

# Entomological studies on Dengue fever vectors in Brazil: the epidemics of Boa Vista, Roraima, 1982, Niteroi, Rio de Janeiro, 1986, and Ceara State, 1986, 1994<sup>1</sup>

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## RESUMO

No Brasil, o vírus Dengue é transmitido pelo mosquito urbano *Aedes aegypti*. Foi na ocasião dos primeiros isolamentos realizados a partir de casos humanos em Boa Vista (RR) que, o vírus foi também isolado - sorotipos DEN 1 (1 amostra) e DEN 4 (2 amostras) - a partir de mosquitos naturalmente infectados. Durante o segundo episódio epidêmico, em Niterói (RJ), foram isoladas 3 amostras de DEN 1 a partir de mosquitos fêmeas coletadas com isca humana ou em repouso. Durante essa epidemia, nos locais não tratados por inseticidas, o índice de Breteau era de 102. A dissecação de uma amostragem dos mosquitos mostrou que (1) as fêmeas agressivas eram mais velhas que as coletadas em repouso e (2) a proporção de repastos interrompidos ou múltiplos era elevada. Inquéritos entomológicos foram realizados durante as epidemias de 1986 e 1994 no Ceará. Três amostras de DEN 1 e 16 amostras de DEN 2 foram isoladas a partir de *Ae. aegypti* coletados em Cascavel e Caucaia, respectivamente. A suscetibilidade à infecção oral dos mosquitos sobre pacientes com viremia pelo vírus DEN 2 foi testada. Positividade dos mosquitos apareceu a partir do terceiro dia após repasto. 44 % dos mosquitos foram infectados após ter sido alimentados com sangue contendo um título de vírus (Log TCD<sub>50</sub>) igual a 3,5. Tentativas de isolamento a partir de mosquitos machos, imaturos ou outras espécies foram negativas.

## INTRODUCTION

*Aedes aegypti*, the urban vector of Dengue fever (and urban Yellow fever), was declared as eradicated from Brazil in 1958 (Slosek, 1986). However, it was found again in this country in 1967 (Fraile, 1968; Bezerra, 1987). Since then, and repeatedly, it has been detected in various places, mostly situated along the frontier of the country. This may be explained by the high infestation rates existing in some neighboring countries, e. i. Bolivia, Peru, Venezuela, Surinam, French Guiana, etc. The reinfestation of the cities bordering the Atlantic Ocean has been more probably maritime than aerial (Marques, 1985).

This species is represented in Brazil by strains (= populations) which may differ by ecological, physiological and vectorial characteristics (Tabachnick, 1991; Tardieux *et al.*, 1990; 1991). The study of these parameters is necessary to understand better the epidemiology of Dengue fever and develop more efficacious control methodologies.

The present work reports observations done on this species when Dengue viruses have been isolated at the same time from it and from man in Brazil. They concern three regions located in the north (Roraima State), in the southeast (Rio de Janeiro) and in the northeast (Ceará State) of the country. In the last cited region, field studies have been done in 1986 and 1994.

## MATERIAL AND METHODS

The virological methods have been described in details by Travassos da Rosa *et al.* (1994), only entomological procedures will be related here.

Catches of adult mosquitoes were done during epidemics in a variety of ways: on human bait (volunteers whose immunity against yellow fever has been serologically confirmed) inside and outside human dwellings, at rest inside houses, and with "CDC miniature light - traps" in the peri-domestic area. In the case of the epidemics of Niterói and Ceará State, only the heads and thorax of the collected adult have been inoculated for isolation attempts. Thus, any antibody-containing blood from engorged females would not interfere with the virus eventually present in the salivary glands. Furthermore and in the case of the Niterói survey, a sample of the abdomens has been dissected for determination of the parous rate, following the technique of Potovodova (Detinova, 1963).

Larvae and pupae have been collected in their natural breeding places, reared until hatching of adults which were then kept alive one week before being processed for inoculation. Eggs have been collected either in ovitraps or laid by gravid females, and reared until hatching of adults.

