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Risk Presented by *Copernicia prunifera* Palm Trees in the *Rhodnius nasutus* Distribution in a Chagas Disease-endemic Area of the Brazilian Northeast

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Abstract. With the aid of live-bait traps, we studied the risk that *Copernicia prunifera* palm trees, present in both periurban and rural localities of an endemic Brazilian northeast Chagas disease region, represent to domestic infestation by *Rhodnius nasutus*. In this area, this important vector has been encountered harboring and transmitting *Trypanosoma cruzi*, the etiologic agent of the American trypanosomiasis, to mammals, possibly including humans. Results indicate that this bug colonizes *C. prunifera* palm trees of both regions, mainly in dry seasons, and is infected with high levels of *T. cruzi*. Although more triatomines were captured in rural areas, proportionally the number of infected bugs from peri-urban regions was much higher. Herein we address the epidemiologic implications and challenge for the Brazilian health authorities to control the disease in this region, where the native palm trees have been largely destroyed causing a severe disturbance in the environmental equilibrium.

INTRODUCTION

Since the Brazilian scientist Carlos Chagas published the first paper on his discovery of American trypanosomiasis,[1] the importance of the Reduviid bugs of the subfamily Triatominae, the natural vectors of *Trypanosoma cruzi*, has been emphasized by many authors. Until recently, the impact of wild and semi-wild triatomine species was underestimated or even ignored. However, in the last few years, there has been an ever increasing interest in these triatomines, because some sylvatic species are frequently found inhabiting domiciles.[2–5] As a consequence of the anthropic activities and the reduction of the food source in the natural system, several triatomine species from wild populations have been forced to invade human dwellings, making the long-term control of American trypanosomiasis difficult. This problem has become increasingly evident since *Triatoma infestans*, formerly the main species responsible for *T. cruzi* transmission, has been controlled in Brazil as well as in other countries of the South Cone.[6,7] Nowadays, public health authorities have turned their attention to the wild triatomines because they are the key to our understanding of the process leading to the initial colonization of human habitations and recolonization after vector control interventions.[3,7,8]

Among the triatomines, several *Rhodnius* species are the most important vectors of *T. cruzi* in various South and Central Latin American countries. All of these species have been reported to breed in arboreal habitats, mainly in palm trees of different genera.[3,9–11]

Rhodnius nasutus Stall is a wild and semi-wild triatomine species, naturally colonizing the *Copernicia prunifera* palm tree (Carnauba),[12,13] among other palm trees.[14] Nevertheless, it is frequently found in peridomiciles of rural areas in the States of Ceará, Piauí, Rio Grande do Norte, Paraíba, and Maranhão situated in the Brazilian northeast.[12] This triatomine has likely been involved in the transmission of human Chagas disease in the State of Ceará, where it has been found to colonize peridomiciles of rural areas, together with *Triatoma brasiliensis* and *T. pseudomaculata*, which commonly harbor high levels of *T. cruzi*.[15] In this State, the *C. prunifera* palm tree is endemic and flourishes in all regions, although it has been intensively cut down and the soil substituted for tillage and herbage. To study the role of the *C. prunifera* palm tree as an ecotope of wild *R. nasutus*, an entomologic survey was carried out in rural and peri-urban localities of Jaguaruana county, Ceará, with the aim of increasing the knowledge of the ecology and dispersion of this species and of evaluating the risks that the *C. prunifera* palm represents for Chagas disease in this region.

