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# The effect of relative humidity on the behaviour and development of *Triatoma brasiliensis*

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**Abstract.** The preference for relative humidity (RH) and suitability of different levels of this environmental parameter were investigated in the haematophagous bug *Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae). The hygropreference of *T. brasiliensis* was studied using a RH gradient and the effect of different RHs on the egg hatching, nymph mortality and moulting success was also analysed. The results show that egg hatching in first-instar nymphs of *T. brasiliensis* was lower at extreme RHs and, particularly, it was lowest at 9.3% RH. The survival of starved nymphs was not affected by RH, but the percentage of engorged nymphs and the ecdysis success of these nymphs once fed was diminished strongly by high humidity. Fourth-instar nymphs preferred to stay at the lowest RH during the first 5 days after feeding and during ecdysis. This preference changed markedly during starvation. Fifteen days after ecdysis, the bugs moved towards intermediate humidities, and 30 days after ecdysis they even preferred the most humid sectors of the gradient. Females preferred to lay eggs in dry environments, suggesting that they may not have a particular hygropreference for oviposition, but that they simply lay their eggs at the RHs where they prefer to stay.

**Key words.** Behaviour, haematophagous, microclimate, relative humidity, Triatoma.

## Introduction

*Trypanosoma cruzi*, the aetiological agent of Chagas disease, is a flagellate parasite, which is transmitted by triatomine bugs. Such a disease affects approximately 17 million people in Latin America, where another 100 million people are at risk of contracting it (Schofield & Dias, 1999).

*Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae) is now the main vector of *T. cruzi* in Brazil. This species can be found in the north-eastern region of this country, in an area characteristically hot and dry, with absence of rain for 8–9 months of the year. *Triatoma brasiliensis* lives inside cracks in rocky formations that are typical of that landscape. In these shelters, the insect is found in association with rodents, marsupials and bats (Alencar, 1987). It can also be found colonizing domestic and peridomestic environments, where it feeds on man and domestic animals (Alencar, 1987).

Relative humidity (RH) is an environmental factor which may affect different aspects of insect life (Willmer, 1982). Research work on *T. infestans*, formerly the main vector of Chagas disease, demonstrated that both nymphs and adults of *T. infestans* prefer to stay, regardless of their nutritional status, at a RH of approximately 0% (Roca & Lazzari, 1994). In addition, females of this species also showed a preference to lay eggs at a low RH (Roca & Lazzari, 1994). Lorenzo & Lazzari (1999) showed that *T. infestans* prefers experimental shelters with a low RH, and that the natural shelters inside chicken-coops, in a region in which this species is endemic, present RH levels that tend to be lower than that of the external environment.

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To better characterize the effects of environmental RH on the biology of Triatominae, the impact of this environmental parameter on egg hatching, nymph mortality and moulting success of *T. brasiliensis* was studied. The RH preference of *T. brasiliensis*, particularly in relation to resting, ecdysis and oviposition, was also studied. Finally, the effect of starvation on the hygropreference was also analysed.

## Materials and methods

### *Insects*

The insects used for all assays were collected in Piauí state, Brazil, and reared in the insectary of the Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, Centro de Pesquisas René Rachou, FIOCRUZ (Brazil). Insects were reared under a natural regime of temperature (27 ± 3 °C), relative humidity (65 ± 10%) and illumination, and fed weekly on chicken.

### *Control of Relative Humidity*

Different salt solutions or distilled water were used to control the RH to which eggs or insects were exposed. As indicated by Winston & Bates (1960), Roca & Lazzari (1994) and Dambach & Goehlen (1999), acid or inorganic (non-volatile) salt solutions are adequate for this purpose, as their water vapour pressure remains constant for long periods. The liquids used to establish the RHs were distilled water, saturated solutions of NaCl, $Ca(NO_3)_2$, $MgCl_2$ and $H_2SO_4$ 97%. The RHs were measured using a thermo-hygrometer (Testo 625, Lenzkirch, Germany, accuracy ±3% RH).

