ARTIGO/ARTICLE

Life cycle and reproductive patterns of *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera: Reduviidae) under constant and fluctuating conditions of temperature and humidity

Ciclo de vida e características reprodutivas de *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera: Reduviidae) em condições constantes e variáveis de temperatura e umidade

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ABSTRACT

The aim of this study was to evaluate the temperature and relative humidity influence in the life cycle, mortality and fecundity patterns of Triatoma rubrovaria. *Four cohorts with 60 recently laid eggs each were conformed. The cohorts were divided into two groups. In the controlled conditions group insects were maintained in a dark climatic chamber under constant temperature and humidity, whereas triatomines of the ambiental temperature group were maintained at room temperature. Average incubation time was 15.6 days in the controlled conditions group and 19.1 days in the ambiental temperature. In group controlled conditions the time from egg to adult development lasted 10 months while group ambiental temperature took four months longer. Egg eclosion rate was 99.1% and 98.3% in controlled conditions and ambiental temperature, respectively. Total nymphal mortality in controlled conditions was 52.6% whereas in ambiental temperature was 51.8%. Mean number of eggs/ female was 817.6 controlled conditions and 837.1 ambiental temperature. Fluctuating temperature and humidity promoted changes in the life cycle duration and in the reproductive performance of this species, although not in the species mortality.*

Key-words. *Life cycle. Reproductive characteristics. Temperature. Humidity.* Triatoma rubrovaria.

RESUMO

O presente trabalho teve como objetivo avaliar a influência da temperatura e umidade relativa sobre o ciclo biológico, mortalidade e fecundidade de Triatoma rubrovaria. *Formaram-se quatro coortes, cada uma de 60 ovos recentemente colocados. As coortes foram divididas em dois grupos: O grupo condições controlada foi mantido em estufa com temperatura e umidade constantes, e o grupo temperatura ambiental foi mantido em condições variáveis de temperatura e umidade. O tempo de incubação dos ovos foi de 15.6 condições controlada e de 19.1 dias temperatura ambiental. O período de desenvolvimento de ovo a adulto foi de dez meses em condições controlada e 4 meses mais extenso em temperatura ambiental. A taxa de eclosão foi 99.1% em condições controlada e 98.3% em temperatura ambiental. A fecundidade foi 817.6 condições controlada e 837.1 temperatura ambiental. O percentual de mortalidade ninfal foi 52.6% no grupo condições controlada e 51.8% no grupo temperatura ambiental. As variações de temperatura e umidade relativa exercem influência no ciclo biológico e em alguns padrões reprodutivos desta espécie, mas não em sua mortalidade.*

Palavras-chaves: *Ciclo biológico. Padrões reprodutivos. Temperatura. Umidade.* Triatoma rubrovaria.

Triatoma rubrovaria (Blanchard, 1843) is widely distributed in the Argentina Mesopotamia, Uruguay and South of Brazil[8]. Its presence in the province of Corrientes (Argentina) was verified in basaltic outcrops of the Mercedes department[4]. In Brazil it has been occasionally encountered in domestic and peridomestic environments in rural areas of the Paraná and Río Grande do Sul States, increasing its presence in human dwellings where *Triatoma infestans* has been controlled[1]. *T. rubrovaria*

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is a generalist species feeding from a wide variety of hosts including humans, and its competence as *Trypanosoma cruzi* vector has been experimentally verified[2 20 23].

Higher temperature and lower relative humidity are climatic factors that decrease the development time from egg to adult of Chagas disease vectors[7], promoting a higher population density, an increase in the feeding frequency and an expansion of the geographic distribution[9 10 21].

The influence of temperature on the biology of *Triatoma rubrovaria* as well as in other Triatominae species has been reported[6 11 13 18 19 22]. Studies on cold resistance showed that *T. rubrovaria* was the most affected among a number of Triatominae species[5]. However, there is little information concerning the development of Chagas disease vectors under fluctuating temperature and relative humidity[15].

The aim of the present study was to evaluate the temperature and relative humidity influence on the life cycle and mortality and reproductive patterns of *T. rubrovaria*.