During the epidemic of Ceará State in 1994 (Vasconcelos *et al.*, 1995), preliminary investigations have been done on the susceptibility to oral infection of the natural *Ae. aegypti* population. Mosquitoes reared from immatures collected in Caucaia, CE, have been allowed to feed until repletion on the arms of sick volunteers (vaccinated against yellow fever) whose infection by dengue virus has been confirmed by isolation and titration

of the virus. The proportion of infected mosquitoes vs. days after the infecting meal have been estimated by their individual inoculation in C6/36 cell culture.

### Geographical situation and climate

Few locations in South America are unsuitable for *Aedes aegypti* establishment. Only places above 2200 m and/or away from the tropics do not allow the installation of this species (Dégallier *et al.*, 1988a; 1988b). In fact, most of Brazilian territory is situated between the tropics and offers a climate very suited for the transmission of Dengue epidemics by *Ae. aegypti*.

#### Boa Vista (Roraima)

This town has already been described by Travassos da Rosa *et al.* (1982) and Osanai (1984). It is located at 2°48' N and 60°48' W, and 85 m above sea level. The mean annual rainfall is 1822 mm (1017 mm in 1982), with a peak of rains from May to August.

In 1981, the urban zone accounted for 66 % of the 71492 inhabitants.

#### Niterói (Rio de Janeiro)

The epidemic outbreak covered all Rio de Janeiro urban area, including neighboring cities as Niterói, located at 20 km from RJ. The coordinates of this town are: 22° 53' S and 43° 07' W. Excepted some hills with very cliff slopes ("morros") and not inhabited, mean altitude is 2,5 m above sea level. The town is situated on a 130 km<sup>2</sup> peninsula. Mean annual rainfall is 1029 mm with rainy season months from december to march. A census made in 1985 accounted for 442706 inhabitants.

#### Ceará: Fortaleza and various cities

In 1986, the entomological studies have been conducted mainly in a small town of 20,000 inhabitants named Cascavel (3° 43' S; 38° 32' W), located at 56 km southeast of Fortaleza. Its area is about 9 km<sup>2</sup>.

In 1994, entomological and epidemiological studies have been conducted in a city west of Fortaleza: Caucaia (3° 42' S; 38° 39' W).

Annual rainfall in Fortaleza (the nearest weather station) is between 1,000 and 1,500 mm, with rainy season from January to May.

## RESULTS

### BOA VISTA

Three entomological surveys have been done in this city: 29 April to 5 May 1982, 16 to 21 June 1982 and 1 to 10 June 1983. The last two surveys have been done after the control measures.

Table 1 shows the culicids species that have been collected during the first survey. It is noteworthy that indoor collecting on human bait have furnished also exophilic species as *Aedes scapularis*, *Ae. serratus* and *Psorophora albipes*, as already noted by Forattini *et al.* (1978). Some male mosquitoes other than *Ae. aegypti* have also been collected, suggesting that this work has probably not been done exclusively indoor and on human bait but also by netting in the gardens<sup>4</sup>.

Despite the fact that indoor and outdoor collections could not be separated we will make some comments on the species associated with human dwellings. *Ae. aegypti* and *Cx. quinquefasciatus* represented 23 % (1,438/6,368) and 36 % (2,320/6,368) of the total, respectively, and almost 75 % (4,757/6,368) of the mosquitoes were *Culex* mosquitoes.

From the female *Ae. aegypti* collected indoor on human bait, one strain of Dengue 1 and two strains of Dengue 4 have been isolated. The minimum infection rates for these viruses were thus 0.24 % and 0.48 %, respectively.

66 % (955/1438) of *Ae. aegypti* individuals were male. This rate is higher than the rate of male *Ae. aegypti* reared from larvae and pupae. No virus strain has been isolated from either male or immature mosquitoes.

