

Laboratory analysis of the flight of *Rhodnius brethesi* Matta, 1919, potential wild vector of *Trypanosoma cruzi* in the Brazilian Amazon. (Hemiptera:Reduviidae:Triatominae)

Avaliação em laboratório da atividade de voo de *Rhodnius brethesi* Matta, 1919, potencial vetor silvestre do *Trypanosoma cruzi* na Amazônia Brasileira. (Hemiptera: Reduviidae:Triatominae)

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ABSTRACT

The flight ability of *Rhodnius brethesi* was evaluated through laboratory observations, and it was correlated with the quantity of blood ingested, gender and fasting period. A total of 65 insects were analyzed: 27 males and 38 females; their mean survival period was 17.8 and 22.3 days, respectively. Most of the insects started flying after 13 days.

Keywords: *Rhodnius*; Chagas Disease; Triatominae; Insect Vectors; Behavior; Animal; Flight, Animal.

INTRODUCTION

Triatominae bugs (Hemiptera: Reduviidae) are the vectors of Chagas disease. A total of 141 species, grouped into five tribes and 18 genera, are currently known^{1,2,3}, including the first fossil species, which was recently described⁴.

Rhodnius brethesi, known locally as "piolho da piaçaba" (piaçaba louse), is a sylvatic species found in the native palm tree piaçaba *Leopoldina piaçaba*, in Amazonas State, Brazil. Transmission of *Trypanosoma cruzi* to families

working on the extraction of piaçaba fibers has been recorded in the Municipality of Barcelos, north of the Negro River, in the natural ecotope of *R. brethesi*^{5,6,7,8}.

Infection of humans, reservoirs and vectors with *T. cruzi* and autochthonous cases of Chagas disease have been reported in most countries in the Amazon Basin. Interest in Chagas disease has increased over the past two decades in this area. The Amazon Region is characterized by the absence of vector domiciliation and dispersal of native species, mostly within the genus *Rhodnius*. This situation may change due to continuous deforestation and the immigration of human populations from other regions.

Passive dispersal of triatomines depends on the habits and behavior of their hosts. Several authors have reported the involuntary transport of triatomines in clothing, utensils and vehicles⁹. The feathers of migratory birds also act as excellent vehicles for the dispersal of eggs and even nymphs^{10,11,12,13}. Active dispersal may occur by walking, for nymphs and adults, but mostly occurs by adult flight.

Abrahan et al¹⁴ have recently analyzed active dispersal (by flying and walking) and the role of chickens as passive

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Translated by / Traduzido por / Traducido por:
American Journal Experts

carriers of *Triatoma infestans* in Argentina. For the first time, the authors reported the recapture of females that dispersed by walking and suggested that it is unlikely that chickens passively transport the insects. From an epidemiological perspective, dispersal is of great importance to the spread of Chagas disease, especially in treated areas that may experience recolonization.

Flight initiation in triatomines may be induced by environmental factors, such as temperature or nutritional status. Field and laboratory experiments performed by Lehane et al¹⁵ have shown that flight initiation in triatomines is primarily associated with nutritional status and increased environmental temperature. However, Soares and Santoro¹⁶ have observed that some species do not initiate flight under any conditions. During the imaginal ecdysis of triatomines, flight ability appears to be a result of morphological changes and the adaptation of muscles directly involved in this activity.

The flight activity of *R. brethesi* was evaluated in the laboratory and correlated with sex, the amount of blood ingested and fasting period duration.

MATERIALS AND METHODS

The experiment was performed using stage-5 specimens of *R. brethesi* from a colony reared from insects captured in piassaba trees by the Aracá River, a tributary of the Negro

River (Amazonas State, Brazil), in 2004 and acclimated to the laboratory. The specimens selected for the rearing of adults were fully engorged after a 4-hour feeding period. After the imaginal ecdysis, the insects (27 males and 38 females) were weighed before and after feeding to determine the amount of blood ingested and were individually marked with gouache on the pronotum and scutellum using a color code based on the one proposed by MacCord et al¹⁷. All insects were then placed inside a device designed by Galvão et al¹⁸ for the observation of triatomine flight. The insects were observed on a daily basis until they died, and the flights of flying specimens were recorded. The entire experiment was conducted at $26 \pm 5^\circ \text{C}$ and $70 \pm 5\%$ relative humidity (RH). Wingless insects were used as a control group. The insects were fed on pigeons (*Columba livia*) that were properly immobilized and anesthetized in accordance with protocol L-081-08, which was approved by the Ethics Committee on the Use of Animals (CEUA-Fiocruz). Statistical analyses were performed using unpaired t tests with equal SDs and the GraphPad InStat 3.06 software.

