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Protocol & Techniques

Rearing the green-belly stink bug, *Diceraeus furcatus* (Fabricius, 1775) (Hemiptera: Pentatomidae) in laboratory

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Abstract. The green belly stink bug, *Diceraeus furcatus* (Fabricius, 1775) (Heteroptera: Pentatomidae) is an emergent Neotropical crop pest, whose status has progressively changed in the last few years. *Diceraeus furcatus* is a polyphagous stink bug and its feeding behavior damages corn seedlings, wheat and oat spikes, and soybean pods, causing economic losses. Establishing stock populations of *D. furcatus* is difficult, and a suitable rearing method is lacking. The establishment of stock colonies is essential to study many aspects of a species, especially economically important ones. In this paper, a method to keep viable stock populations of *D. furcatus* in the laboratory is described. The periodic asepsis of cages (48 hours), periodical change of diet (48 hours), and mixed diet consisting of bean pods plus peanut and soybean grains, allowed us to obtain adequate biological parameters and population increase of this species. Fertility (251 eggs/female), fecundity (225.91 nymphs/female), and R_0 (43 times of increase) show a consistent increase in the population. Additionally, adult survivorship/longevity and weight support the suitability of the method applied. The establishment of stock populations is the first step for a mass-rearing method that is essential to the development of biological control programs and other studies on this emergent pest.

Keywords: Emergent Neotropical pest, biology, asepsis, fertility life table, stock colony.

Within the stink bug complex of economic importance, the green belly stink bug, *Diceraeus furcatus* (Fabricius, 1775) (Heteroptera: Pentatomidae), is an emergent Neotropical pest species. Considered a secondary pest of soybean, *Glycine max* L. Merrill (Gamundi & Sosa 2007; Panizzi et al. 2012), in recent years the pest status of this species has progressively changed. Adults and nymphs of *D. furcatus* have become a serious problem not only to soybean but also to crops such as maize *Zea mays* L. and wheat *Triticum aestivum* L. (Panizzi et al. 2016; Jacobi et al. 2022). The change of the pest status in the species has been attributed to the crop rotation practice, which forms a "green bridge", and the no-tillage cultivation system, which provides shelter to overwinter, leading to the dramatic economic increase of *D. furcatus* in South America in the last decades (Jacobi et al. 2022).

Diceraeus furcatus is a polyphagous stink bug and has been recorded on 32 plant species, including cultivated and non-cultivated plants (Smaniotto & Panizzi 2015). Among the cultivated plants, *D. furcatus* was reported on soybean, sunflower, corn, common oat, wheat (Panizzi et al. 2016, and references therein), and canola (Bianchi et al. 2019). Feeding damage is reported for nymphs and adults causing economic losses when in high population densities (Roza-Gomes et al. 2011). The major crops associated with *D. furcatus* in South America are soybean and corn, which are cultivated in approximately 50 and 25 million hectares, respectively, in Brazil (https://www.conab.gov.br), and Argentina (http://datosestimaciones.magyp.gob.ar).

Rearing insect pest species in the laboratory has become an important issue, and rearing methods seek to maintain insects under conditions that simulate nature providing all factors necessary for the complete life cycle in continuous generations (Cohen 2018). In this way, a rearing method that allows the establishment of laboratory stock populations of *D. furcatus* is still lacking. *Diceraeus furcatus* has 3 developmental stages (egg, nymph, adult), and its life cycle has been presented recently under different aspects (Chiaradia 2015; Panizzi et al. 2018; Possebom et al. 2020). However, published studies are usually based on field-collected insects (e.g., Roza-Gomes et al. 2011; Somavilla et al. 2020; Jacobi et al. 2021) or demand short periodical colony replenish from field-collected insects (e.g., Panizzi et al. 2016). Field collection of *D. furcatus* can yield the appropriate number of specimens for specific experiments, but it may result in uncertainty with respect to age, fitness, and life history, thus limiting the research and mass rearing possibilities under laboratory conditions.

