

## COLONIZATION OF PALM TREES BY *Rhodnius neglectus* AND HOUSEHOLD AND INVASION IN AN URBAN AREA, ARAÇATUBA, SÃO PAULO STATE, BRAZIL

Vera Lúcia Cortiço Corrêa RODRIGUES(1), Clovis PAULIQUEVIS JUNIOR(2)\*, Rubens Antonio da SILVA(3), Dalva Marli Valério WANDERLEY(4), Marlucci Monteiro GUIRARDO(2), Lilian Aparecida Colebrusco RODAS(2), Claudio CASANOVA(1), Marcio L. PACHIONI(2), Wilson A. SOUZA(2), Abílio Jose Batista COSTA(2), Delvo BAITELO(1) & Vera Lúcia Braga TONIETTI(1)

### SUMMARY

The objective of this study is to report on the colonization of palm trees by *Rhodnius neglectus*, its invasion in an urban area, in Araçatuba - São Paulo, and the control and surveillance measures that have been put in place. Domiciliary triatomine searches occurred in apartments upon the inhabitants' notification. The collected insects were identified and examined for natural infection and food sources with a precipitin test. To search the palm trees, tarps were used to cover the floor, and a "Munck" truck equipped with a tree-pruning device was utilized. Chemical control was performed with the utilization of a manual compression. In 2009, 81 specimens of *Rhodnius neglectus* were collected from the domiciles by the population. The precipitin test revealed a presence of human blood in 2.7% of the samples. Entomological studies were carried out in these domiciles and in those located within a radius of 200 meters. The search performed in the palm trees resulted in the capture of 882 specimens of triatomines, negative for tripanosomatids. Mechanical and chemical controls were carried out. New searches conducted in the palm trees in the same year resulted in the capture of six specimens. The mechanical and chemical controls of the palm trees, together with the population's work, proved to be effective, therefore preventing these insects' colonization of the city's domiciles.

**KEYWORDS:** *Rhodnius neglectus*; Urban area; Vector control; Entomological surveillance.

### INTRODUCTION

*Rhodnius neglectus* is a triatomine species that is present in 12 Brazilian States (Maranhão, Paraíba, Piauí, Pernambuco, Tocantins, Goiás, Bahia, Minas Gerais, Mato Grosso, Mato Grosso do Sul, São Paulo and Paraná) and in the Federal District, in latitudes between 2° e 3° N and 25° S, from sea level to altitudes of approximately 1,000 meters<sup>21,23,27,30,46</sup>.

According to BARRETO *et al.*<sup>3</sup>, the discovery of nymphs of *R. neglectus* in artificial biotopes in the State of São Paulo was first mentioned by CORRÊA & LIMA<sup>8</sup>, who reported on the capture of 414 adult specimens and nymphs in dovecotes in the municipality of Tapiratiba. Populations of this species frequently inhabit palm trees of the genera *Attalea*, *Acrocomia*, *Oenocarpus*, *Syagrus* and *Mauritia*, and they can be found in nests of birds and mammals, like the opossums of the genus *Didelphis*<sup>1,7,19</sup>. The first demonstration that palm trees are natural foci of American trypanosomiasis was made by FREITAS *et al.*<sup>14</sup>, who showed that the crowns of macaubeiras (genus *Acrocomia*) are refuges for opossums and bats, and they shelter triatomines that feed on these mammals. Subsequently, other authors<sup>3,5,6,39</sup> highlighted the importance of palm trees as natural ecotopes of triatomines, mainly of species of the genus *Rhodnius*, and they can function as ecological indicators of risk

areas for Chagas disease, due to the presence of these vectors and hosts of the *Trypanosoma cruzi* and *Trypanosoma rangeli* transmission cycle<sup>24</sup>.