MATERIALS AND METHODS

The field study was carried out in Jaguaruana (4°50′90″ S, 37°46′48″ W; 20 m mas; population, 30,965), a small county located in a hot, dry region in eastern Ceará State, ~180 km from the capital Fortaleza, in the Jaguaribe River Valley. The average annual temperature ranges from 23°C to 33°C, and rainfall is ~850 mm/yr. Generally, the rainy season extends from January to June and the dry season from July to December. Surroundings consist of loamy and sandy soil in the domain of Caatinga (scrublands), a characteristic vegetation of the semi-arid Brazilian northeast region. The sparse forests are situated ~1 km away from domiciles; nevertheless, it is common to find the *C. prunifera* palm tree growing very near the dwellings, mainly in the peri-urban areas. This palm tree is the most typical tree of the area, amply spread amid *Licania rigida* (Oiticica), *Ziziphus joazeiro* (Joazeiro), *Auxemma oncocalyx* (pau-branco), and *Boumelia sertarum* (quixabeira), among other natural trees of the Caatinga. However, there has been an accelerated reduction of this palm, as well as other native trees, because of continuous deforestation for construction of houses, fences, corrals, and for agricultural purposes. The study was conducted in four rural and six peri-urban areas of the county, peri-urban areas defined as transitional zones or surroundings where urban and rural activities are juxtaposed, and landscape features are subject to rapid modifications, induced by anthropogenic activity.

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The presence of triatomines in the randomly selected carnauba palm trees was studied with the aid of a very efficient live-bait adhesive trap appropriated to capture triatomines,[10] which consists of a small plastic bottle (12 cm length × 10 cm diameter) containing a 7-day-old chick. The experimental protocol was submitted to the Animal Research Ethics Committee of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (Protocol number P-0402/07). The traps were fixed to 30-cm-long forceps tied to the end of a 10-m-long shaft that was set in the palm tree crowns at 6:00 PM and removed at 8:00 AM. For each capture, 40 live-bait traps were used, and the trap effort was 120 trap-nights in rural areas and 80 trap-nights in peri-urban areas, for a total of 200 trap-nights. No palm trees were felled or damaged.

The study was performed in both the dry and rainy seasons. In each season, three captures were conducted from 2001 to 2004 in six different localities of rural areas and two captures from 2004 to 2005 in four different localities of peri-urban areas. Each studied site had an approximate area of 1 km^2.

The captured triatomines were stored in labeled plastic containers and forwarded to the laboratory for identification of triatomine species, developmental stages, examination for *T. cruzi* infection, and assessment of nourishment. Parasitic protozoa in the insect feces were detected directly with a conventional light microscope. The bloodmeal type in the gut contents of nymph and adult triatomines was identified by the indirect enzyme-linked immunosorbent assay (ELISA).[16,17] We used the following antisera: armadillo (*Dasypus novencinctus*), chicken (*Gallus gallus*), cat (*Felis domestica*), cockroach (*Periplaneta americana*), cow (*Bos taurus*), dog (*Canis familiaris*), opossum (*Didelphis marsupialis*), horse (*Eqqus caballus*), human, lizard (*Tupinambis meriane*), rodent (*Rattus norvegicus*), goat (*Capra hyrcus*), and sheep (*Ovis aries*). Results are presented as a total number of positive meals with respect to each host (i.e., a triatomine with positive reactions to the antisera of two or more hosts was counted as having consumed host blood from two or more sources).

Statistical analyses were performed using the Epi Info program, and results are expressed as descriptive statistics. The proportion for different groups was compared by χ^2 test, and different means were compared by Student t test. Statistical significance was accepted at $P \leq 0.05$.

RESULTS

Table 1 shows all data related to the studied palm trees present in both rural and peri-urban study areas for both the dry and rainy seasons. A total of 1,134 *C. prunifera* palm trees were studied, with 70.1% from the rural areas and 29.9% from the peri-urban areas. In all study localities, in rural as much as in peri-urban regions, palm trees were found to be infested by *R. nasutus*, the unique triatomine species captured. The remains of birds (e.g., nests or feathers) were frequently found in the palm trees where the triatomines were captured. Several species of insects proved to be available as a food source; however, no opossum or rodent was seen in the palms. In peri-urban areas, the number of infested palms from the dry seasons was significant compared with the number of infested palms from the rainy seasons ($P < 0.001$). For palm trees in rural areas, there was no observable difference ($P > 0.05$).

Correlating the total amount of studied palm trees with the total number of captured *R. nasutus*, in the two study regions of Jaguaruana, in dry and rainy seasons, there was an average of 0.73 triatomines per palm; considering only the infested palm trees, the average was 2.79 triatomine per palm, in all stages of development, including both adult males and females.