Considering the economic importance of *D. furcatus*, the difficulties to establish stock populations, and the lack of standard rearing methods, we established and described a set of procedures to overcome this challenge. The biological parameters obtained are discussed.

Insects used in this work were collected on wheat plants in the state of Rio Grande do Sul, in the municipality of Coxilha, experimental area II of Embrapa Trigo (28°11'42.8''S, 52°19'30.6''W, 710 m) in September of 2010.

The adults collected (n = 30) were transported to the entomology laboratory of Embrapa Trigo (Passo Fundo, Rio Grande do Sul, Brazil). The field-collected insects were kept in screened cages (50 × 40 × 30 cm) with a substrate for oviposition (filter paper was placed at the bottom of the cage). Cages were kept in a walk-in chamber under 25 ± 1°C and 65 ± 5% RH, with a photoperiod of 14:10 (light: dark). To the insects were offered green bean pods, *Phaseolus vulgaris* L., raw peanut grains, *Arachis hypogaea* L., raw soybean grains, *G. max*, and water *ad libitum*. Special attention was given to asepsis, every two days cages were cleaned, and diet and water were replaced by new ones. The standard cleaning and diet change adopted is generally once or twice a week.

Field-collected insects were identified based on the dichotomy key from Grazia (1978). Voucher specimens are deposited at the Entomological Collection of Museum of Entomology Luiz de Queiroz

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Approximately 350 eggs (25 egg masses) from the field-collected insects were kept in a Petri dish (1.0 cm high with a radius of 4.5 cm) with moistened filter paper, which was changed every two days. After hatching nymphs were kept in the dish until reaching the second instar (first instar nymphs are gregarious and do not feed). After, nymphs were transferred to a translucent plastic box with screened lid (11 x 11 x 3.5 cm) lined with filter paper until reaching adulthood. Diet and laboratory conditions were the same as described above giving special attention to the asepsis of the colony (cleaning and diet change every 48 h).

One day old emerged adult insects were sexed, and 40 (forty) couples were assembled in translucent plastic boxes with screened lid (11 x 11 x 3.5 cm), one couple per box, lined with filter paper and egg-laying substrate, and the diet. The abiotic conditions, the special asepsis, the diet offered to the couples and their offspring (eggs and nymphs) followed the laboratory and rearing conditions described above.

The biological parameters development time and mortality of each immature phase (egg-adult), adult pre-oviposition time, number of egg masses, average number of eggs per egg mass, fertility, fecundity, the average number of days of oviposition, sex ratio (♀/ ♀+♂), weight, and adult longevity of *Diceraeus furcatus* in a controlled environment (as described above) were accessed. Adult longevity box plots were generated in R (RStudio Team 2022) with the package "ggplot2". The survival time of the females and males were submitted to a Log-rank test in R (RStudio Team 2022) with the package "nph". Subsequently, we determined the parameters i) net reproduction rate (R_0), defined by: $\mathbf{R_0 = \sum lx\ mx}$ in which *lx* is the number of adults still alive at age *x* and *mx* is the age-specific fertility; ii) T is the mean interval between generations and can be calculated from: $\mathbf{T = \sum lx\ mx\ x\ /\ lx\ mx}$; iii) the intrinsic growth rate (r_m) estimated by the equation $\mathbf{r_m = Ln(R_0)\ /\ T}$; **iv)** λ which is the finite increase ratio that is determined by: $\mathbf{\lambda = e\text{^}r_m}$; and v) DT, the doubling time can be calculated from $\mathbf{DT = Ln\ (2)\ /\ (r_m)}$. Equations proposed by Maia et al. (2000).

Immature *D. furcatus* kept under laboratory conditions and fed on the natural diet completed its life cycle (egg-adult) in 36.12 days with an average of 69.02% of mortality (Tab. 1). The development time from 2nd instar to adult (25.4 days) was similar when feed exclusively on crop plants or winter cereals, which ranged from 21-32 days; and the 2nd instar to adult mortality (57.73 %) found here was higher when compared with spring cereals and soybean and lower when compared to canola or maize (Chiaradia 2015; Panizzi et al. 2018; Possebom et al. 2020). The fifth instar showed the highest mortality and the longest development time, suggesting that it is the critical phase for *D. furcatus*.