### *The effect of RH on egg hatching, mortality of nymphs, feeding and moulting success in* T. brasiliensis

Batches of eggs of *T. brasiliensis* laid over a 3-day period were randomly assigned to five groups corresponding to five different RH levels. Each group was placed in a Petri dish surrounded by the solution kept in a larger Petri dish that was hermetically closed. The observed RH values in each Petri dish were: 9.3, 37.3, 46.7, 73.8 and 99.9%. The dishes were put into a thermostatized chamber at 28 ± 1 °C, and in a light cycle of LD 12 : 12 h. During the light phase, the chamber was illuminated by a fluorescent lamp (7 V), which rendered a diffuse illumination intensity of 10 lux. The dishes were kept under these conditions until hatching. The percentage of hatched eggs was then calculated. The eclosed nymphs were maintained under the same conditions for 10 days. After this period, their mortality was determined. The surviving bugs were fed on Swiss mice, previously anaesthetized with Thionembutal injected intraperitonially. The percentage of insects fed to repletion was then determined. Afterwards, they were put in the same RH conditions until moulting. The moulting success was determined for each of the five RH levels tested. Five replications of the complete assay were conducted for each group ($k = 5$). Although within a replicate the five groups had always the same number of eggs ($n$), such number varied between replicates (between 25 and 74). The total amount of eggs used per RH level was 219 ($N = 1095$).

### *Hygropreference of* T. brasiliensis *nymphs. The effect of nutritional status*

A group of 50 fourth-instar nymphs was fed on live chicken 15 days after moulting and on the next day put in the centre of a RH gradient established inside an arena made of glass. This chamber (24 × 13 × 5 cm) contained vials in a lower compartment where the solutions were deposited and in an upper one, a piece of white chiffon acted as substrate for the bugs (Fig. 1). The gradient was established using the five solutions described above. The following RH values were observed at each sector of the gradient: 16.4, 35.9, 56.8, 71.7 and 87.5%. The gradient was maintained in a closed chamber at 28 ± 1 °C, illumined on a LD 12 : 12 h cycle.

An IR-sensitive video camera located above the arena allowed the position of the bugs to be recorded, even during complete darkness: the insects do not perceive wavelengths of this type of IR illumination (Reisenman *et al.*, 1998). For 5 days, the positions of the bugs were recorded every 3 h. This procedure was repeated with the same insects under four different situations: recently fed; at the time of ecdysis (between 10 and 14 days after feeding); 15 days after ecdysis; and 30 days after ecdysis. The positions of the exuviae along the gradient were also recorded.

### *The hygropreference of* T. brasiliensis *for oviposition*

A group of 17 females was fed on chicken and on the next day put into the same RH gradient conditions described in the previous section. The white chiffon that acted as substrate was loosely glued to establish small concavities on each RH sector to keep the eggs from rolling to the side.

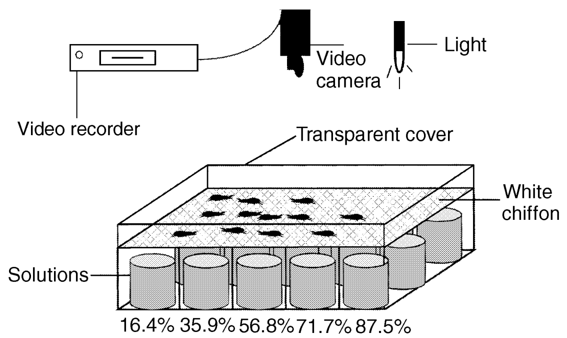


**Fig. 1.** Experimental arena presenting a relative humidity gradient to test the hygropreference of *Triatoma brasiliensis*.

**Table 1.** Influence of relative humidity (mean ± SE (*n*)) on the development of early stages of *Triatoma brasiliensis*

| %RH | Eclosion success (%) | Mortality (%) | Fed insects (%) | Ecdysis (%) | Cumulative effect of %RH* |
|---|---|---|---|---|---|
| 9.3 | 68.9 ± 7.2 (5) | 2.71 ± 2.23 | 88.1 ± 4.5 (4) | 69.0 ± 15.9 (4) | 69.5 ± 7.3 (4) |
| 37.3 | 92.1 ± 2.9 (5) | 0 | 94.8 ± 0.8 (3) | 93.0 ± 4.7 (3) | 20.9 ± 1.9 (3) |
| 46.7 | 95.9 ± 1.7 (5) | 0 | 91.7 ± 4.4 (4) | 88.9 ± 3.4 (4) | 26.7 ± 5.9 (4) |
| 73.8 | 91.5 ± 2.5 (5) | 0 | 95.2 ± 2.7 (4) | 94.0 ± 2.5 (4) | 19.6 ± 3.8 (4) |
| 99.9 | 82.5 ± 5.7 (5) | 1.33 ± 1.3 | 69.0 ± 4.9 (3) | 45.7 ± 4.9 (3) | 71.7 ± 1.5 (3) |

* Proportion of insects that reached the second instar relative to the total number of eggs submitted to that RH level.