The *R. neglectus* biological cycle is completed in a short period of time, when compared to other triatomine species, due to its shorter duration and the absence of differences between the periods of permanence in several stages, and the adult triatomines who are able to abandon their ecotopes more quickly<sup>13</sup>. Under laboratory conditions, this species is more active and aggressive, moving quickly and abruptly when compared to other species of triatomines<sup>27</sup>. Concerning the age structure of *R. neglectus* populations observed in palm trees of the Federal District in wild ecotopes, no difference was observed between the dry and wet seasons, which indicates an absence of marked seasonality<sup>18</sup>.

*R. neglectus* has already been found naturally infected by *T. cruzi* with different indexes of natural infection, probably due to different circumstances and contacts with infectious sources<sup>9,10,34</sup>. The species is capable of inhabiting ecotopes in open areas, that is, those which resulted from man's interference in the natural environment<sup>11</sup>. Although it is a sylvatic triatomine whose habitat is preferably on palm trees, it may invade human habitations and the peridomiciles and form colonies in them<sup>3,20,22,42,45</sup>. The invasion of residences and their peridomiciles

(1) Secretaria de Estado da Saúde de São Paulo, Superintendência de Controle de Endemias, Laboratório de Chagas de Mogi Guaçu. Mogi Guaçu, SP, Brazil. E-mails: veracorrea@dglnet.com.br, casanovaclauss@gmail.com, sr05.guassu@sucen.sp.gov.br, verabragatonietti@yahoo.com.br

(2) Secretaria de Estado da Saúde de São Paulo, Superintendência de Controle de Endemias, Serviço Regional de Araçatuba. Araçatuba, SP, Brazil. E-mails: mmguirardo@gmail.com, lilianrodas@sucen.sp.gov.br, mlpachioni@yahoo.com.br, aswilson@ig.com.br, setorabilio@yahoo.com.br

(3) Secretaria de Estado da Saúde de São Paulo, Superintendência de Controle de Endemias, Departamento de Combate a Vetores. E-mails: rubensantoniosilva@gmail.com

(4) Secretaria de Estado da Saúde de São Paulo, Superintendência de Controle de Endemias. São Paulo, SP, Brazil. E-mail: dalva.wanderley@gmail.com

\*in memoria

**Correspondence to:** Vera Lúcia Cortiço Corrêa Rodrigues, Superintendência de Controle de Endemias, Laboratório de Chagas de Mogi Guaçu, R. Afonso Pessini 86, Bairro Pedregulhal, 13845-206 Mogi Guaçu/SP, Brasil. Tel/Fax: +55.19.3861 1233. E-mail: veracorrea@dglnet.com.br

by this species has already been observed in the Brazilian States of Tocantins, Goiás, Piauí, Pernambuco, Bahia, Minas Gerais, São Paulo and Paraná<sup>2,20,22,29,42</sup>, generally accompanied by low indexes of infection by *T. cruzi*. In São Paulo State, in the period 2007-2009 1059 specimens of *R. neglectus* were collected with infection rates by *T. cruzi* of 0.1%, therefore being the third species in copies to be captured<sup>38,43,44</sup>.

The entomological surveillance strategy that has been in force in São Paulo includes the population's participation in the notification of suspected triatomines insects to the Superintendência de Controle de Endemias (SUCEN - Superintendence of Endemics Control) - an agency of the Health Department of the State of São Paulo. From 2004 onwards, through spontaneous notifications constantly performed by the population, the presence of triatomines of the species *R. neglectus* has been observed in domiciles located in the urban area of the municipality of Araçatuba - São Paulo<sup>36,38</sup>. This study aims to report the presence of *Rhodnius neglectus* in buildings and houses, and the colonization of palm trees located in the urban area, as well as to evaluate its control in these environments.