In places with secondary forest, the culicid fauna was different than that in the city. *Ae. aegypti* was no more collected and some sabethine were present. Specimens in the genus *Culex* represented only 42 % (293/700) of the total. The other mosquitoes were mainly diurnal in habits (*Aedini* and *Sabethini*). *Haemagogus celeste* was reported for the first time in Brazil (Travassos da Rosa *et al.*, 1982).

<sup>4</sup> This conclusion has been confirmed by the technician who supervised the collecting sessions.

The two subsequent surveys did not allow to collect any more *Ae. aegypti* among 8,177 mosquitoes. This was without any doubt due to the effectiveness of control measures.

Table 1 : Mosquitoes collected during an epidemic of Dengue in Boa Vista, Roraima, Brazil, and inoculated for virus isolation (1982).

Species	Nr. inoculated	pools
<b>Human bait: indoor</b>		
<i>Aedes aegypti</i> <sup>a</sup>	409	19
<i>Ae. aegypti</i> (male)	820	32
<i>Ae. (Ochlerotatus) species</i>	12	1
<i>Ae. scapularis</i>	6	1
<i>Ae. serratus</i>	77	6
<i>Ae. serratus</i> (male)	11	1
<i>Psorophora albipes</i>	11	1
<i>Culex species</i>	1035	27
<i>Cx. species</i> (male)	149	9
<i>Cx. (Culex) species</i>	54	4
<i>Cx. (Cux.) species</i> (male)	699	22
<i>Cx. coronator</i>	29	3
<i>Cx. declarator</i>	13	2
<i>Cx. quinquefasciatus</i>	1104	43
<i>Cx. quinquefasciatus</i> (male)	865	31
<i>Cx. (Melanoconion) species</i>	57	5
	<b>5351</b>	<b>207</b>
<b>Human bait : peri-domestic</b>		
<i>Aedes aegypti</i>	74	4
<i>Ae. aegypti</i> (male)	135	6
<i>Ae. scapularis</i>	8	1
<i>Ae. serratus</i>	25	1
<i>Ae. serratus</i> (male)	14	1
<i>Psorophora albipes</i>	9	1
<i>Culex species</i>	83	3
<i>Cx. species</i> (male)	38	3
<i>Cx. (Culex) species</i>	17	2
<i>Cx. (Cux.) species</i> (male)	263	8
<i>Cx. quinquefasciatus</i>	277	12
<i>Cx. quinquefasciatus</i> (male)	74	1
	<b>1017</b>	<b>43</b>
<b>Human bait : rural &amp; sylvatic</b>		
<i>Aedes (Ochlerotatus) species</i> (male)	43	2
<i>Ae. scapularis</i>	54	2
<i>Ae. serratus</i>	79	3
<i>Psorophora albipes</i>	152	5
<i>Ps. ferox</i>	26	2
<i>Ps. ferox</i> (male)	21	1
<i>Haemagogus celeste</i>	7	1
<i>Culex species</i>	65	2
<i>Cx. species</i> (male)	27	1
<i>Cx. (Culex) species</i>	39	2
<i>Cx. (Cux.) species</i> (male)	105	4
<i>Cx. coronator</i>	19	1
<i>Cx. coronator</i> (male)	33	1
<i>Cx. (Melanoconion) species</i>	5	1
<i>Limatus species</i>	6	1
<i>Wyeomyia species</i>	19	1
	<b>700</b>	<b>30</b>
<b>CDC miniature light-trap</b>		
<i>Culex species</i>	5	1
<i>Cx. (Culex) species</i>	5	1
	<b>10</b>	<b>2</b>
<b>Total</b>	<b>7078</b>	<b>282</b>

## Rio de Janeiro : Niterói

The entomological survey has been conducted from 10 to 27 May 1986 in a residential district (Dr. Sardinha and Pr. Otacílio streets in Santa Rosa district) in which no control measures had previously been applied.