The females showed a high reproductive capacity under these laboratory conditions and diet (Tab. 1). Females achieved reproductive maturity in 12 days, in average each female laid eggs for 85 days and laid approximately 251 eggs, 89% of the eggs where viable, and a balanced sex ratio of the offspring was found (0.46). These results are similar or higher than reported for *D. furcatus* (on natural diet or fed on crop hosts), and related phytophagous stink bug species e.g., *Nezara viridula* (L., 1758), *Diceraeus melacanthus* Dallas, 1851, *Euschistus heros* (Fabricius, 1798), *Euschistus taurulus* Berg, 1878, and *Euschistus crenator* (Fabricius, 1794) on standardized diets (natural or artificial) or fed on crop hosts (Fortes et al. 2006; Chocorosqui & Panizzi 2008; Mendoza et al. 2016; Cingolani et al. 2019; Hickmann et al. 2019; 2021). The short pre-reproductive period and high fecundity reported here possibly can be related to the balanced diet and multiple copulations, which usually are positively correlated (Cohen 2004; Cingolani et al. 2019).

Both females and males lived more than 150 days on average (females: 159.55 ± 7.1, range 88-349 days; males: 156.75 ± 8.19, range 84-397 days), and we did not find significant differences between males and females' survival probability over time (Fig. 1A and B; Log-rank: p = 0.58). The similar longevity between males and females indicates that the method/diet is appropriate for both sexes. In addition, adult females are heavier when compared to males (Tab. 1). The adult longevity found is higher than reported for *D. furcatus* feeding on maize (Chiaradia 2015), and for other species reared in laboratory feeding on crop hosts or diet, such as *D. melacanthus*, *Chinavia impicticornis* (Stål, 1872), *Chinavia ubica* (Roslton, 1983), and *E. heros* (Chocorosqui & Panizzi 2008; Silva et al. 2015; Mendoza et al. 2016). Moreover, *D. furcatus* females' longevity is negatively corelated to reproductive costs [e.g., virgin females live more than mated females] (Cingolani et al. 2019). Thus, in our study females were prone to multiple copulations and they lived longer than reported in Cingolani et al. (2019), suggesting that the increase in the longevity may be also attributed to the rearing method.

Fertility life table parameters allow us to estimate the suitability of a diet or the environmental condition for insect rearing. The parameter R_0 indicates an increase of the population in each generation more than 43 times; the T parameter shows that on average *D. furcatus* needs 81 days from the parents' birth until the birth of the offspring; the time span necessary for doubling the initial population (DT) found was 38 days; the r_m (0.020) means the innate capacity of increase in number; and (finite rate of increase) = 1.020 meaning the number of individuals added to the population per female that will generate a female (Tab. 2). According to our data, *D. furcatus* biological parameters still are lower when compared to other species (e.g., *E. heros*, and *N. viridula*); however, they are similar to species, such as for *C. impicticornis* and *C. ubica* (Tab. 2) (Fortes et al. 2006; Silva et al. 2015; Mendoza et al. 2016). Thus, the diet and methods employed here proved to be promising to rear *D. furcatus*, however, still need adjustments especially for the nymphs.

Here, we propose adjustments to the standard rearing methods to successfully rearing *D. furcatus* under laboratory conditions. The resulting biological parameters suggest the effectiveness of the procedures adopted. The periodic asepsis (every 48 hours cages were cleaned and diet replaced) summed with the diet consisting of green bean pods, raw peanut, and raw soybean grains offered the environment conditions and diet suitability for *D. furcatus* develop and reproduce. In general, insect fecundity and survival are positively affected by the consumption of a nutritionally well-balanced diet (Cohen 2004).