## MATERIAL AND METHODS

**The municipality:** Araçatuba is located in the Northwestern region of the State of São Paulo (SP), latitude 21° 12' 32", longitude 50° 25' 58" (Fig. 1). It occupies an area of 1170.5 km<sup>2</sup> with an altitude of 390 meters. Its climate is semi-humid tropical, with an average temperature of 27 °C<sup>49</sup>. Belonging to the northern urban center, the city presents isolated buildings, affordable housing, industrial areas and housing nobles. The region is especially known for having historically turned to cattle ranching, which resulted in a sparse occupation of the territory. The city was influenced in its growth due to its land relief and by the early 1960s went through a process of vertical integration with the construction of apartment buildings spread along the main avenues, lined with different plant specimens. The population density which was initially 20 people per hectare in the central region became 60 hab./ha in the 1990s<sup>4,15</sup>. Most recently, cattle ranching was replaced by sugar cane plantations and a consequence of this was deforestation.

### Triatomines search:

**In the domiciles:** A Triatomines search in the domiciles of the urban area of Araçatuba was carried out in the apartments, in 2009, upon the



Fig. 1 - Location of the municipality of Araçatuba. State of São Paulo, Brazil.

inhabitants' notification, according to the technical norm of the Chagas Program that has been in force in the State of São Paulo<sup>41</sup>. After the verification of the presence of the insect vector in the household that triggered the notification, an educational initiative was launched in the neighboring households. The insects collected by the inhabitants were sent to SUCEN, Regional Service of Araçatuba and, subsequently, to the Chagas Disease Laboratory of SUCEN, in the city of Mogi Guaçu - SP, for identification, examination of the intestinal contents through abdominal compression to detect natural infection by trypanosomatids<sup>33</sup>, and performance of the precipitin test, using the technique proposed by SIQUEIRA<sup>47</sup>, with human, canine, feline, rodent, marsupial and avian antisera, to verify the food source.

**On the palm trees:** The urban area palm trees, located within a radius of 200 meters from the households where the notifications came from, were searched by SUCEN technicians, Regional Service of Araçatuba, and by technicians from the municipality of Araçatuba and from the Chagas Laboratory of the SUCEN of Mogi Guaçu. During the search of the palm trees, tarpaulins were used to cover the floor, and a "Munck truck" equipped with a tree-pruning device was also used, lent by the municipal government of Araçatuba, to remove dry leaves and bunches. The materials removed from the palm trees were thoroughly examined. The collected insects were put into bottles labeled with information on the name of the street and the number of the palm tree, and these data were included in sketches. The insects collected on the palm trees were submitted to the same laboratory procedure of those sent by the population. The traffic had to be blocked on the streets to search the palm trees, and this was the responsibility of the Municipality's Traffic Department.

The searches conducted in June 2009 and revised in September of the same year included 100 palm trees, located on Street A, Square A and Street B. In September 2010, new entomological searches were conducted on the same palm trees.

**Mechanical and chemical control of the palm trees:** Mechanical control consisted of cleaning the palm trees, that is, removing dry leaves, bunches and animals' nests. Chemical control was performed with the utilization of the manual compression T-jet 8002, insecticide Alphacypermethrin in a dosage of 40 mL of the commercial product and 60 mL of adhesive spreader Polyoxyethylene alkylphenol ether in eight liters of water<sup>35</sup>. In the work carried out in June 2009, all palm trees were sprayed independently of being infested, based on the supposition that nymphs at the early stages and/or eggs might not be noticed in the mechanical search. In September 2009, in the revision of the initial control evaluation, only the infested palms were sprayed.

In September 2010, after the entomological search was performed on the palm trees, regardless of the presence of the triatomine, the chemical control was not carried out, only the mechanical control.

All the materials collected in all the searches (nests, leaves and bunches) were burned at a place destined for garbage disposal by the municipality.

**Entomological indicators:** The following indicators were calculated: - palm tree infestation = number of infested palm trees/total number of searched palm trees x 100; - triatomine density on the palm trees = number of triatomines collected on the palm trees/total number of

searched palm trees; - infection index for triatomines = number of positive triatomines for *T. cruzi*/number of captured triatomines; - average number of triatomines per positive palm tree = number of collected triatomines/number of positive palm trees; - colonization = number of palm trees infested by nymphs/number of palm trees sampled.