MATERIAL AND METHODS

A total of 93 *T. rubrovaria* (6 N1; 15 N2; 17 N3; 25 N4 and 30 N5) were captured in an area of natural rock piles, located 37km from Mercedes City (29º 23' S, 57º 50' W) in the province of Corrientes, Argentina. The investigation was carried out with the first generation offspring from October 2000 to August 2003.

Four cohorts with 60 recently laid eggs (0-48 hours old) each were conformed. The eggs were kept in 60cm^3 plastic containers until all those that were viable hatched. The cohorts were divided into two groups: controled conditions (CC) and ambiental temperature (TA). The nymphs were transferred into 300cm^3 glass containers. The jars were covered with nylon mesh and provided with vertically placed strips of Whatman paper n° 4 to allow insects access to the food resource and for absorption of excess humidity. The specimens of both groups were fed weekly on chickens during 30 minutes.

The CC group insects were maintained in a dark climatic chamber under constant temperature and humidity. The triatomines of the TA group were maintained under room temperature and humidity with a photoperiod of 12L:12D hr. The temperature and humidity were measured with a Weatherlink-Monitor II –Davis weather station.

Adult couples were placed in individual containers to evaluate fecundity. Egg hatching was recorded daily and ecdysis, mortality, fecundity and fertility were noted weekly.

The rate of total nymphal mortality (TNM) was calculated as the ratio between the number of dead nymphs of all stadia (d_t) and the number of living nymphs at time t (N_t), and it was expressed as a percentage using the formula: $TNM = d_t/N_t \times 100$.

The absolute nymphal mortality (ANM) was estimated as the ratio between the number of dead nymphs of a specific nymphal stage (d_x) and the total number of nymphs of that stage (N_x) x 100: $ANM = d_x/N_x \times 100$.

Longevity was measured as the time elapsed since each individual moulted to the adult stage until its death.

The fecundity was obtained as the ratio between all laid eggs and the total number of females. The fertility was calculated as the number of hatched eggs of the total number of laid eggs.

Statistical analysis. Analysis of variance (ANOVA) was used to compare developmental time, mortality, longevity, number of eggs/female/week and number of reproductive weeks between groups. The variables were transformed to log (x+1) or hyperbolic arcsine. Post-hoc comparisons were made using the Duncan test. Male-female ratio was compared using chi-square test. Fecundity, fertility and first oviposition age between groups were statistically measured by the Kruskall-Wallis test. The test for difference of proportions was used to compare the egg survival between groups. Pearson coefficient (r) was used to analyze the correlation between monthly fecundity of the TA couples and monthly mean temperature. All data obtained were analyzed at a 5% significance level. Statistical analysis was carried out with the program INFOSTAT 2002, version 1.1[12].

RESULTS

The temperature in the climatic chamber was 28° ± 2.2°C (mean ± standard deviation), mean relative humidity was 63% ± 6.2%. The mean room temperature was 26.1° ± 2.8°C. Over 3 years, the temperature fluctuated between 20.6°C and 30.8°C, the absolute minimum was 16.9°C and the maximum 33.1°C (Figure 1). Mean relative humidity was 55.8% ± 5.6%, variations ranged from 44.3% to 70%, the absolute minimum was 30% and the maximum 79% (Figure 2).

Triatoma rubrovaria of the TA group completed one egg to adult cycle from October 2000 to December 2001. During this period, the mean room temperature fluctuated between 22.1° and 28.2°C. In nine of these 15 months, the temperature was less than 26°C and the relative humidity lower than 56%. The lowest absolute temperature values (22.1°C and 23.6°C) were recorded from May to September 2001.

Development time. The average incubation time was 15.6 days in CC group and 19.1 days in TA (Table 1). Seventy-five percent of the eggs hatched between 15 and 16 days in the CC, while those in group TA took between 18 and 21 days.

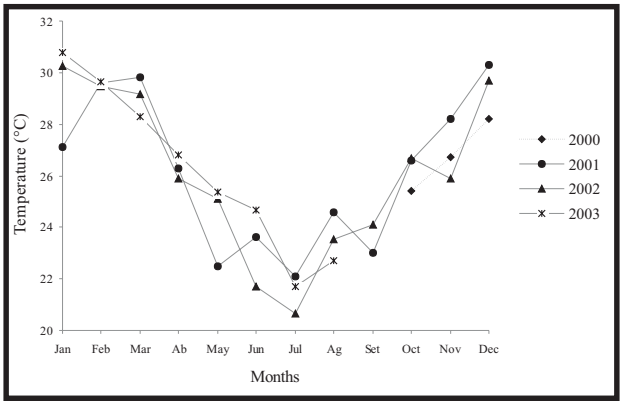

Figure 1- Monthly mean room temperature from October 2000 to August 2003.