Females were kept in the arena for 15 days after feeding and subsequently fed again so that the experiment could be repeated. This treatment was repeated three times to attain a significant number of eggs. In the experimental arena, females could freely choose a place to rest or oviposit, therefore showing their RH preference. The number of eggs present in each sector was counted at the end of each of the three replicates.

### *Statistics*

The software STATISTICA version 5.0 (Statsoft Inc., 1996) was used for the analysis of the hygropreference of *T. brasiliensis* nymphs and for the analysis of the effect of RH on the egg hatching, nymph mortality and moulting success. Analysis of variance (ANOVA) and *post hoc* comparisons (Scheffé test) were made to test the null hypothesis of a lack of effect of RH on the different variables assessed.

## Results

### *The effect of RH on egg hatching, mortality of nymphs and moulting success of* T. brasiliensis

The results presented in Table 1 show that RH affected egg hatching in *T. brasiliensis* (ANOVA, $P < 0.05$). The lowest RH caused a significant decrease in egg hatching when compared with the three intermediate RH levels (Scheffé test, $P < 0.05$), but not with the highest RH (Scheffé test, NS). However, the mortality of first-instar nymphs was not significantly affected by RH (Table 1, ANOVA, NS). Feeding success was affected by RH (Table 1, ANOVA, $P < 0.05$). There was a significant decrease in the percentage of insects fed at the highest RH when compared with the three intermediate levels (Scheffé test, $P < 0.05$), but not with the lowest RH (Scheffé test, NS). In addition, the ecdysis success of the engorged nymphs was strongly impaired by the highest RH (Table 1, ANOVA, $P < 0.05$).

### *Hygropreference of* T. brasiliensis *nymphs. The effect of nutritional status*

In each assay, a brief exploratory interval of *c.* 8 h during which data were not collected was allowed after the release of the insects. Subsequently, the insects invariably stayed in their preferred RH sector for the rest of the assay.

Fourth-instar nymphs of *T. brasiliensis* preferred to stay in the driest sector of the arena during the first 5 days after feeding (Fig. 2a, ANOVA, $P < 0.05$). The same preference was also observed during ecdysis, with a slight increase in the number of bugs present in more humid sectors (Fig. 2b, ANOVA, $P < 0.05$). The preference of the insects 15 days after ecdysis changed markedly. In this situation, the bugs were distributed along the whole gradient, with a maximum in its central sectors (Fig. 2c, ANOVA, $P < 0.05$). Finally, 30 days after ecdysis, the insects preferred the most humid sectors of the gradient (Fig. 2d, ANOVA, $P < 0.05$). The exuviae left by moulted insects inside the RH gradient were distributed as shown in Fig. 3. In all cases, the preference of the bugs did not show any daily variation (ANOVA, NS).

### *The hygropreference of* T. brasiliensis *for oviposition*

Females of this species exhibited the oviposition pattern depicted in Fig. 4. They preferred to lay their eggs in drier environments such that the number of eggs per experimental sector decreased with increasing RH. According to our direct observation during this experiment, as starvation ensued females showed a change in their hygropreference similar to that of the nymphs in the previous experiment (data not shown).

## Discussion

Roca & Lazzari (1994) showed that *T. infestans* has a strong hygropreference of approximately 0%, which does not depend on starvation. Here we show that nymphs of *T. brasiliensis* exhibit a similar hygropreference but, in contrast to *T. infestans*, *T. brasiliensis* modulates its hygropreference depending on its nutritional status. We hypothesize that the gradual changes of hygropreference shown by fourth- and fifth-instar nymphs represent a behavioural mechanism of water loss regulation.

Triatomines experience an intense diuresis during the first 4 h following a blood meal. As a consequence they eliminate up to 40% of the ingested weight as urine (Wigglesworth, 1931). In addition, triatomines show a five-fold increase in their metabolic rate after feeding. Such an increase lasts for a period of *c.* 15–20 days (Wigglesworth, 1931). Some days