**Community mobilization:** In conjunction with the search for triatomines, an educational initiative was launched in the urban area, with the distribution of informative leaflets, providing the inhabitants with information on Chagas disease, its vectors, prophylaxis and the importance of the population's participation in the notification of suspected triatomine insects, as well as the correct way of collecting and sending them to the municipality's health care unit.

The research protocol was submitted to and approved by the *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA - Brazilian Institute of the Environment and Renewable Natural Resources) and by SUCEN.

## RESULTS

The 56 notifications of triatomines sent by the inhabitants of the city of Araçatuba in 2009 totaled 81 specimens of *R. neglectus*: 70 adults (42 females and 28 males) and 11 nymphs (ten 1<sup>st</sup> stage nymphs and one 5<sup>th</sup> stage nymph). These notifications tested positive in four domiciles, and 11 triatomine specimens of the same species were collected (five male adults and six female adults), located in three peridomiciles (palm trees) and on one balcony of an apartment located on the 8<sup>th</sup> floor. The precipitin test showed, among insects examined, which 59.5% had drunk the blood of birds, 2.7% human blood, 2.7% rodents and 35.1% no reagents. The result of the examination of the intestinal content of these triatomines was negative for flagellate forms of trypanosomatids.

The 100 palm trees that were searched belonged to the genera

*Roystonea* (93), *Syagrus* (6) and *Acrocomia* (1) and had an average height of 12 meters. At the first stage of the search (June 2009), 63 palm trees were infested by *R. neglectus* (63.0%). A total of 882 specimens was collected: 246 adults (27.9%) and 636 nymphs (72.1%) (Table 1). Triatomine density on the palm trees was 8.82. The average number of triatomine specimens per positive palm tree was 14.2. On these palm trees, there were bird nests, mainly *Pionus maximiliani*, and in one of them, bats were found. The precipitin test indicated that 82.1% of the triatomines fed on bird's blood; 10.9%, marsupial; 2.0%, rodent; and 5.0% were not reactive to the tested antisera. The examined specimens were negative for flagellate forms of trypanosomatids.

In the control revision of the 100 searched palm trees (September 2009), live triatomine specimens were found on two palm trees (4.0%) located on Street A (two 4<sup>th</sup> stage nymphs and one 5<sup>th</sup> stage nymph, as well as three adult specimens), representing a palm tree infestation of 2.0%. Triatomine density on the palm trees was of 0.06. The average number of triatomine specimens per palm tree was 3.0. The infested palm trees presented a large quantity of bird nests.

In September 2010, the palm trees were assessed again. It was observed that, of the 100 palm trees, 15 were infested with *R. neglectus*, which represents a palm tree infestation of 15.0%. A total of 77 specimens of *R. neglectus* was collected: 10 adults (seven males and three females) and 67 nymphs (thirteen 5<sup>th</sup> stage nymphs, twelve 4<sup>th</sup> stage nymphs, twenty 3<sup>rd</sup> stage nymphs, eleven 2<sup>nd</sup> stage nymphs, and eleven 1<sup>st</sup> stage nymphs). The triatomine density on the palm trees was 0.77. The average number of triatomine specimens per palm tree was 5.1. All the triatomines were examined to detect natural infection by trypanosomatids, with negative results. The test to verify the food source indicated bird's blood. It is important to emphasize that the positive palm trees had already been observed as being infested in the research that was carried out in June 2009.