RESULTS

The mean lifespan of adult *R. brethesi* individuals was 17.8 days for males and 22.3 days for females, and the difference was statistically significant according to the unpaired t test performed (Table 1).

Table 1 – Flight activity of *Rhodnius brethesi* Matta, 1919 in the laboratory

	Min	Max	Mean	S	S ²
Lifespan (days)					
(m)	7	29	17.8	35	5.81
(f)	7	42	22.3	63.9	7.89
N° of flights					
(m)	1	6	3.7	2.684	1.600
(f)	1	7	4	4.032	1.975
Initial weight after ecdysis (mg)					
(m)	0.0729	0.1477	0.1154	0.0004	0.0206
(f)	0.0849	0.2079	0.1299	0.0006	0.0255
Final weight after death (mg)					
(m)	0.0326	0.0970	0.0576	0.0002	0.0155
(f)	0.0304	0.1069	0.0570	0.0002	0.0143
Flight initiation after feeding (days)					
(m)	1	19	11.04	17.95	4.139
(f)	2	30	15.32	54.49	7.261
Total weight loss (mg)					
(m)	0.0301	0.0806	0.0571	0.0001	0.0134
(f)	0.0301	0.1589	0.0729	0.0005	0.0240
% weight loss (1 st meal)					
(m)	15.2	52.2	34.14	101.08	9.811
(f)	11.5	54	34.22	135.72	11.46

(m) = males (f) = females

There was no significant difference in the number of flights between male and female individuals, despite the significantly greater initial weight of the females.

Females ingested significantly more blood than male individuals and also exhibited higher weight loss than male insects during their significantly longer lifespan ($P = 0.0035$).

The insects required an average of 13 days after the imaginal ecdysis to start flying. Flight initiation took significantly longer in female individuals (15 days) than males.

There were significant differences in flight time, initial weight and time to first flight between male and female individuals. Flight time was 3.58% longer in females than in males; initial weight was 11.53% higher in females than in males; and time to first flight was 4.7% higher in females than in males. Females that flew more frequently were initially heavier and initiated flight earlier than males.

Plots showing the correlation between the number of flights and total weight loss; flight time and initial weight; % weight loss to first flight and time to first flight; and flight time and time to first flight are displayed in figures 1, 2, 3, and 4, respectively, and the Pearson coefficients and P values for the comparative analyses are shown in table 2.

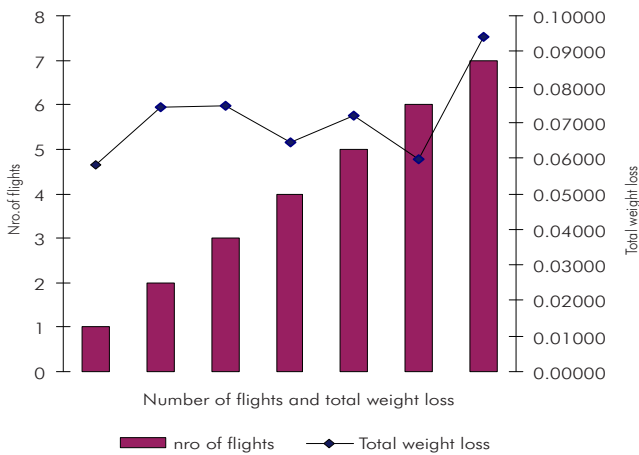


Figure 1 – Number of flights and total weight loss in *Rhodnius brethesi* Matta, 1919 in the laboratory

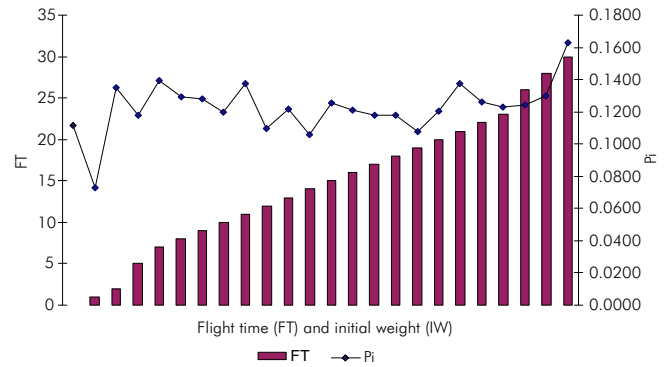


Figure 2 – Flight time and initial weight in *Rhodnius brethesi* Matta, 1919 in the laboratory