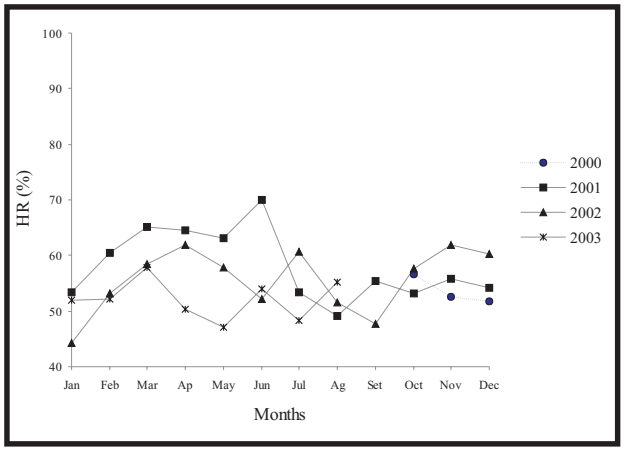

Figure 2 - Monthly mean ambient relative humidity from October 2000 to August 2003.

Table 1 - Life cycle of* Triatoma rubrovaria *under different conditions of temperature and relative humidity. Corrientes, Argentina. 2000-2003.

Age structure	Development time*	
	CC	TA
	Mean±SD	Mean±SD
Egg	15.6 ± 0.2	19.1 ± 1.6
N1	26.5 ± 0.6	34.8 ± 3.5
N2	31.9 ± 1.9	39.0 ± 2.5
N3	41.6 ± 1.4	59.5 ± 1.1
N4	67.7 ± 7.6	91.2 ± 11.0
N5	121.1 ± 11.9	190.4 ± 17.0
Duration of cycle egg-adult	304.5 ± 19.7	434.1 ± 6.8

*Data expressed in days. SD: standard deviation. CC: controled conditions. TA: ambiental temperature.

The mean development time of each nymphal stage was significantly different within the groups ($F_{5, 12}$= 390.30, p< 0.0001), as well as between groups ($F_{1, 12}$= 89.88, p<0.0001). The highest variation was observed in the third (N3), fourth (N4) and fifth nymphal development (N5) (p< 0.05). In group TA the fifth nymphal instars took 69 days longer than the CC group to reach the adult stage. In group CC the time from egg to adult development lasted 10 months while group TA took four months longer.

Nymphal recruitment differed by one week between groups until the third nymphal stage. From the fourth stage the recruitment rate was lower in group TA. Fifth stage recruitment took 16 weeks in CC group while group TA took 39 weeks.

In CC the fifth nymphal instars took 41 weeks to reach the adult stage, whereas in TA they moulted to adults in just 10 weeks.

The male to female ratio was 1:1.5 in CC and 1:1.2 in TA, no significant difference was observed ($\chi 2$ = 0.04, p= 0.8404).

Survival–Mortality. The egg eclosion rate was 99.1% and 98.3% in CC and TA, respectively (p>0.10).

The total nymphal mortality in CC was 52.6% ± 2.5 and in TA was 51.8% ± 7.9 (ANOVA, $F_{1, 10}$= 0.08, p= 0.7790). The lowest mortality rate in CC group was observed in the second nymphal instars, and in TA in the fifth nymphal instars (Figure 3). Before moulting to the last nymphal stage, 33.3% nymphs died in CC, while 48.3% nymphs in the TA group did not reach this stage.

The highest mortality rate occurred during the fifth stage in group CC and during the third stage in group TA, when maximum room temperature reached 31.7°C and humidity 79%. Significantly different mortality between groups was noted in N1, N2 and N5 (Duncan test, p< 0.05).

Longevity. The mean adult survival was 11.9 months (CC, n = 54) and 16.2 months (TA, n = 46). Males had a longer life span, mean survival was 12.9 months and 18.3 months in CC and TA, respectively (ANOVA, $F_{1, 109}$= 10.45, p = 0.0016). Male longevity in TA was significantly higher (Duncan test, p< 0.05).