**Table 1**

Results of the searches in palm trees and number of triatomines collected according to the evolutionary stage in three moments of the triatomine search. Araçatuba, June 2009 to September 2010

Period	Venue	No. palms sampled	Palms infested (%)	Palms colonized (%)	Nymphs (%)	Adults (%)	No. bugs/infested palms
June 2009	Street A	50	66.0	64.0	69.6	30.4	18.6
	Square A	28	50.0	46.4	83.0	17.0	7.1
	Street B	22	72.7	72.7	74.5	25.5	11.3
	Total	100	63.0	61.0	72.1	27.9	14.2
September 2009 (revision)	Street A	50	4.0	4.0	50.0	50.0	3.0
	Square A	28	0.0	0.0	0.0	0.0	0.0
	Street B	22	0.0	0.0	0.0	0.0	0.0
	Total	100	2.0	2.0	50.0	50.0	3.0
September 2010 (evaluation)	Street A	50	14.0	14.0	85.0	15.0	8.6
	Square A	28	3.6	3.6	100.0	0.0	2.0
	Street B	22	31.8	31.8	93.3	6.7	2.1
	Total	100	15.0	15.0	87.0	13.0	5.1

## DISCUSSION

This study had some difficulties and limitations, such as: the height of the palm trees, which meant a special “Munck” truck was required for research and control; the traffic prohibition on the streets where the study was being conducted; and the period during which the study was conducted - June to September. This was indicated by the IBAMA as a non-breeding period of the birds that live in these places, which had an effect on the mechanical control actions, the removal of nests and dry material from the palm trees, and on the chemical control actions.

The invasion of *R. neglectus* in vertical buildings (from the 2<sup>nd</sup> to the 12<sup>th</sup> floor) in the urban zone of the city of Araçatuba is an unprecedented fact in the State of São Paulo. BARRETO *et al.*<sup>3</sup> reported that finding adult forms in human habitations is sporadic, even though they occur relatively frequently in areas where the species is present in wild biotopes, represented mainly by palm trees. This fact led the authors to research nests of *R. neglectus* in artificial ecotopes near the areas where triatomines had been found in wild environments. This resulted in the discovery of 699 specimens; of these, 12 adults and 25 nymphs were in human habitations. In the study conducted by these authors<sup>3</sup>, all the houses where the insects were found were wattle and daub houses covered with thatch grass or palm leaves. SILVEIRA *et al.*<sup>45</sup> observed the colonization of houses by *R. neglectus* in routine operations in the Program for Chagas Disease Control in the State of Goiás. Also in Goiás, in a study about geographical distribution, a large amount of *R. neglectus* was collected in the domiciliary environment<sup>29</sup>. The triatomine colonization in artificial ecotopes has been demonstrated by several authors<sup>16,25,31,40,48</sup>.

In answering the notifications in the State of São Paulo<sup>41</sup>, it was observed that the species was captured in the peridomicile and that a higher number of specimens was captured in the intradomicile, a fact that points to this species' great capacity for dispersal and mobility, and shows that the domiciliary environment is subject to invasions from colonies located near the habitations.

The presence of this species in an urban area meant that the municipality of Araçatuba, reflected on the alterations to the environment caused by monocultures and by the clearing of forest areas. The disturbance of natural ecotopes, caused by man, or even their complete destruction, events that occur in economic development projects, may cause the dispersal of the species in search of new habitats, like, for example, the nearest domicile, thus influencing the ecoepidemiology of the American trypanosomiasis. This makes us suspect that animals, like the birds *P. maximiliani*<sup>37</sup>, which have started to be present in the urban area of the city, could possibly transport eggs that remain adhered among their feathers, and maybe even *R. neglectus* nymphs, contributing to the species dispersal and reinfestation that has also been observed by other authors<sup>11,12,17,32</sup>. Another factor is the reduction in the sylvatic fauna, a result of deforestation, which forces the triatomines that are called sylvatic to look for new food sources<sup>28</sup>. Therefore, these triatomines can bring *T. cruzi* from the wild environment to the urban zone through passive transportation, and this should be considered a transmission risk for man and domestic animals. This event is what HOARE<sup>26</sup> calls “potential epidemiological threat”. It is noteworthy that in this study, the food source of human blood was observed in triatomines notified by residents. In vectors collected in entomological research by field teams, this was not observed, verifying the high percentage of bird blood as a

source food, which somehow minimizes the risk posed by this species in this environment.