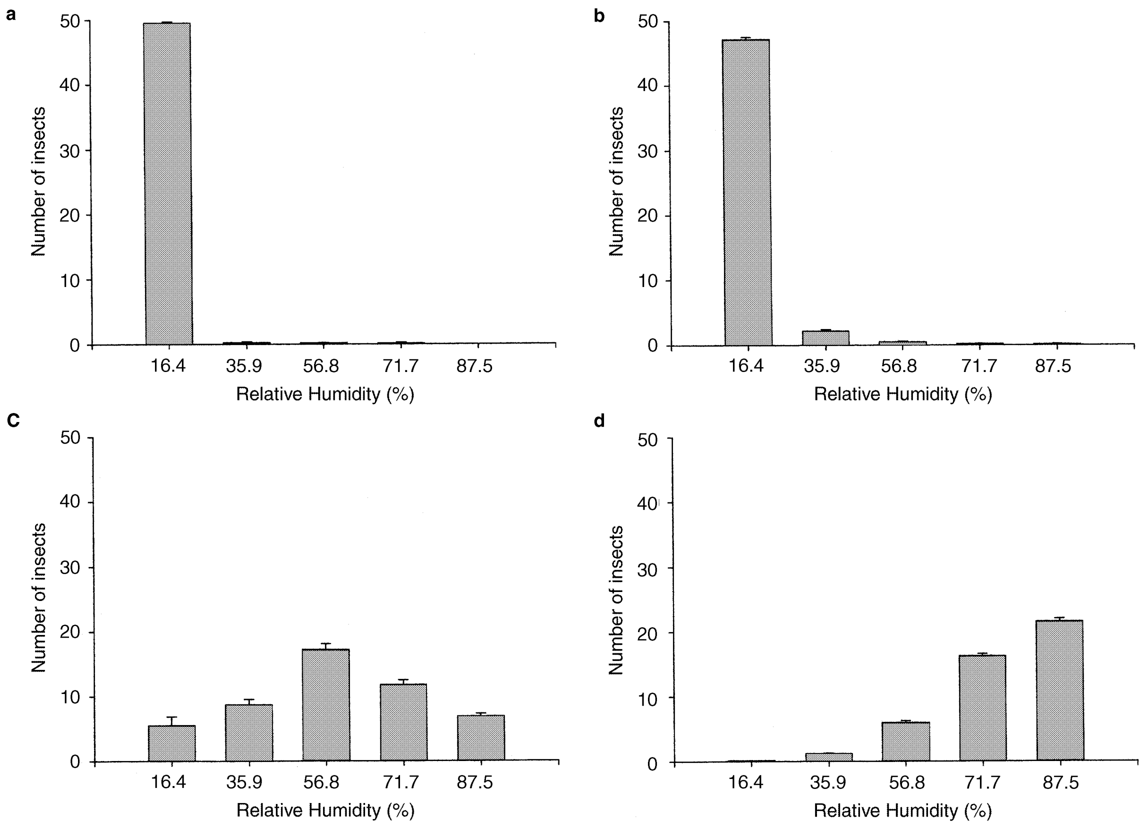


**Fig. 2.** The hygropreference of nymphs of *Triatoma brasiliensis* as a function of the nutritional status: (a) recently fed ($n = 50$); (b) during ecdysis ($n = 50$); (c) 15 days after ecdysis ($n = 50$); and (d) 30 days after ecdysis ($n = 45$). Bars represent mean ± SE.

