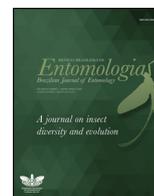




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Postembryonic development and reproductive parameters of the grasshopper pest *Borellia bruneri* (Acrididae: Gomphocerinae) under controlled conditions

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ABSTRACT

Borellia bruneri, a common grasshopper in much of the grasslands of Argentina and Uruguay, is considered, according to the categories widely accepted for defining the pest status of grasshopper species, a “Frequent plague of importance”. In order to determine fundamental aspects of its biology and reproduction, three cohorts of *B. bruneri* were monitored under controlled conditions (30° C, 14L: 10D, 40% RH). The total duration of nymphal development was 50.6 days, both males and females having five nymphal instars. There was a significant difference in the duration of the different stages within each cohort. In the three cohorts, the first instar duration (12.87 days) was longer than the rest, approximately 5.6 days more than the second that was the shortest (7.26 days). The average longevity of female adults was 56.6 days, and in males, 54.4 days. The number of egg-pods *per* female was 3.5 and the amount of eggs *per* egg-pod was 10.8. Mean fecundity was 37.9 eggs *per* female with an oviposition rate of 1.20 eggs/female/day. Finally, knowing the life cycle of *B. bruneri* is relevant in order to optimize the control measures for this species.

Borellia bruneri is one of the most common species of the Gomphocerinae subfamily inhabiting the Pampas grasslands of Argentina and Uruguay (Mariottini et al., 2012; Miguel et al., 2014) (Fig. 1). This grasshopper shows a wide geographic distribution, occurring in southernmost Brazil, much of Argentina, Chile, and Uruguay (Carbonell et al., 2017). It is a gramminivorous and univoltine species with obligatory embryonic diapause. Following the categories widely accepted for defining the pest status of grasshopper species (COPR, 1982), Carbonell et al. (2017) have categorized *B. bruneri* as a “Frequent plague of importance”.

To date chemical insecticides still remain as the only available option for grasshopper control in Argentina. These may produce negative impacts on social, economic, and environmental levels. A better knowledge of the biological parameters of the involved grasshopper species would possibly allow to reduce the number of applications and improve its timing so that they would be done at the optimal moment of the cycle life (ideally IV and V nymphal stage), reducing population densities, forage consumption, and female oviposition.

In recent years, several studies carried out in Argentina and Uruguay have contributed valuable information regarding *B. bruneri*, such as its distribution, density, sex ratio, and nymphal morphometry (De Wysiecki et al., 2011; Mariottini et al., 2012, 2015; Miguel et al., 2014; Zerbino et al., 2016). Nevertheless, important aspects related to the biology and ecology are still unknown. Thus, the aim of this work was to study different biological and reproductive parameters of *B. bruneri* under controlled laboratory conditions.

Individuals used in this experience belonged to the first laboratory generation (F1) of specimens originally collected in grasslands at southern of Buenos Aires province, Argentina (60° 44' 13.3", 37° 34' 31.4"), and were maintained in a rearing room under controlled conditions (30°C, 14L: 10D, 40% RH) as was described in a previous study (Mariottini et al., 2010). Three cohorts of 25 individuals each were monitored daily, recording the number of nymphal instars, the duration of each instar, the total duration of nymphal development, the mortality in the nymphal cycle, and adult longevity. The individuals of each cohort were maintained in cages with wire-screened walls and a transparent acrylic slide opening (20 x 20 x 30 cm). They were fed

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a variety of wild grasses, and flakes of wheat bran. Immediately after moulting to adults, forty females and males were separated as 20 couples (1♂, 1♀). Each couple was placed in a wire-screened, aluminum cage (12x12x16cm) and was provided with a substrate for egg-pod laying that consisted of a plastic container (10 cm deep) filled with sterilized sand. Mating and thermoregulation were stimulated with 75W bulbs suspended 15 cm above each cage. The average fecundity (number of eggs/female) and the oviposition rate (eggs/female/day) were recorded. In order to compare the different parameters estimated the Infostat software (Di Rienzo et al., 2015) was used. The duration of each instar within the cohorts and between the cohorts was compared from the non-parametric Kruskal & Wallis test, and for later comparisons the pairwise comparisons was used. The total duration of the nymphal cycle of each cohort was evaluated from a univariate analysis of variance

(ANOVA), the Shapiro-Wilks normality test was previously performed. Results are expressed as mean values \pm standard error (SE)

The total duration of nymphal development was 50.6 ± 0.44 (47-63) days; no significant differences were registered in the duration of the nymphal state between different cohorts (Table 1). Both males and females had five nymphal instars as recorded by Miguel et al. (2014) in populations from the Uruguay Pampas. The mortality rate during the nymphal cycle was low of 4% in each of the three cohorts, 24 of the 25 initial individuals of each cohort reached to adult's stage.

There was a significant difference in the duration of the first three stages of development between the cohorts (Table 1). In addition, there was a significant difference in the duration of the different instars within each cohort (Cohort 1: H: 69.83, $p < 0.0001$, N: 24; Cohort 2: H 55.02, $p < 0.0001$, N: 24; Cohort 3: H 72.74, $p < 0.0001$, N: 24), in the



Figure 1. a-b, nymphs in first stage breeding under controlled conditions, c- nymphs of five stage, d- male and female adults.