The houses are mostly individual with gardens, swimming pools and outbuildings. Some buildings of less than 5 floors have also been visited.

3,099 mosquitoes or 79 pools (plus 1,718 eggs, or 2 pools) have been inoculated for isolation attempts (Table 2). Some biocological data are reported as follows.

Table 2 : Mosquitoes collected during an epidemic of Dengue in Niterói, Rio de Janeiro, Brazil, and inoculated for virus isolation (1986).

Species	Nr. inoculated	pools
<b>Human bait</b>		
<i>Aedes aegypti</i> <sup>a</sup>	230	9
<i>Ae. aegypti</i> (male)	231	5
<i>Culex quinquefasciatus</i>	118	2
<i>Cx. quinquefasciatus</i> (male)	134	2
	<b>713</b>	<b>18</b>
<b>Resting mosquitoes</b>		
<i>Aedes aegypti</i>	224	8
<i>Ae. aegypti</i> (engorged females, reared until egg laying <sup>b</sup> ) <sup>c</sup>	49	2
<i>Ae. aegypti</i> (male)	231	5
<i>Culex quinquefasciatus</i>	654	15
<i>Cx. quinquefasciatus</i> (male)	387	9
	<b>1545</b>	<b>39</b>
<b>CDC miniature light-trap</b>		
<i>Aedes aegypti</i>	18	1
<i>Ae. aegypti</i> (male)	11	1
<i>Culex quinquefasciatus</i>	196	5
<i>Cx. quinquefasciatus</i> (male)	89	1
	<b>314</b>	<b>8</b>
<b>Breeding from immatures collected indoor and outdoor</b>		
<i>Aedes aegypti</i>	140	5
<i>Ae. aegypti</i> (male)	224	5
<i>Culex quinquefasciatus</i>	44	1
<i>Cx. quinquefasciatus</i> (male)	76	1
<i>Limatus species</i>	26	1
<i>Li. species</i> (male)	17	1
	<b>527</b>	<b>14</b>
<b>Total</b>	<b>3099</b>	<b>70</b>

<sup>a</sup> Mosquitoes from which have been isolated two strains of DEN 1 (AR 450170 and AR 450172)

<sup>b</sup> Egg batches inoculated in two pools of 1026 and 692 eggs, respectively (total : 1718)

<sup>c</sup> Mosquitoes from which has been isolated one strain of DEN 1 (AR 450117)

**ovitraps.** 54 traps with two paddles each have been put during four weeks (total = 216 paddles x weeks). Only ten paddles have been positive for *Ae. aegypti* eggs (86 eggs). The mean prevalence index was thus 4.6 % and the mean number of eggs by paddle and by week was 0.4.

***Ae. aegypti* indexes.** 193 dwellings (plus four closed houses = 2.0 %) have been surveyed in the Santa Rosa district. Adults of *Ae. aegypti* have been found in 100 houses (51.8%). 486 potential breeding recipient (with water) have been counted, of which 197 contained *Ae. aegypti* larvae. Various indexes were thus estimated as follows:

- **house index:** Nr. of houses among 100 houses, with positive breeding containers = 35.23% (8/193);
- **container index:** Nr. of positive containers among 100 potential breeding containers = 40.53% (197/486)
- **Breteau index:** Nr. of positive containers among 100 houses = 102.07 (197/193);
- **mean** Nr. of potential breeding containers (with water) by house containing putative breeding container (without water): 4.26 (486/114);
- **mean** Nr. of positive containers by house with putative breeding containers: 1.72 (197/114).

**Adult mosquitoes reared from immatures:** Only three species were present as immatures in the domestic and peri-domestic containers. *Ae. aegypti* was the most abundant (364/527 or 69%) with much more male than females (224/364 or 62 %). This contrasts with the other collecting methods where the two sexes were in similar proportions (50 % and 46 % of males for the human bait and resting mosquitoes collections, respectively)

Despite the noteworthy presence of *Cx. quinquefasciatus* (23 % of the collected individuals) in little domestic containers it has not been possible to determine if its presence was or was not associated with *Ae. aegypti*.

