males and females was greater in the treatments with *E. urophylla* and mealworm pupae feeding, than with only the prey (Table 3). Beyond this, *B. tabidus* females with prey and eucalyptus lived longer than those fed only prey. Females fed only prey, and those fed prey and plant material had a 60 % and 80 % survival rate after 25 days of the adult stage, respectively. All females of the treatment fed only prey died after 45 days, while 60 % of those fed on prey and plant material were still alive at the end of this period (Fig. 1).

Table 2 Weight (mg) of *Brontocoris tabidus* males and females (mean ± SE, standard error) fed on *Tenebrio molitor* pupae after zero (T2) (n = 25), five (T3) (n = 20), and 10 (T4) (n = 22) days from the beginning of the fifth instar on *Eucalyptus urophylla* or without plants (T1) (n = 23) (Tr. = Treatments) at 23 ± 6 °C, 76 ± 9 % RH, and 13:11 (dark:light) h photoperiod

Tr.	Weight (mg)	
	Males	Females
T1	103.2 ± 1.56 a (90.4-118.9)	128.3 ± 3.32 a (79.8-152.1)
T2	102.6 ± 3.29 a (81.0-132.3)	135.4 ± 5.03 a (104.7-205.3)
T3	95.8 ± 3.94 a (64.4-133.4)	131.5 ± 3.69 a (100.8-188.3)
T4	92.1 ± 4.65 a (47.2-134.1)	111.3 ± 4.74 b (69.7-157.4)

Means followed by the same letter, per column, do not differ between them by the Tukey's honest significant difference (HSD) test ($p > 0.05$).

Table 3 Characteristics (Char.) of pre-oviposition (days) (Pr), oviposition (days) (Ov), post-oviposition (days) (Po), number of egg masses (Em), number of eggs (Eg), number of eggs/egg mass (Eg/Em), number of nymphs (Ny), number of nymphs/egg mass (Ny/Em), longevity of females (days) (Lf), longevity of males (days) (Lm), and nymphs hatching (%) (Nh) of *Brontocoris tabidus* (mean ± SE, standard error, and variation interval) fed on *Tenebrio molitor* pupae after zero (T2), five (T3), and 10 (T4) days from the beginning of the fifth instar on *Eucalyptus urophylla* or without plants (T1) at 23 ± 6 °C, 76 ± 9 % RH, and 13:11 (dark:light) h photoperiod

Char.	Treatments			
	T1	T2	T3	T4
Pr	10.8 ± 0.97a (6.0-22.0)	12.6 ± 1.35a (6.0-29.0)	13.2 ± 1.42a (3.0-26.0)	13.5 ± 1.39a (6.0-30.0)
Ov	11.8 ± 1.94b (1.0-33.0)	48.0 ± 4.14a (2.0-86.0)	49.6 ± 5.38a (7.0-108.0)	45.0 ± 5.48a (1.0-84.0)
Po	5.8 ± 1.05a (1.0-17.0)	7.0 ± 1.02a (1.0-18.0)	5.3 ± 0.65a (1.0-16.0)	5.5 ± 1.38a (1.0-14.0)
Em	3.6 ± 0.45b (1.0-8.0)	9.4 ± 1.00a (2.0-18.0)	12.9 ± 1.65a (2.0-29.0)	9.5 ± 1.26a (2.0-20.0)
Eg	110.0 ± 14.59b (19.0-205.0)	410.4 ± 40.41a (57.0-815.0)	452.8 ± 49.87a (82.0-890.0)	332.5 ± 40.43a (40.0-633.0)
Eg/Em	32.2 ± 3.47a (9.0-52.0)	40.4 ± 2.18a (24.3-70.6)	39.4 ± 3.78a (20.5-95.0)	38.5 ± 3.16a (10.0-88.5)
Ny	86.4 ± 14.32b (23.0-180.0)	259.8 ± 33.77a (42.0-609.0)	299.2 ± 33.07a (54.0-600.0)	201.5 ± 21.23a (54.0-366.0)
Ny/Em	29.8 ± 3.36a (11.5-60.0)	26.0 ± 2.77a (5.2-67.6)	26.4 ± 2.76a (13.5-57.8)	26.0 ± 2.76a (8.2-62.0)
Lf	29.0 ± 2.37b (8.0-51.0)	62.0 ± 4.85a (23.0-111.0)	65.0 ± 6.06a (8.0-110.0)	63.0 ± 7.02a (17.0-114.0)
Lm	29.0 ± 2.97b (6.0-52.0)	66.0 ± 6.50a (11.0-135.0)	55.0 ± 6.65a (23.0-122.0)	62.0 ± 7.50a (5.0-127.0)
Nh	79.6 ± 2.90a (51.8-96.0)	62.4 ± 4.50b (16.5-95.5)	66.8 ± 2.65ab (40.9-85.8)	64.0 ± 4.04b (27.4-85.7)

Means followed by the same letter, per line, do not differ between them by the Tukey's honest significant difference (HSD) test ($p > 0.05$).