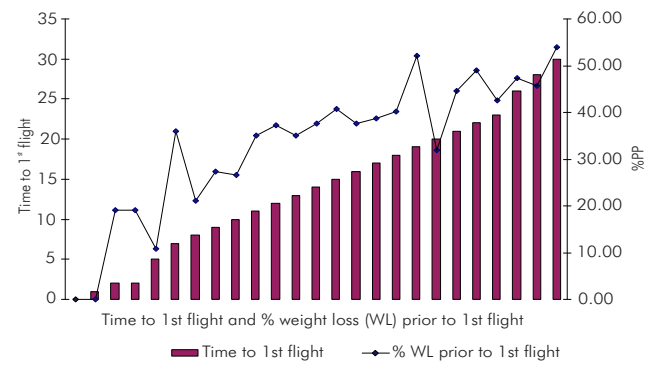


Figure 3 – Time to first flight and weight loss prior to first flight in *Rhodnius brethesi* Matta, 1919 in the laboratory

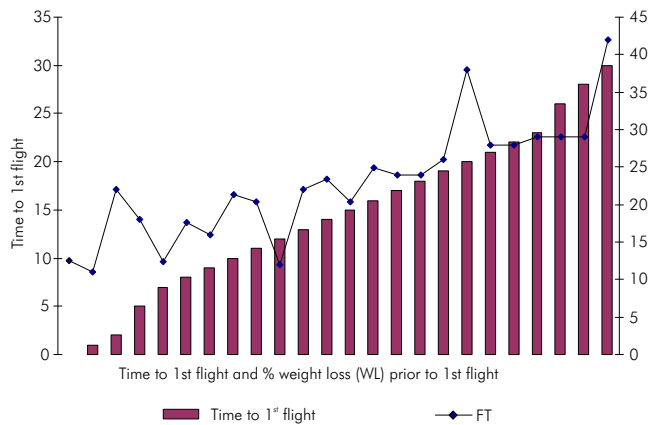


Figure 4 – Time to first flight (days) and flight time (days) in *Rhodnius brethesi* Matta, 1919 in the laboratory

Table 2 – Pearson linear correlation

	Time to 1 st flight and FT	Time to 1 st flight and % WL prior to 1 st flight	FT and initial weight	No. of flights and total weight loss
Pearson Coeff. (r)	0.8373	0.8802	0.4133	0.4775
P Value	<0.0001 Extremely sig.	<0.0001 Extremely sig.	0.0447 Slightly sig.	0.2280 NS

The Pearson coefficient (r) ranges from +1 to -1, and the closer the results are to these values, the stronger the association between the variables tends to be.

Individuals who took longer to initiate the first flight also flew more frequently. Moreover, these individuals also exhibited a higher percentage of weight loss. The time to first flight \times lifespan and time to first flight \times percentage weight loss to first flight variables were strongly associated.

Individuals who flew longer exhibited higher initial weight than other individuals, but the difference was not significant ($P = 0.4133$). Furthermore, total weight loss was higher in individuals who flew more frequently, but this difference was not statistically significant ($P = 0.2280$).

DISCUSSION

The small supply of hosts in its natural ecotope may have caused adaptations to the feeding behavior of *R. brethesi*, which has previously been observed feeding on piassaba collectors during the day. Rocha et al¹⁹ studied the life cycle of the species in the laboratory and found that the insects were extremely efficient in using blood meals, with only one meal needed for ecdysis to the fifth nymph stage. This swift and efficient feeding mode would enable the insects to feed during the day using humans as a host. In 1922, Matta²⁰ reported on the aggressiveness of *R. brethesi* for the first time; these insects sucked on the entire body of piassaba collectors, causing secondary inflammation in their skin.

The mean lifespans in this study (17.8 days for males and 22.3 days for females) were much lower than the lifespans observed by Mascarenhas^{21,22}, (51.5 days for males and 57.5 days for females) under similar conditions (26° C, 75% RH). Silva and Silva²³ also observed a longer lifespan (45.2 days for males and 38.9 days for females), although at a higher temperature (30° C, 70 \pm 5% HR). These results suggest that the short lifespan recorded in this study was caused by the nutritional restriction the individuals experienced and fatigue due to flight attempts.