**Resting adult mosquitoes.** One third and two third of the mosquitoes were *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. These rates, on the contrary to those shown by diurnal human bait collecting are probably the result of important behavioral differences between the two species, although both are anthropophilic and endophilic.

**Adult mosquitoes collected on human bait.** When compared with the population that has been collected at rest, it appears that the aggressive females represent an older population (parity rate = 70.8 % versus 50.5 % (Table 3).

Table 3. Physiological age-grading of *Aedes aegypti* females collected at rest and with human bait in Niterói RJ: Follicles development stage, ovaries physiological age and presence vs. absence of dark blood (DB) in the stomach. The female mosquitoes with ovarian follicles at stage V (with eggs ready to be laid) have been considered parous.

Collected at rest	Stage of Christophers									Total
	IIId	IIIm	IIIf	IIIId	IIIIm	IIIIf	IV	V	?	
Nr. dissected	56	17	15	12	20	14	28	20	5	187
Nulliparous	33	10	3	-	-	2	-	-	3	51
Parous	18	4	6	-	2	-	-	20	2	50
parturity not stated	5	3	6	12	18	12	28	-	-	84
parous rate (%)	35.3	28.6	66.6	-	-	-	-	-	10	50.5
Stomach with DB	2	2	7	12	17	12	19	9	-	78
<b>Human bait</b>										
Nr. dissected	20	7	26	-	14	1	22	17	-	107
Nulliparous	11	5	5	-	-	-	-	-	-	21
Parous	9	2	21	-	2	-	-	17	-	51
parturity not stated	-	-	-	-	12	1	22	-	-	35
parous rate (%)	45	28.6	80.7	-	-	-	-	-	-	70.8
Stomach with DB	-	-	3	-	9	-	19	1	-	32
<b>Total dissected</b>	<b>76</b>	<b>24</b>	<b>41</b>	<b>12</b>	<b>34</b>	<b>15</b>	<b>50</b>	<b>37</b>	<b>5</b>	<b>294</b>

The high rate (32/107 or 30 %) of mosquitoes with dark blood in their stomach suggest that multiple blood feeding and/or interrupted feeding may be common for *Ae. aegypti*. Most of them have ovarioles that attained the IVth Christophers' stage.

Adult mosquitoes collected at light. Unexpectedly, *Ae. aegypti* represented near ten percents (29/314) of the light-trap material.

*Cx. quinquefasciatus* was the most numerous (91 %).

#### Fortaleza : Cascavel

Adult mosquitoes and immatures have been collected in resting sites and breeding containers, respectively. Numbers of collected and inoculated mosquitoes are shown on table 4.

Three strains of Dengue 1 have been isolated from *Ae. aegypti*, collected indoor at resting sites. No strain has been isolated either from males, immatures, or the other sinanthropic species, *Cx. quinquefasciatus*.

Adult *Ae. aegypti* have been found in 50 of 56 surveyed houses (89 %) in the central district. No data have been available on breeding containers.

Table 4 : Mosquitoes collected during an epidemic of Dengue in Cascavel (region of Fortaleza, Ceara), Brazil, and inoculated for virus isolation (1986).

Species	Nr. inoculated	pools
<b>Mosquitoes at rest</b>		
<i>Aedes aegypti</i> <sup>a</sup>	188	8
<i>Ae. aegypti</i> (male)	203	5
<i>Culex quinquefasciatus</i>	30	1
<i>Cx. quinquefasciatus</i> (male)	37	1
	<b>458</b>	<b>15</b>
<b>Adult mosquitoes reared from immatures collected indoor and outdoor</b>		
<i>Aedes aegypti</i>	1805	43
<i>Ae. aegypti</i> (male)	1103	23
<i>Culex quinquefasciatus</i>	4	1
<i>Cx. quinquefasciatus</i> (male)	13	1
	<b>2925</b>	<b>68</b>
<b>Immatures collected indoor and outdoor</b>		
<i>Aedes aegypti</i> (larvae)	1200	24
<i>Ae. aegypti</i> (pupae)	77	2
	<b>1277</b>	<b>26</b>
<b>Total</b>	<b>4660</b>	<b>100</b>