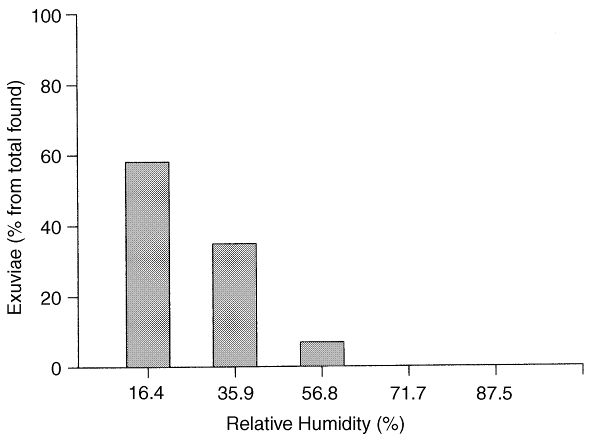


**Fig. 3.** Distribution of exuviae of *Triatoma brasiliensis* through the RH gradient ($n = 50$).

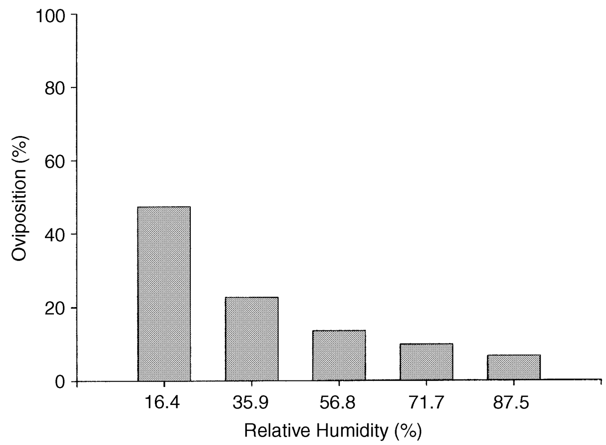


**Fig. 4.** The hygropreference for oviposition of females of *Triatoma brasiliensis* ($n = 17$).

after completion of the moulting process, the metabolic rate decreases to a lower level and remains at that state until the next meal. The intense post-feeding metabolism results in the production of metabolic water (Chapman, 1998). Additional metabolic water comes from the gradual digestion of the blood meal. Therefore, an alternative mechanism other than diuresis is necessary to eliminate water without costs. We propose that *T. brasiliensis* regulates the amount of water lost through the cuticle and ventilation by actively searching for an appropriate environmental RH that corresponds to its nutritional status. It is important to realize that *T. brasiliensis* inhabits extremely arid microecotopes, where they endure dehydrating conditions (Lorenzo *et al.*, 2000). Thus, such a sensitive preference for dry environments that starts with engorgement and extends up to ecdysis, and that can be reversed as a consequence of starvation, may have evolved as a behavioural adaptation to haematophagy. Accordingly, the insects would not withstand long starvation periods during the dry season due to the lack of humid shelters. Conversely, during the rainy season, they would tolerate prolonged fasting.

Our results with females of *T. brasiliensis* show a heterogeneous oviposition pattern that results in most eggs being laid at low RHs. Nevertheless, the daily direct observation of the experimental arena revealed that these insects vary their position with increasing starvation, a fact that is consistent with the conclusions detailed earlier in this paper. The females had an initial clear xeropreference and, after 15 days, they exhibited a preference for intermediate RH levels. The effect of RH on oviposition indicates that females may not have a particular hygropreference for oviposition, but that they simply lay their eggs at the RHs where they prefer to stay.

Our results show that the eggs were affected by extremely low and by high RHs. Low RH is reported to be particularly deleterious for eggs of some triatomine species (but not for all, see Roca & Lazzari, 1994) at hatching time (Clark, 1935). Desiccation could affect the embryo or prevent larval release from the eggshell by a loss of lubrication or sufficient cuticular softness. A way to overcome the last effect, even in arid zones, would be to hatch at dawn, when the RH reaches its daily maximum. Indeed, this has been demonstrated to occur in other triatomines (Lazzari, 1991; Schilman, 1998). Nevertheless, the hatching success of the eggs of *T. brasiliensis* was sensibly higher than that of *Rhodnius prolixus* at low RHs. *Rhodnius prolixus* eggs do not hatch at 0% RH and only 45% of them hatch at 30% RH (Clark, 1935; Schilman, 1998). This suggests that either embryos have a higher tolerance to desiccation or the chorion of the eggs of both species differ in their permeability to water. On the other hand, the lower eclosion success observed for *T. brasiliensis* eggs exposed to 99.9% RH, even though not statistically significant, may be related to fungal pathogens growing in such conditions. Indeed, *R. prolixus* eggs exposed to 100% RH are heavily attacked by fungal pathogens that probably affect their normal development and the survival of embryos (Schilman, 1998). In the present experiments, RH did not affect the survival of starved nymphs. Moreover, mortality was fairly low at all RHs. However, the percentage of engorged insects after a blood meal was lowest for those nymphs exposed to the highest RH, indicating that such a RH level may have somehow affected the physiology of the insects. The ecdysis success was also affected only at the highest RH level, even though no fungal attack was observed by direct observation.

The set of results presented in this work supports the previous findings on xeropreference in triatomines (Roca & Lazzari, 1994), but additionally suggests that such a preference can be modulated by the metabolic-nutritional status of the insects. As a consequence, more detailed studies of the physiological processes related to the water balance of this species at different nutritional stages are necessary. These studies would help to gain understanding of the mechanisms of hygropreference as well as the kind of signals that trigger the behavioural changes. Studies on the permeability to water of the eggshell of different species of triatomines may also explain the differences observed between the hatching success of the eggs of *T. brasiliensis* and *R. prolixus* at low RHs. Further studies on these aspects of the physiology, behaviour and ecology of triatomines are fundamental to understand their distribution, microclimatic needs and physiological restrictions in relation to climate changes and thus to control efficiently their action as disease vectors.

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