Fecundity–Fertility. These variables were studied in 25 and 22 couples in CC and TA, respectively. Fecundity (H = 0.03, p = 0.86) and fertility (H = 1.56, p = 0.21) values were not significantly different between groups (Table 2).

Female age at first oviposition was 7.2 ± 2.8 days in group CC and 10.1 ± 4.6 days in TA (H = 21.94, p< 0.0001).

Mean number of eggs/female/week (ANOVA, $F_{1,101}$= 4.43, p = 0.0378) and number of reproductive weeks ($F_{1,45}$= 6.53, p = 0.0141) were different between groups.

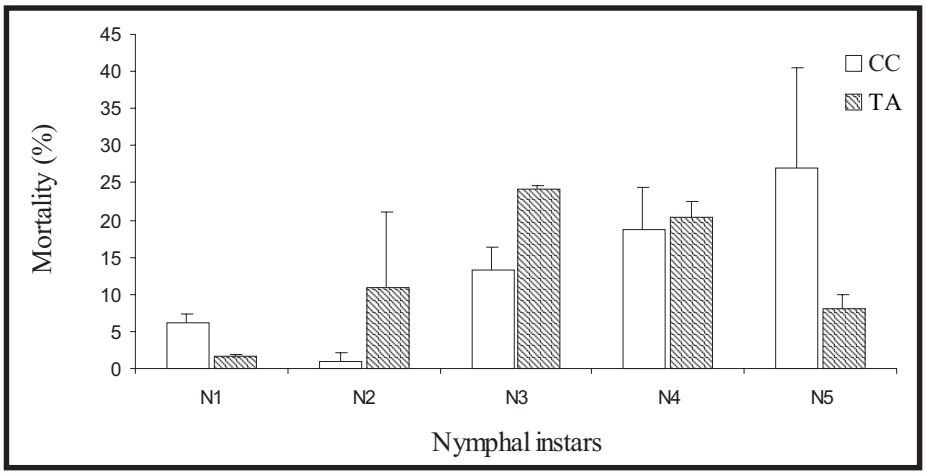

Figure 3 - Mortality pattern (%) of* Triatoma rubrovaria *nymphal stages under fluctuating (TA) and controlled conditions (CC).

Table 2 - Reproductive patterns of* Triatoma rubrovaria *for each group tested. Corrientes, Argentina. 2001-2003.

	Groups			
	CC		TA	
	minimum	maximum	minimum	maximum
	(Mean±SD)		(Mean±SD)	
Age of first reproduction(data in weeks)	22	63	56	64
	(41.1±14.1)		(60.2±2.4)	
Eggs/female (nº)	211	1362	416	1301
	(817.6±331.1)		(837.1±212.2)	
Reproductive weeks/female (nº)	11	63	20	72
	(37.7±14.2)		(49.3±14.6)	
Eggs/female/week (nº)	13.9	30.7	10.6	32.1
	(22.5±4.3)		(16.8±4.2)	
Fertility (%)	30.9	98.1	20.6	93.7
	(75.1±18.3)		(69.7±18.9)	

SD: standard deviation. CC: controled conditions. TA: ambiental temperature.

The highest number of eggs/female/week in TA was observed during the hottest months. A direct correlation between fecundity and temperature was verified ($r = 0.56$, $p < 0.001$, $n = 251$).

An interruption period of up to six weeks in the oviposition occurred in all the TA females during cold as well as warm months. This interruption was only observed in 5 CC females and the longest duration was 3 weeks. After the interruption, the females continued oviposition until they died.

DISCUSSION

Most studies on life cycles in Triatominae have been carried out under controlled conditions of temperature and relative humidity, although these factors show daily and seasonal variations in natural habitats[15].

Although no assessments of the natural biotope of *T. rubrovaria* have been carried out, studies on microclimatic conditions within a similar habitats of rock piles where *T. brasiliensis* is found, demonstrated that temperature and relative humidity reaches lower ranges than the external condition (especially on the rock surface)[14].