#### Fortaleza : Caucaia

Adult mosquitoes and immatures have been collected in resting sites and breeding containers, respectively. Numbers of collected and inoculated mosquitoes are shown on table 5. A total of 1,984 mosquitoes (331 pools) have been inoculated for isolation attempts.

No strain has been isolated either from males, immatures, or the other sinanthropic species, *Cx. quinquefasciatus*. Sixteen strains of DEN 2 have been isolated from naturally infected females of *Ae. aegypti*. The MIR was 7,8 % (16/204).

Twenty-nine strains of DEN 2 have been isolated from *Ae. aegypti* that have been fed on sick volunteers. At the time of experiment, one patient (F.P.B.) was positive, with a level of circulating virus ( $\log_{10} \text{TCD}_{50}$ ) equal to 3.5. Figure 1 shows the results of the isolation attempts from individual mosquitoes drawn at successive

days after engorgement. Twenty-three mosquitoes show inconclusive results due to bacterial and/or fungal contamination of the inoculums. Thirty-three and twenty-six were negative and positive for DEN 2 virus, respectively. Virus was detectable in mosquitoes since the third day (no mosquito has been tested on the second day) until the last day of experiment. If we consider only the interpretable results, 44 % (26/59) of the mosquitoes have been susceptible to oral infection.

Table 5: Mosquitoes collected during an epidemic of Dengue in Caucaia (region of Fortaleza, Ceara), Brazil and inoculated for virus isolation (1994).

Species	Number	Pools	strains
<b>Adults reared from eggs (oviposition traps)</b>			
<i>Ae. aegypti</i>	23	4	
<i>Ae. aegypti</i> (male)	37	3	
<b>Indoor resting mosquitoes</b>			
<i>Ae. aegypti</i>	17	1	
<i>Ae. aegypti</i> (male)	5	1	
<i>Cx. quinquefasciatus</i>	822	20	
<b>Indoor human bait</b>			
<i>Ae. aegypti</i>	187	13	
<i>Ae. aegypti</i> (male)	120	12	
<i>Ae. fluviatilis</i>	1	1	
<i>Cx. quinquefasciatus</i>	293	14	
<i>Cx. quinquefasciatus</i> (male)	185	12	
<b>Adults reared from larvae or pupae (susceptibility tests)</b>			
<i>Ae. aegypti</i>	294	250	29
<b>Total</b>	<b>1984</b>	<b>331</b>	<b>29</b>

At the beginning of the second experiment, the titre of circulating virus was  $10^{5.5}$  but only 17 mosquitoes engorged on the patient (M.S.R.). Of these, only 7 gave interpretable results, with 4 negative on the 2d day after bloodmeal and 3 positive on the 5th and 6th days. On other days, mosquitoes have not been tested or tests have been inconclusive.