During the dry seasons in the peri-urban areas, we found an average of 3.9 triatomines per palm versus 1.8 in the rainy seasons. In rural areas, the correlation was 3.1 triatomines per palm versus 1.7 triatomines per palm in dry and rainy seasons, respectively. These results show that there was no difference in the density of triatomines in the palm trees with respect to the rural or peri-urban areas. However, in both rural and peri-urban regions, most of the triatomine captures were strongly correlated with the dry seasons versus the rainy seasons (64.3% and 35.7%, respectively). Proportionally, the insects were captured in higher numbers in rural areas than in peri-urban areas (66.8% and 33.2%, respectively). Nevertheless, the density of specimens per infested palm tree was 3.2 in peri-urban areas and 2.6 in rural areas. In all seasons, independent of rural or peri-urban region, the number of adults captured was much lower than that of nymphal instars (Table 2).

To study the natural *T. cruzi* infection rates, only 403 specimens of *R. nasutus* were examined of the 829 captured: many had starved to death or arrived completely dehydrated in the laboratory, making dissections or the acquisition of feces impossible. Among the examined triatomines, 196 (71.3%) were from peri-urban areas and 207 (42.5%) were from rural areas. Twenty-five percent of individuals from the dry seasons and 17.9% from the rainy seasons were infected (Table 3). Results show that, although 70.5% of the triatomines were captured

TABLE 1

Number of studied palm trees (*C. prunifera*) and the percentage of infestation found in different localities, four of which were peri-urban and six of which were rural areas of Jaguaruana/Ceará, Brazil, during both the dry and the rainy seasons from 2001 to 2005

	Peri-urban areas		Rural areas	
Seasons	Palm trees investigated/ infested (%)	Palm stem height (mean ± SD)	Palm trees investigated/ infested (%)	Palm stem height (mean ± SD)
Dry	160/58 (36.3)*		510/133 (26.1)†	
Rainy	160/28 (17.5)	6.0 ± 1.2	304/78 (25.7)	5.7 ± 0.9

* Significant.
† Not significant.

TABLE 2

Developmental stage of *R. nasutus* found in *C. prunifera* palm trees situated in rural and peri-urban areas of Jaguaruana, Ceará, Brazil

Triatomines	Rural areas (season dry/rainy *N*)	Peri-urban areas (season dry/rainy *N*)	Total (season dry/rainy *N*)
Nymphs			
First instar	60/13	77/2	137/15
Second instar	87/18	24/6	111/24
Third instar	92/36	17/10	109/46
Fourth instar	89/37	38/11	142/38
Fifth instar	71/27	53/19	124/46
Adults			
Male	16/2	9/1	25/3
Female	3/3	6/2	9/5
	418/136	224/51	
Total	554	275	642/187

TABLE 3
Rhodnius nasutus captured and investigated for infection with *T. cruzi* in *Copernicia prunifera* palm trees found in rural and peri-urban areas of Jaguaruana, Ceará, Brazil

	Rural areas		Peri-urban areas	
Seasons	Captured [*N* (%)]	Inquired/infected [*N* (%)]	Captured [*N* (%)]	Inquired/infected [*N* (%)]
Dry	418 (85.8)	134/23 (17.2)	224 (81.5)	151/48 (31.8)*
Rainy	136 (27.9)	73/12 (16.4)	51 (18.5)	45/11 (24.4)†
Total	554	207/35 (16.9)	275	196/59 (30.1)

* Significant.
† Not significant.

in rural areas versus only 29.5% from peri-urban areas, the proportion of infected triatomines captured in peri-urban areas was much higher than those captured in rural areas ($P < 0.001$).

Table 4 presents the results of bloodmeal assays. A total of 61 (12.5%) triatomines from rural areas and 143 (52%) from peri-urban areas were analyzed. We found a low number of positive reactions for all hosts tested, and no differences were detected between the rainy and dry periods versus detectable differences between the rural and the peri-urban areas. Hemolymph was the most frequent source of food in both rural and peri-urban areas, followed by opossums and rodents. Fifty-five percent of the triatomines from rural areas and 66.4% from peri-urban areas were negative for one or more of the hosts tested.