Table 1. Biological parameters development time and mortality of each immature phase (egg–adult), adult pre-oviposition time, average number of egg masses per female, average number of eggs per egg mass, fertility, fecundity, average number of days of oviposition, sex ratio, and weight of *Diceraeus furcatus* in controlled environment [25 ± 1ºC, 65 ± 5% RH, 14: 10h (light: dark)] and special asepsis.

Immature biological parameters								
Parameter / Phase	**Egg**	**1st instar**	**2nd instar**	**3rd instar**	**4th instar**	**5th instar**	**2nd–adult**	**Egg–adult**
Development time (days ± SE)	6.84 ± 0.07*	3.88 ± 0.11	6.28 ± 0.017	5.08 ± 0.16	5.72 ± 0.15	8.32 ± 0.17	25.4 ± 0.37	36.12 ± 0.41
Mortality (% ± SE)	10.93 ± 2.52	0.36 ± 0.35	3.91 ± 1.28	13.91 ± 3.98	11.17 ± 2.37	28.74 ± 3.88	57.73 ± 3.74	69.02 ± 3.14
	♀ reproductive parameters						Adult mass (mg)	
Pre-oviposition (days ± SE)	**Egg masses / ♀ (mean ± SE)**	**Eggs / egg mass (mean ± SE)**	**Fertility (eggs / ♀) (mean ± SE)**	**Fecundity (nymphs / eggs) (mean ± SE)**	**Oviposition days** (mean ± SE)**	**Sex ratio (♀ / (♀ + ♂))**	**♀ weight (mean ± SE)**	**♂ weight (mean ± SE)**
12.28 ± 0.84	21.15 ± 1.80	11.63 ± 0.24	251.85 ± 22.83	225.91 ± 20.47	85.43 ± 4.22	0.46 ± 0.04	79.87 ± 3.19	68.82 ± 3.10

* Mean followed by the standard error.