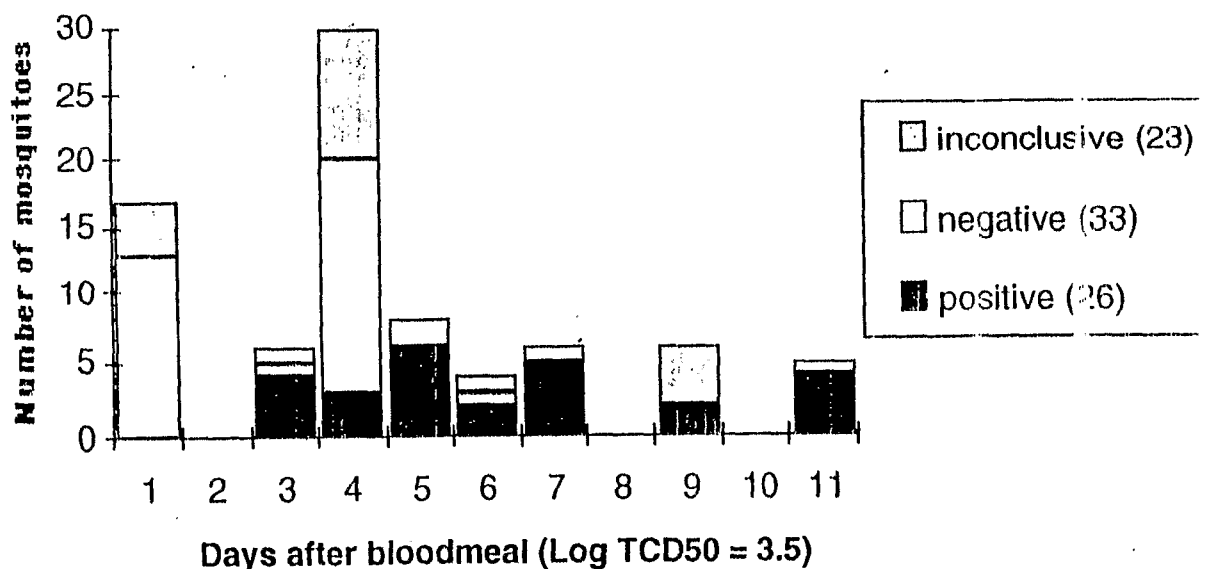


Figure 1: Test of susceptibility to oral-infection by DEN 2 virus of *Aedes aegypti* mosquitoes collected in Caucaia, CE, Brazil, June 1994: number of mosquitoes positive, negative or inconclusive for virus presence vs. day after bloodmeal; days 2, 8 and 10 not tested.



## DISCUSSION

As suggested by old descriptions of urban epidemics of Yellow fever or Dengue fever, *Ae. aegypti* has probably been introduced in Brazil by ships coming from Africa. Since that time, it has been hypothesized that this mosquito was responsible for the transmission of these diseases (Finlay, 1892), and many works have been done for eradicating and later, controlling its populations, with more or less success (Franco, 1967; Reiter, 1993).

Nowadays, both epidemiological and entomological situations appear much more complicated, with the presence of three of the four serotypes of Dengue viruses, the emerging of Hemorrhagic Dengue Fever syndrome (Nogueira *et al.*, 1993; Gubler & Trent, 1994) and the acclimatization in Brazil of a second potential vector, *Aedes albopictus* (Araújo *et al.*, 1988; 1994; Miller & Ballinger, 1988; Mitchell, 1991; Moreira & Almeida, 1994). Despite the fact that its role in the transmission of Dengue and/or other arboviruses in Brazil has not yet been established<sup>5</sup>, and contrary to *Ae. aegypti* that is a strictly urban mosquito in S.A., *Ae. albopictus* appears as a potential link between sylvatic and urban Yellow fever and between urban and sylvatic Dengue, the latter yet undetected in South America (Hawley, 1988). Thus, both vector species should be studied at the same time where they are both present because of the complicated ecological interactions between them and their high genetic plasticity. These interactions seem to be different in the neotropics than in Asia, as reported by Hawley & Craig Jr. (1989).

During epidemics, control measures are in most cases quickly undertaken, which prevent from collecting infected mosquitoes. However, when the cities are great and the control measures not simultaneous in all districts, it is possible to do some entomological works on mosquito populations that represent the vector population. Such opportunities will permit to develop and test models that will allow to decide which are the best control measures in each situation (Reiter, 1993).

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<sup>5</sup> For epidemiological reasons, the report by Serufo *et al.* (1993) of vertical transmission of Dengue by this species in nature is not supported by the authors.

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