DISCUSSION

As has been stated by several epidemiologists, information on the distribution and behavior of autochthon triatomines is essential in the control of Chagas disease vectors because of possible reinvasion of dwellings by the insects flying from their natural habitats a few months after insecticide spraying. The Brazilian northeast is one of the most important regions in our country for vector control, where triatomine control demands strong efforts to inhibit human transmission, especially in the rural areas.[7,18] In this study, we investigated the presence of triatomines in carnauba palm trees in six rural and four peri-urban areas of the Jaguaruana municipality. In at least two of the peri-urban areas, researchers could not distinguish structural differences between rural and peri-urban regions, although the peri-urban dwellings were slightly less primitive than the rural ones, containing a higher prevalence of mud walls with plaster.

TABLE 4
Type of blood* in *R. nasutus* captured in the palm trees *C. prunifera* from rural and peri-urban areas

Type of blood	Rural areas (%)	Peri-urban areas (%)
Reactive	45.0	33.6
Bird	7.1	12.5
Opossum	17.9	16.7
Haemolymph	85.7	31.3
Rodent	10.7	16.7
Multiple feed		
Bird/rodent/hemolymph	1.6	—
Opossums/hemolymph	4.9	1.4
Bird/rodent	1.6	0.7
Rodent/hemolymph	1.6	—

* Identified by the indirect ELISA.

In both areas, the endemic carnauba palm tree was largely distributed both far and near dwellings, although the trees in rural areas did tend to be a bit more preserved, forming denser groups than in the peri-urban regions. Many domestic animals such as chickens, goats, sheep, pigs, dogs, and cattle usually circulate freely in the peridomiciles of both rural and peri-urban localities. The presence of opossums, rats, and other synanthropic mammals was reported to be frequent, according to inhabitants of both regions. Indeed, in surveys carried out in these areas, we captured several of these small mammals, most of them carrying *T. cruzi* I (unpublished data).

The studied palm trees, even those from the rural areas, were located in anthropic landscapes. It was not very common to find organic material in the trees, perhaps because of the architecture of this palm tree species, besides the dry and windy climate, which is prevalent throughout a major part of the year. In the rainy season, only few epiphyte plants appeared growing around the trunks. We found no observable correlation between these characteristics and the prevalence of infestation, such as has been reported by other researchers.[3,11] However, triatomines showed a preference for palms with bird nests, feathers frequently being present in the palm trees that contained *R. nasutus.*

Similar to observations related to *T. brasiliensis* and *T. pseudomaculata* in northeastern Brazil,[19] *R. nasutus* was captured in high numbers during the dry season, primarily in a fasting state. Several authors have observed that starved triatomine bugs are more likely to be attracted by live bait, suggesting that the nutritional status of sylvatic bugs is generally very poor.[10,20] In fact, the study of the type of blood ingested by *R. nasutus* in their natural habitat showed that many specimens had fed on hemolymph. Indeed, during our inquiry, we verified that other species of insects co-inhabited with the bugs, because several larvae and adult insects similar to the cockroach were captured in the live bait traps either with or without the bugs. Ants, arachnids, stick insects (an insect of the order Phasmatodea—*Phibalosoma phyllinum*), and other Hemiptera were also frequently seen in the palm trees or close to them. We could not find any studies describing the development of *R. nasutus* exclusively feeding on other insects as it has been observed for *Triatoma circummaculata* and *Triatoma rubrovaria.* These triatomines have been shown to complete their life cycle feeding on cockroach hemolymph.[21] A colony of *Belminus herreri* collected from human dwellings in Colombia also exhibited a strong propensity toward hemolymphagy.[22]

According to Gaunt and Miles,[23] nymphs of the triatomine *Eratyrus mucronatus* can feed on the hemolymph of large arachnid when they live in the same hollow trees. After citing several examples of feeding behavior of triatomine, these authors[23] suggested that the adaptation of these insects to specialist niches is commonplace, not exceptional. Other authors have stated that hematophagy is derived from the predatory of behavior in other Reduviidae, nevertheless, whereas the assassin bugs prey on arthropods by pre-digesting their tissues to kill their prey, the Triatominae seem only to immobilize their prey and suck their fluids in the process of hemolymphagy.[4]

In our study area as well as in all regions of The Jaguaribe River Valley, *R. nasutus* is the third most frequent vector of *T. cruzi* after *T. brasiliensis* and *T. pseudomaculata*, respectively.