Experimental studies revealed that higher temperatures cause a reduction in the developmental period of *T. rubrovaria*[22]. Higher temperature and lower relative humidity also decrease the development time of eggs and nymphs of *T. rubrofasciata*, *T. melanosoma*, *R. neglectus* and *R. robustus*[6 11 18 19]. Extremely low humidity can interrupt the insect development[15], although no influence of humidity on egg development time of *R. neglectus* was found[18].

Although average temperature and relative humidity were similar in the two experimental situations under which *T. rubrovaria* were reared, the variability was higher under the ambient conditions compared with the condition inside the climatic chamber. Room temperature was similar and relative humidity was slightly lower than records obtained within a palm trees community in the San Roque department of Corrientes[3].

Development time in the TA group was longer than in the CC group, corroborating studies on the same species[22] and on other triatomines, describing longer life cycles under changing conditions of temperature and relative humidity[16].

Adult emergence in the CC group occurred almost all year long (February to December 2001) at an approximately constant rate, whereas all nymphs of the TA group reached the adult stage between October and November 2001. In the last experimental group, no recruitment occurred during the colder months (April to September 2001).

Sex rate revealed a slight predominance of females in the two experimental groups, suggesting a higher mortality risk during the preadult development in males, as indicated for *T. infestans*[17]. Male longevity in the TA group was longer, coinciding with *Triatoma rubrofasciata*[6].

Changing conditions of temperature and relative humidity did not affect egg survival and total nymphal mortality, as no difference was observed between the two experimental groups. However, the high mortality rate in the third stage in TA occurred during a period when the highest temperature and relative humidity were recorded. At 33°C and 70% a complete mortality for *Rhodnius neglectus* fifth nymphal instars was also reported[18].

Fecundity and fertility did not differ greatly between the two experimental groups, coinciding with the results reported by Silva[22] when the species was kept at 25°C and 30°C. Date of first reproduction (from initial day of the study) began earlier in the CC group with higher variability, as the result of the long recruitment period to the adult stage in this group.

Constant temperature and humidity were the most optimal conditions for development, the life cycle of *T. rubrovaria* reared under these conditions was shorter. Although females under room conditions had a longer reproductive period, the number of eggs/week was lower and oviposition interruptions were constantly observed. Developmental time in the group exposed to fluctuating temperature and relative humidity took over a year to complete. As the insects had no food restriction, this might be considered as the species minimum under natural climatic conditions.

This study indicates that fluctuating temperature and humidity have an important effect on the life cycle duration of *T. rubrovaria*. A difference of 2°C in the mean temperature under which the two experimental groups were developed, promoted changes in the life cycle duration and in the reproductive performance of this species.