When flight initiation and feeding were examined, some individuals were observed initiating flight on the same day as feeding. This result was unexpected, as several authors have associated flight initiation with low nutritional status and high temperatures^{15,24}. Moreover, the time to first flight \times lifespan and time to first flight \times percentage weight loss to first flight were strongly associated.

Of the 65 individuals studied, 12 (18%) did not fly under any conditions, thus indicating that intraspecific characteristics may also influence flight behavior in

triatomines, as reported by Soares and Santoro¹⁶. Several studies have shown that each species has a specific profile, and further characterization may help to identify species with greater active dispersal ability. Several authors have also associated the absence or reduction of flight muscles with the lack of flight initiation^{24,25}.

A greater number of flights by females was also observed in *Triatoma infestans* by Gurevitz et al²⁵, who found that the absence of flight muscles was 2.4 times more frequent in males than in females.

Rocha et al¹⁹ observed an average of seven blood meals during the adult stage of *R. brethesi*, which corresponds to approximately 50% of all flight attempts in the species. Weight loss measurements indicated that no individual initiated flight before losing at least 11.5% body weight. The average weight loss necessary to initiate flight was 34%. In this study, 81.53% of all *R. brethesi* individuals exhibited flight behavior.

Knowledge about triatomine species that actively disperse by flight is useful for entomological surveillance programs in the Amazon. During an international meeting on the implementation of the intergovernmental initiative on the surveillance and prevention of Chagas disease in the Amazon, particular transmission situations were reported (including the involvement of the people working on the extraction of piassaba fibers) during the sylvatic cycle of *T. cruzi* in its natural ecotope. Increasing deforestation rates; the construction of houses closer to the forest where many palm tree species can be found; the presence of vectors, domestic and sylvatic reservoirs; and night lighting of households, which attracts the insects, all increase the risk of human contact with triatomine species in the Amazon.

CONCLUSION

The flight activity of *R. brethesi* was similar in the laboratory and in nature, as discussed in other studies that described reports of piassaba collectors being bitten by the species in its natural environment^{5,6,7}.

According to the results of the experiments, *R. brethesi* usually disperses by flight between 11 and 15 days after the last blood meal, with a 34% weight loss from its initial weight occurring during this period. Moreover, *R. brethesi* required a minimum of 11% weight loss to initiate flight and meet its nutritional requirements. Increasing deforestation and the subsequent elimination of their usual food sources force the insects, which can be attracted by the presence of light in households close to deforested areas, to undergo a fasting period that leads them to disperse by flight after a week or two.



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RESUMO

A partir de observações realizadas em laboratório, foi avaliada a capacidade de voo de *Rhodnius brethesi*, correlacionando esta atividade a fatores como quantidade de sangue ingerida, gênero e período de jejum. Foram observados 27 machos e 38 fêmeas. O período médio de sobrevivência foi de 17,8 e 22,3 dias para machos e fêmeas, respectivamente. A maioria dos insetos necessitou em média de 13 dias para iniciar o voo.

Palavras-chave: *Rhodnius*; Doença de Chagas; Triatominae; Insetos Vetores; Comportamento Animal; Voo Animal.

Evaluación en laboratorio de la actividad de vuelo de *Rhodnius brethesi* Matta, 1919, potencial vector silvestre del *Trypanosoma cruzi* en la Amazonía Brasileña. (Hemiptera: Reduviidae: Triatominae)

RESUMEN

A partir de observaciones realizadas en laboratorio, fue evaluada la capacidad de vuelo de *Rhodnius brethesi*, correlacionando esta actividad a factores como cantidad de sangre ingerida, género y período de ayuno. Fueron observados 27 machos y 38 hembras. El período medio de supervivencia fue de 17,8 y 22,3 días para machos y hembras, respectivamente. La mayoría de los insectos necesitó un promedio de 13 días para iniciar el vuelo.

Palabras clave: *Rhodnius*; Enfermedad de Chagas; Triatominae; Insectos Vectores; Comportamiento Animal; Vuelo Animal.



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Received / Recebido em / Recibido en: 3/3/2011
Accepted / Aceito em / Aceito en: 28/6/11