Discussion

The survival of fifth instar *B. tabidus* nymphs was higher even after 10 days without mealworm pupae than that for those fed daily on pupae and river red gum seedlings, *Eucalyptus camaldulensis*, flooded gum, *Eucalyptus grandis*, and *E. urophylla* (Zanuncio *et al*., 2000) or only mealworm pupae (Jusselino-Filho *et al*., 2001). This suggests that asopine nymphs can store reserves in the fifth instar to reach adult stage even with prey shortages during the final instar. Beyond this, these results indicate that *Eucalyptus* spp. trees can be a better food source for *B. tabidus* than its seedlings. *Supputius cincticeps* and *P. nigrispinus* also presented higher survival rates with leaves of upland cotton, *Gossypium hirsutum* (Malvaceae) and *E. urophylla* in their diets when fed on cotton leafworm caterpillars, *Alabama argillacea* (Lepidoptera: Noctuidae) (Lemos *et al*., 2001).

The increased duration of *B. tabidus* fifth instar nymphs that became males and the decrease of female weight without prey show the importance of food quality for predatory asopines. This was also shown for *P. nigrispinus* (as *Podisus connexivus*) with longer nymphal period when fed on lighter (3.1 and 11.6 mg) than on heavier (72.4 and 171.0 mg) *A. argillacea* caterpillars (Santos *et al*., 1995). The similar duration of the fifth instar *B. tabidus* nymphs between treatments was greater than that for this predator fed only mealworm pupae (Jusselino-Filho *et al*., 2001) or domesticated silk moth caterpillars, *Bombyx mori* (Lepidoptera: Bombycidae) (5.8 days) (Oliveira *et al*., 1999). The temperature in the field may have contributed to increasing the duration of the fifth instar *B. tabidus* nymphs. The duration of this instar was shorter while mean minimum and maximum temperatures were 16.67 ± 3.03 °C and 27.61 ± 2.99 °C with an average of 23.0 ± 6.56 ºC during the period when *B. tabidus* nymphs were

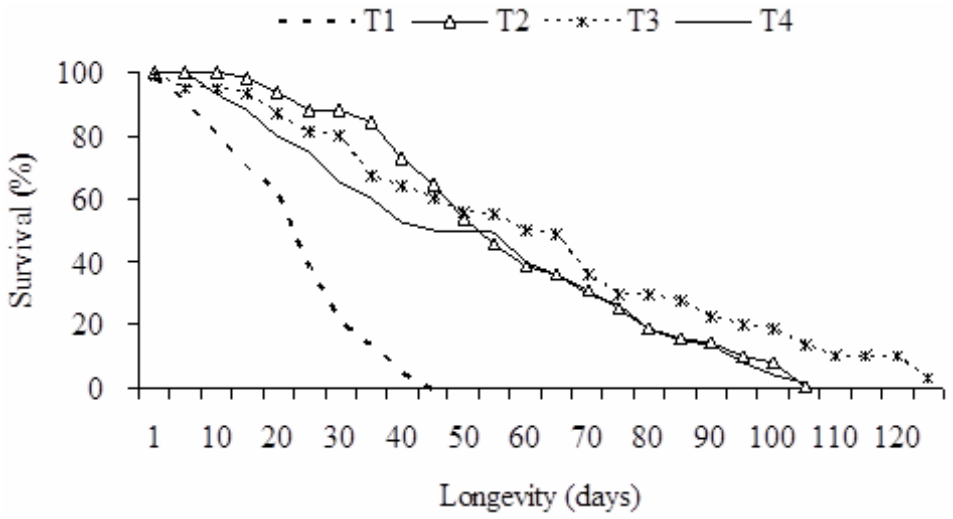

Fig. 1 Survival (%) and longevity (days) (mean) of *Brontocoris tabidus* females on *Eucalyptus urophylla* fed on *Tenebrio molitor* pupae after zero (T2), five (T3), and 10 (T4) days from the beginning of fifth instar or without plants (T1) at 23 ± 6 ºC, 76 ± 9 % RH, and 13:11 (dark:light) h photoperiod.

maintained on plants in the field. Temperature affects *B. tabidus* metabolism, reproduction, longevity, and behavior (Zanuncio *et al*., 2006, 2011a). The duration of the fifth instar of *S. cincticeps* nymphs varied from 26 to 28 days at 15 °C and from 6.3 to 6.8 days at 28 °C when fed on house fly larvae, *Musca domestica* (Diptera: Muscidae) (Zanuncio *et al*., 2005). The duration of fifth instar *P. nigrispinus* nymphs fed on *A. argillacea* caterpillars varied from 15.3 to 17.5 days at 17 °C and from 6.3 to 8.0 days at 28 °C (Medeiros *et al*., 2003). This predator had a longer duration of the fifth instar at 10 and 20 °C, 13 and 23 °C and 15 and 25 °C than at a constant temperature of 27 °C (Torres *et al*., 1998). The longer duration of the fifth instar found in this study for *B. tabidus* may be due to lower temperature and/or to temperature variations in the field.