Table 1. Mean duration in days (\pm SE) of the different nymphal instar of three cohorts of *Borellia bruneri* under controlled conditions. Different letters denote significant differences between the cohorts in the same column according to pairwise comparison (Kruskal & Wallis) or LSD Fisher (ANOVA) ($p < 0.05$).

| Nymphal Instar | First instar | Second instar | Third instar | Fourth instar | Fifth instar | Total duration of nymphal cycle |
|--------------------------------------|---|---|---|--|--|---|
| Cohort 1 | 12.98 b \pm 0.11 (CV: 4.47), N: 24 | 7.83 a \pm 0.10 (CV: 6.15), N: 24 | 9.88 a \pm 0.25 (CV: 12.42), N: 24 | 9.83 a \pm 0.35 (CV: 17.66), N: 24 | 10.08 a \pm 0.33 (CV: 16.26), N: 24 | 51.3 a \pm 0.48 (CV: 4.65), N: 24 |
| Cohort 2 | 12.67 b \pm 0.19 (CV: 7.24), N: 24 | 6.96 b \pm 0.32 (CV: 22.24), N: 24 | 8.83 b \pm 0.21 (CV: 11.88), N: 24 | 10.25 a \pm 0.61 (CV: 29.09), N: 24 | 10.33 a \pm 0.58 (CV: 27.47), N: 24 | 50.75 a \pm 0.86 (CV: 8.28), N: 24 |
| Cohort 3 | 13.64 a \pm 0.11 (CV: 4.17), N: 25 | 7.00 b \pm 0.26 (CV: 18.90), N: 25 | 8.75 b \pm 0.33 (CV: 18.54), N: 24 | 9.54 a \pm 0.52 (CV: 26.94), N: 24 | 10.96 a \pm 0.53 (CV: 26.94), N: 24 | 49.71 a \pm 0.84 (CV: 8.32), N: 24 |
| Kruskal Wallis Test (H) or ANOVA (F) | H: 27.24 p: <0.0001 | H: 17.36 p: 0.0001 | H: 9.47 p: 0.0059 | H: 0.96 p: 0.61 | H: 4.02 p: 0.1231 | F: 1.15 p: 0.3218 |

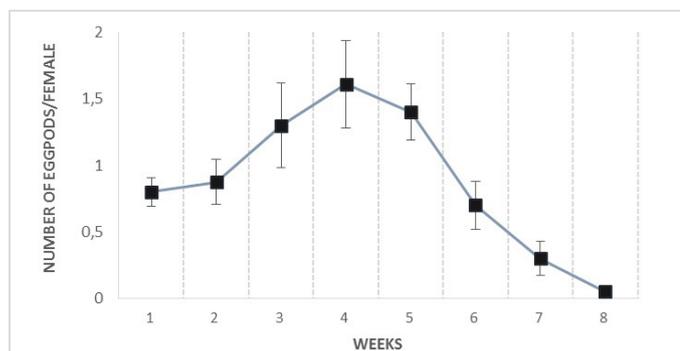


Figure 2. Mean fecundity (\pm SE) of *Borellia bruneri* under controlled conditions (30°C, 14L: 10D).

three cohorts the first stage was longer than the rest ($p < 0.05$) and the second the shortest ($p < 0.05$). Intraspecific variation must play an important role in such differences (Esperk et al., 2007).

The average longevity of female adults was 56.6 ± 1.35 days, and in males, 54.4 ± 0.98 days. There were no significant differences between longevity of males and females (ANOVA= F: 1.77; p : 0.1877 N: 72.). The number of egg-pods per female was 3.5 ± 0.2 (1-5) and the amount of eggs per egg-pod was 10.8 ± 0.6 (7-14). Mean fecundity was 37.9 ± 1.8 eggs per female with an oviposition rate of 1.20 ± 0.2 eggs/female/day.

The reproductive value changed significantly over time, (ANOVA= F: 6.59; $p < 0.0001$, N: 77). The highest reproductive value of females was reached in the fourth week leaving a number of egg-pods significantly higher than registered in the following weeks (LSD Fisher $p < 0.05$) (Fig. 2).

In recent years, different studies on biological and reproductive parameters, mainly in Melanoplineae grasshoppers present in Argentina were conducted (Bardi et al., 2011; Mariottini et al., 2010, 2011a, b, 2015). *Dichroplus maculipennis* and *Dichroplus elongatus* are two of the most common melanoplines of the Pampas and are co-dominant together with *B. bruneri* in the grasshopper community of the southern Pampas (Mariottini et al., 2013). It is important to mention that *D. maculipennis* and *B. bruneri* share the same type of habitat, with halophilous plant communities with prevalence of low-bearing grasses like *Distichlis spicata* (Torrusio et al., 2002; Mariottini et al., 2013). Also, these two species were the dominant grasshoppers in the last outbreak recorded in the south of the Pampas region between 2008 and 2010, with densities that in some places exceeded 75 ind m^2 (Mariottini et al., 2012). Like *B. bruneri*, both *D. maculipennis* and *D. elongatus* are univoltine with obligate embryonic diapause (Mariottini et al., 2011a; Bardi, 2013). Reared in laboratory under the same conditions of photoperiod and temperature it could be observed that *B. bruneri* have a longer nymphal development than that of *D. maculipennis* (34.5 ± 0.47 days) and *D. elongatus* (32 ± 0.7 days) (Mariottini et al., 2011b). Respect to the fecundity of these species, the mean fecundity of *B. bruneri* was lower than that of *D. elongatus* (81.09 ± 14.02 eggs per female (De Wysiecki et al., 1997) and that of *D. maculipennis* (83.3 ± 11.9 eggs per female (Mariottini et al., 2011b) estimated under the same laboratory conditions. Thus, even though *B. bruneri* is considered a pest species it has a comparatively lower fecundity than the two Melanoplineae species that are more abundant in the Pampas region.

Further studies on demographic parameters and estimates on consumption and food preferences of *B. bruneri* are necessary to determine the actual status of this species as pest since some of its biological traits appear to deviate from typical pest species such as *D. maculipennis* and *D. elongatus*.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author's contributions

The four authors have contributed equally to this work.

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