** Mean time interval between the first and last oviposition.

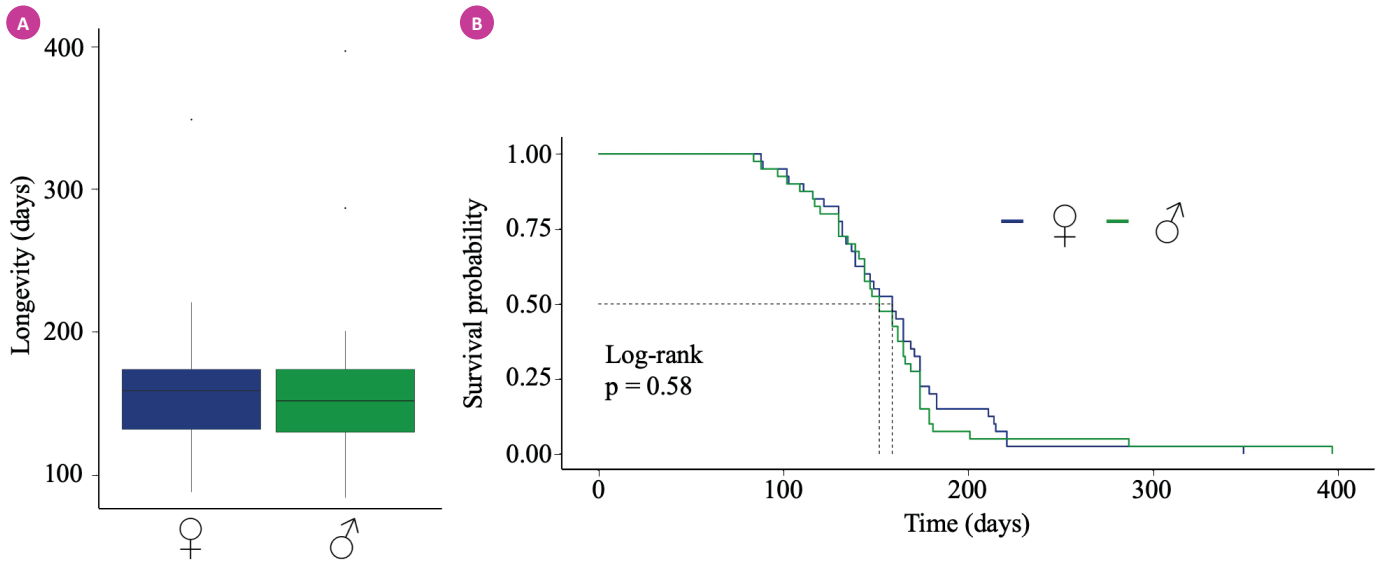

Figure 1. Adult survival of *Diceraeus furcatus* in laboratory under controlled environment [25 ± 1ºC, 65 ± 5% RH, 14: 10h (light: dark)], and extra asepsis. A) Boxplot showing the average longevity in days of females and males of *D. furcatus*. B) Survival probability over time of male and females of *D. furcatus* (Log-rank: p = 0.58).

Table 2. Fertility life table parameters net reproductive rate (R_0), mean interval between generations (T), doubling time (DT), intrinsic rate of increase (r_m), and finite rate of increase (λ) of *Diceraeus furcatus* on natural diet and with periodical cleaning (current work). Additionally, we compiled fertility life table data already published for *Euschistus heros* (on natural and artificial diet), *Nezara viridula* (on natural and artificial diet), and *Chinavia impicticornis* and *C. ubica* (on natural diet).

Species / diet	R_0	T (days)	DT (days)	r_m	λ	References
Diceraeus furcatus / natural diet	43.54	81.42	34.43	0.020	1.0203	Current work
Euschistus heros / natural diet	86.31	58.22	9.04	0.076	1.0790	Mendonza et al. (2016)
E. heros / optimized artificial diet	136.11	57.82	8.14	0.085	1.0890	Mendonza et al. (2016)
Chinavia impicticornis / natural diet	15.90	59.60	14.90	0.040	1.0500	Silva et al. (2015)
C. ubica / natural diet	13.60	48.10	12.70	0.050	1.0600	Silva et al. (2015)
E. heros / natural diet	57.10	70.20	–*	0.074	1.0772	Fortes et al. (1996)
E. heros / optimized artificial diet	53.20	58.10	–	0.068	1.0708	Fortes et al. (1996)
Nezara viridula / natural diet	132.70	63.80	–	0.076	1.0796	Fortes et al. (1996)
N. viridula / optimized artificial diet	24.20	64.20	–	0.049	1.0502	Fortes et al. (1996)

*Data not presented by the authors.

On the other hand, the diet can also represent an excellent nutrient source for fungi, molds and bacteria that could alter the diet quality, and adversely affect the insect's development (Sikorowski & Lawrence 1994; Cohen 2004). Increasing the asepsis of the rearing container and replacing the diet periodically can significantly reduce the proliferation of pathogens harmful to insect feeding, e.g., mold and fungi (Funke 1983; Cohen 2004).

Diceraeus furcatus is recorded on 13 botanical families on different life stages and plant parts (Smaniotto & Panizzi 2015). To reduce nymph mortality an alternative would be to incorporate suitable wild or cultivated plants into the diet. The establishment of a population in the laboratory is the first step to a mass rearing program. Stock colonies are essential to study many aspects of a species such as reproduction, interaction with hosts, estimate the level of economic damage, efficiency and resistance tests with insecticides, as well as biological control studies, all of which are the basis to design and develop management strategies. Although the method described here still requires adjustments, we gave a step further to the development of a rearing method for this emergent pest species in South America.

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Authors' Contributions

S.L., M.S., and P.R.V.S.P., conceived and designed the experiments; S.L., M.S collected data; F.H. performed the statistical analysis; F.H., C.F.S., M.S.L.A., M.S., P.R.V.S.P., interpreted the results and wrote the manuscript; all authors read and reviewed the manuscript.

Conflict of Interest Statement

All authors have declared that there is no conflict of interest.

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