The lower weight of *B. tabidus* females in the T4 than in the T2 and the similar reproduction between treatments suggests that this predator can survive from prey shortages after receiving prey daily. Hence, fifth instar nymphs of this predator can survive in the field even after 10 days without prey from the beginning of the fifth instar. *Brontocoris tabidus* uses the energy from food for development, and resource shortages can increase the duration of nymphal stages and reduce adult weight (Zanuncio *et al*., 2000; Oliveira *et al*., 2005; Lemos *et al*., 2009). Beyond this, the energy obtained can be used for two or more competitive processes, such as reproduction and general survival (Zanuncio *et al*., 1996; Jusselino-Filho *et al*., 2003) and asopine predators with continuous feeding on prey complete their development in a shorter time period with greater weight (Torres *et al*., 2000). This is important, because heavier *Podisus rostralis* (Stål, 1860) females had higher egg production than lighter ones (Zanuncio *et al*., 2002). Conversely, food shortages reduced *P. nigrispinus* oviposition period, viability, and total number of eggs per day (Vivan *et al*., 2003; Ramalho *et al*., 2008; Holtz *et al*., 2009).

The longer pre-oviposition period of *B. tabidus* fed on mealworm pupae and *E. urophylla* trees in this study than for those fed only *B. mori* caterpillars was similar to that for this predator with seedlings of *E. camaldulensis*, *E. grandis*, and *E. urophylla* and mealworm pupae (8.3 days) (Zanuncio *et al*., 2000) or only mealworm larvae (7.6 days) (Jusselino-Filho *et al*., 2001) suggesting that *Eucalyptus* spp. plant substances may increase the pre-oviposition period of this predator. Differences in the oviposition period (period between the first and the last egg mass is laid) of *B. tabidus* with and without *E. urophylla* plants show that this predator has better reproductive potential with plants in its diet. The oviposition period presented by this predator was longer compared to that with seedlings of three eucalyptus species (*E. camaldulensis*, *E. grandis*, and *E. urophylla*) in the laboratory (22.1, 26.0, 22.5 days, respectively) (Zanuncio *et al*., 2000) indicating that eucalyptus (an exotic plant in the New Word) may be appropriate for *B. tabidus*. This predator can reproduce across almost its entire lifespan on *Eucalyptus* spp. plants with short post-oviposition period (Zanuncio *et al*., 2000; Coelho *et al*., 2009).

High *B. tabidus* survival on *E. urophylla* branches results in a higher number of eggs, nymphs, and egg masses per female of this predator than those reported with only mealworm larvae (98.2 eggs and 2.5 egg masses per female) (Jusselino-Filho *et al*., 2001) or *B. mori* caterpillars (Oliveira *et al*., 1999). Similar results were found for *P. nigrispinus* fed mealworm pupae and plants (*G. hirsutum* cv. precocious CNPA1 or tomato, *Lycopersicum esculentum* cv. IPA5, Solanaceae) (Oliveira *et al*., 2002) and *S. cincticeps* with *E. cloeziana* leaves (Zanuncio *et al*., 2004). These findings reinforce the hypothesis that *Eucalyptus* spp. plants increase the reproductive capacity of predatory stink bugs.

The longevity of *B. tabidus* males and females with *E. urophylla* branches and mealworm pupae was shorter than that found for this predator fed *B. mori* caterpillars (104.5 days for males and 93.8 days for females) (Oliveira *et al*., 1999), indicating the better quality of the later for this predator. However, the longevity of *B. tabidus* fed on mealworm pupae and eucalyptus plants was 4 times greater than that of those fed only on this prey in the laboratory (Jusselino-Filho *et al*., 2001). The high *B. tabidus* female survival in the treatments with prey and plant material is significant, because it allows this predator to reproduce over longer periods of time with a high egg production in the field.

Brontocoris tabidus is a generalist predator of eucalypt pests used in classical biological control in Brazil (Menezes *et al*., 2013; Guanabens *et al*., 2014). Eucalypt plants could be a food source to rear this predator for release in plantations of these species (Zanuncio *et al*., 2000; Pereira *et al*., 2008; Pires *et al*., 2011). The interval of up to 10 days without prey from the beginning of the fifth instar with eucalyptus plants did not affect the adult stage duration and survival for *B. tabidus*. This plant increased the longevity and reproductive capacity of *B. tabidus,* suggesting that this predator should be reared on eucalyptus plants in the field.

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