This vector is autochthonous and primarily associated with *C. prunifera* palm trees, which is its natural habitat.[12,24] In this region, there are usually large colonies of *T. brasiliensis* and *T. pseudomaculata* in peridomiciles, these species easily being able to develop intradomicile colonies. *R. nasutus* is frequently encountered colonizing hen houses or other artificial peridomicile ecotopes. However, because of the proximity of the palm trees and the man-made ecotopes in the dwellings, winged specimens, probably attracted by light, have become increasingly common inside houses.[15] *Rhodnius* genus triatomine has a history of evolution in Latin American palm tree habitats.[23] We agree, especially as several investigators have reported a relationship between this genus and several palm tree species. Notwithstanding, our previous studies in the Jaguaruana areas led us to believe that the *R. nasutus* behavior indicates that this species is undergoing a rapid process of adaptation to the peridomiciles as well as to other types of sylvatic ecotopes, perhaps as a consequence of ever increasing environmental alterations taking place in the Jaguaribe Valley region. Actually, in one of the peri-urban areas studied in this paper, we have encountered this species colonizing the *L. rigida* (Oiticica) tree, a large leafy tree of the Chrysobalanaceae family, native to and extremely common in Ceará State.[25] Possibly this could be the result of the *R. nasutus* adults being captured in much lower numbers than nymphs in the palm trees in our study areas, mainly in the wet season when the scarcity of birds and mammals may contribute to the dispersion of this hematophagous. In this region, the predominant landscape is the "caatinga," characterized by the presence of the carnauba palm trees (*C. prunifera*), the main sylvatic ecotope of *R. nasutus*; this tree, together with other typical trees of the Caatinga, has been largely destroyed. Disturbance in the environmental equilibrium forces wild triatomines to seek other habitats,[4,26] a fact frequently seen in our study localities where *R. nasutus* has been captured in man made structures of the peridomiciles, carrying high infection levels of *T. cruzi*.[13–15] Therefore, the risk of *R. nasutus* to invade and colonize houses in localities with denser population concentration cannot be ignored. Furthermore, considering the biological behavior, extensive infestation area and high degree of peridomiciliar presence in the study areas, we believe that hereafter *R. nasutus* may be responsible for sporadic outbreaks of acute Chagas disease transmitted orally in the Brazilian Northeast, remembering that oral transmission predominates where the vectors have sylvatic habits.

Originally, American trypanosomiasis used to be exclusively an enzootia, becoming an anthropozoonosis on man's invasion of wild ecotopes. Currently, it is a zoonosis affecting ~15–16 million people in South America, with 75–90 million people being exposed to infection.[27] It is a neglected illness related to poverty, involving excluded social groups, conditions present in rural and peri-urban areas of the Brazilian northeast. Campaigns against the disease are mainly based on vector control, because drug treatments or vaccines as of yet are not accessible. In our country as well as in other Latin American countries where *T. infestans* was introduced, the greatest achievement has been the progressive control of this species, which is the triatomine most commonly associated with human habitations and is the principal vector for Chagas infection throughout the Southern Cone countries. Insects with wild habitats cannot readily be controlled and tend to reinvade peridomiciles previously sprayed with chemical insecticides. Therefore, the control of native triatomine species such as *T. brasiliensis, Panstrongylus megistus, Triatoma sordida, T. pseudomaculata,* and *R. nasutus*, which prevail in different ecosystems, offer a greater operational challenge to health authorities,[5] because from their natural ecotope they can invade houses, initiating a new cycle of *T. cruzi* infection.[11] In ecologic and epidemiologic terms, our observations suggest the importance of defining rational surveillance control interventions in this area where sylvatic *R. nasutus* populations are possibly involved in the *T. cruzi* transmission to mammals,[25] possibly including humans, indicating that strategies of surveillance must be intensified to prevent its adaptation to the domiciles.

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