As for the palm trees, in the municipality of Araçatuba, they are located approximately 20 meters away from the invaded buildings and practically at the same height as the apartments; thus, the triatomines were attracted by the apartment's light. In these ecotopes, represented by palm crowns, *R. neglectus* has found a food source, as these biotopes offer a good shelter for vertebrates, especially birds. As this triatomine has a cycle with a shorter duration time, it is feasible to admit that the adults, which have great capacity for dispersal, abandon these habitats more quickly and invade others, in this case, artificial ones, as they are mainly attracted by the light during the night period. Taking into account that the extradomiciliary triatomine populations have greater capacity for active dispersion, it is understandable that the females are granted a larger part of this role<sup>13</sup> - in this study, this was observed by the higher number of females captured in the apartments, as a result of the notifications made by the inhabitants.

In this study, the palm trees of the genus *Roystonea* were considered indicators of the presence of *R. neglectus* colonies. Handling the palm trees' dry parts by spraying and then burning this material in an adequate place was one of the steps to control the species in this ecotope. The search in the palm crowns, with the removal of nests followed by spraying leaves and trunks with chemical insecticide complemented the chemical control, and it is a good practice for similar situations.

Aiming to avoid triatomine colonization in the domiciliary environment, one of the surveillance strategies is the incentive for the population to send notification of suspicious insects by sending them to the competent sectors. In addition, a permanent work so that the inhabitant is responsible for the sanitary conditions of his/her habitation has been developed, aiming to avoid the man-vector contact and to prevent the permanence of insects in the domicile.

The notification of insects by the population triggered the control actions. The study did not identify risks for humans due to the presence of these insects in this episode. However, the study should be presented to the local population, so that the individuals can be guided to participate effectively in the surveillance of their domiciles. This should be applied mainly to entomological surveillance and to the provision of preventive and control measures in the Chagas Disease Program, aiming to monitor the infestation of other areas. In a process of decentralization of the control actions, the municipal government should be responsible for cleaning and pruning the palm trees, and for developing educational and guidance actions to the population, aiming at the adequate surveillance of the domiciles; the state government should be in charge of technical guidance, professionals' qualification and, when insects are found, it should be responsible for their identification and for helping in their control.

## RESUMO

### Colonização de palmeiras por *Rhodnius neglectus* e invasão domiciliar em área urbana, Araçatuba, São Paulo, Brasil

O objetivo deste trabalho é relatar a colonização de palmeiras por *Rhodnius neglectus*, sua invasão em área urbana, na cidade de Araçatuba -

São Paulo, o controle e as medidas de vigilância instituídas. As pesquisas de triatomíneos nos domicílios ocorreram nos apartamentos quando da notificação pelo morador. Os insetos coletados foram identificados, examinados quanto à infecção natural e teste de precipitina. Na pesquisa das palmeiras foram utilizados lonas para forração do chão e caminhão Munck equipado com moto poda. O controle químico foi realizado com a utilização de compressor manual. No ano de 2009, foram coletados pela população 81 exemplares de *Rhodnius neglectus* em domicílios. O teste de precipitina acusou presença de sangue humano em 2,7% das amostras. Pesquisas entomológicas foram realizadas nestas edificações e naquelas situadas em um raio de 200 metros onde ocorreram as invasões. A pesquisa realizada nas palmeiras resultou na captura de 882 exemplares de triatomíneos, negativos para tripanosomatídeos. Foi realizado controle mecânico e químico. Novas pesquisas realizadas nas palmeiras avaliadas no mesmo ano resultaram na coleta de seis exemplares. Os controles mecânicos e químicos das palmeiras aliado ao trabalho de participação popular mostraram-se factíveis impedindo a colonização destes insetos nos domicílios da cidade.

### CONFLICT OF INTERESTS

The authors state there is no conflict of interests.

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