REFERENCES

1. Almeida CE, Duarte R, Guerra RN, Pacheco R, Costa J. *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera, Reduviidae, Triatominae). II: Trophic resources and ecological observations of five populations collected in the State of Rio Grande do Sul, Brasil. Memórias do Instituto Oswaldo Cruz 97: 1127-1131, 2002.
2. Almeida CE, Vinhaes MC, Almeida JR, Silveira AC, Costa J. Monitoring the domiciliary and peridomiciliary invasion process of *Triatoma rubrovaria* in the State of Rio Grande do Sul, Brazil. Memórias do Instituto Oswaldo Cruz 95: 761-768, 2000.
3. Bar ME. Triatominos de la comunidad de palmeras en la Provincia de Corrientes. Ecología e importancia epidemiológica. Tesis de Doctorado, Facultad de Ciencias Exactas, Universidad Nacional del Nordeste, Corrientes, Argentina, 2001.
4. Bar ME, Oscherov EB, Milano AM, Damborsky MP. Ciclo de vida de *Triatoma rubrovaria* (Blanchard 1843) (Heteroptera: Reduviidae) bajo condiciones de laboratorio. Revista de la Facultad de Ciencias Exactas FACENA 19: 113-121, 2003.
5. Blaksley JC, Carcavallo RU. La enfermedad de Chagas-Mazza en la Argentina. Ministerio de Salud, Buenos Aires, 1968.
6. Braga MV, Pinto ZT, Lima MM. Life cycle and reproductive patterns of *Triatoma rubrofasciata* (De Geer, 1773) (Hemiptera: Reduviidae), under laboratory conditions. Memórias do Instituto Oswaldo Cruz 93: 539-542, 1998.
7. Carcavallo RU. Climatic factors related to Chagas disease transmission. Memórias do Instituto Oswaldo Cruz 94 (supl I): 367-369, 1999.
8. Carcavallo RU, Casas SIC, Sherlock IA, Galíndez-Girón I, Jurberg J, Galvão C, Mena Segura CA, Noireau F. Geographical distribution and altitudinal dispersion. *In*: Carcavallo RU, Galíndez-Girón I, Jurberg J, Lent H (orgs) Atlas of Chagas' disease vectors in the Americas, Vol. III, Editora Fundação Oswaldo Cruz, Rio de Janeiro, Brasil, p. 747-792, 1999.
9. Carcavallo RU, Galvão C, Rocha DS, Jurberg J, Casas SIC. Predicted effects of warming on Chagas disease vectors and epidemiology. Entomología y Vectores 5: 137, 1998.
10. Casas SIC, Carcavallo RU, Segura CAM, Galindez Girón I. Bioclimatic factors of Triatominae distribution. Useful technique for studies on climate change. Entomología y Vectores I: 51-67, 1994.
11. Galvão C, Rocha SD, Cunha V, Presgrave OAF, Jurberg J, Carcavallo R. Influência da temperatura no ciclo de vida de *Triatoma melanosoma* Martínez, Olmedo & Carcavallo, 1987 (Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz 94: 851-854, 1999.
12. InfoStat. InfoStat versión 1.1. Grupo InfoStat. Facultad de Ciencias Agrarias. Universidad Nacional de Córdoba, Argentina, 2002.
13. Juarez E, Silva EPC. Comportamento do *Triatoma sordida* em condições de laboratório. Revista de Saúde Pública 16 (supl): 1-35, 1982.
14. Lorenzo MG, Guarneri AA, Pires HHR, Diotaiuti L, Lazzari CR. Aspectos microclimáticos del hábitat de *Triatoma brasiliensis*. Cadernos de Saúde Pública 16 (supl II): 69-74, 2000.
15. Luz C, Fargues J, Grunewald J. Development of *Rhodnius prolixus* (Hemiptera: Reduviidae) under constant and cyclic conditions of temperature and humidity. Memórias do Instituto Oswaldo Cruz 94: 403-409, 1999.
16. Pippin WF. The biology and vector capability of *Triatoma sanguisuga texana* (Usinger) and *Triatoma gerstaeckeri* (Stål) compared with *Rhodnius prolixus* (Stål) (Hemiptera, Triatominae). Journal of Medical Entomology 7: 30-45, 1970.
17. Rabinovich JE. Vital statistics of Triatominae (Hemiptera: Reduviidae) under laboratory conditions. I. *Triatoma infestans* Klug. Journal of Medical Entomology 9: 351-370, 1972.
18. Rocha DS, Jurberg J, Carcavallo RU, Cunha V, Galvão C. Influência da temperatura e umidade na biologia de *Rhodnius neglectus* Lent, 1954 em laboratório (Hemiptera, Reduviidae, Triatominae). Revista da Sociedade Brasileira de Medicina Tropical 34: 357-363, 2001.
19. Rocha DS, Jurberg J, Carcavallo RU, Presgrave OAFG, Cunha V, Galvão C. Influência da temperatura e umidade no desenvolvimento ninfal de *Rhodnius robustus*. Revista de Saúde Pública 35: 400-406, 2001.
20. Salvatella R, Calegari L, Puime A, Basmadjian Y, Rosa R, Guerrero J, Martínez M, Mendaro G, Briano D, Montero C, Wisnivesky-Colli C. Perfil alimentario de *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera, Triatominae) en ámbitos peridomiciliarios, de una localidad rural de Uruguay. Revista do Instituto de Medicina Tropical de São Paulo 36: 311-320, 1994.
21. Schofield CJ. Nutritional status of domestic populations of *Triatoma infestans*. Transactions of the Royal Society of Tropical Medicine and Hygiene 74: 770-778, 1980.
22. Silva IG. Influência da temperatura na biologia de triatomíneos. I. *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera, Reduviidae). Revista Goiana de Medicina 31: 1-37, 1985.
23. Silva IG, Silva HHG. Suscetibilidade de 11 espécies de triatomíneos (Hemiptera, Reduviidae) à cepa ¨Y¨ de *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae). Revista Brasileira de Entomologia 37: 459-463, 1993.