

Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 10- Survey of adult behaviour of *Culex nigripalpus* and other species of *Culex* (*Culex*) in South-Eastern Brazil*

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A survey of adult behaviour of *Culex* (*Culex*) species was carried out from August 1992 through December 1993 in a human modified (anthropic) environment in the Ribeira Valley, S. Paulo State, Brazil. *Culex nigripalpus* dominated the catches at several sites and its tendency to increase in the anthropic environment became quite clear. Nevertheless no high level of synanthropy was demonstrated. So it seems that the mosquito may have a restricted role in natural arbovirus cycles. Nonetheless, *Cx. nigripalpus* must be considered a potential vector of arboviruses, especially St. Louis encephalitis virus outside dwellings.

Culex. Ecology, vectors. St. Louis encephalitis, transmission.

Introduction

From the epidemiological point of view *Culex* (*Culex*) is a group of mosquitoes that includes several arbovirus vector species. They are essentially nocturnal-crepuscular tending to be active throughout the night, but most are active just after dark. In general, these mosquitoes are mainly ornithophilic and thus they cycle virus agents among avian hosts. Nevertheless, important differences exist according to weather and peculiar environmental conditions that may influence their role as an arbovirus vector. In North America, species of the *Culex* subgenus are important in the transmission of mosquito-borne arboviruses of public health importance such as eastern (EEE), western (WEE) and St. Louis (SLE) encephalitis. *Culex nigripalpus* is considered to be an important SLE vector in the south-eastern United States, and perhaps EEE virus as well (Chamberlain et al.², 1964; Dow et al.⁴, 1964; Morris²⁷, 1992).

In Central and South America, *Cx. nigripalpus* was considered as a potential vector of EEE virus in

the Dominican Republic (Mitchell et al.²⁵, 1979). In addition, an isolation of vesicular stomatitis New Jersey (VSNJ) virus was obtained from this species in Guatemala (C.H. Calisher apud Webb and Holbrook²⁹, 1988), and isolations of others virus were reported from this mosquito in Panama (Dutary et al.⁵, 1984). Isolations of SLV from *Cx. nigripalpus* were obtained in Jamaica and Guatemala (Belle et al.¹, 1964; Cupp et al.³, 1986). In Argentina, natural infections by SLE virus were reported in *Cx. quinquefasciatus* and undetermined *Cx. (Culex)* specimens (Mitchell et al.²⁶, 1985). In the Brazilian Amazon region, SLE virus was isolated from *Cx. coronator*, *Cx. declarator* and unidentified *Cx. (Culex)* mosquitoes which also provided Mucambo virus isolations (Vasconcelos et al.²⁸, 1991). The relative importance of all these neotropical culicids in the maintenance and transmission of these arboviruses, particularly SLE virus in Latin America, remains to be defined.

Observations on mosquito behaviour were made during a research program in the Ribeira Valley region of S. Paulo State, subsequent to an

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encephalitis epidemic started in 1975-1976 thereafter decreasing until 1983 (Iversson²¹, 1988). Culicidae were studied regarding their dominance, biting habits, synanthropy and geographic distribution (Forattini et al.^{6,7,8,10,11}, 1981, 1986, 1989, 1990). More recently, observations were performed at a rural man-made environmental sites where artificial irrigation is used for rice cultivation, focusing on several species including some *Culex* (*Culex*) found there (Forattini et al.^{12,13,14,15} 1993, 1994). A continuation of these researches in that anthropic environment focused on mosquito biting activities and occurrence at several places. During 1992-1993, adults were collected through the use of human bait and Shannon-type traps. The results regarding the subgenus *Culex* species are presented here for the purpose of revealing epidemiological evidence about their potential hypothetical vector role.

Study areas and methods

Adult sampling followed the same study areas and methods have been described elsewhere (Forattini et al.^{16,17}, 1995). To make it easier to understand the results presented, the sampling sites are noted again.

Shannon traps (Shan) collections:

Period	Site	Place	Shannon trap
January - December 1993	ES	remnant forest A	Shan 1
		remnant forest B	Shan 2
		rice-paddy margin (open land)	Shan E
August 1992 - December 1993	GA	margin of Pariquera-Açu river	Shan GA

As Shan 1 and Shan 2 traps operated alternately once a week, the total number of mosquitoes caught corresponded to the fortnightly rhythm.

Human bait (BC) collections:

Period	Site	Place
August 1992- December 1993	BC	dwelling in the ES area

BC - "Sítio Barra do Capinzal"; ES - Experimental Station; Ga - "Galiléia Farm"

Biting activity was estimated through the Williams' mean (\bar{X}_w), the domiciliation degree estimation was made by the synanthropic index(s) comparing it through the synanthropic ratios(sr). Besides, considering the several sites as representative of development phases, the index of change (IC) was calculated for the abundance comparison at each of these phases.

Data about the climatic conditions during the period were obtained directly from the climatology Section of the Campinas Agronomic Institute (Instituto Agrônômico de Campinas) of S. Paulo State.

Results

A total of 1,880 adult mosquitoes (1,787 females and 93 males) belonging to the subgenus *Culex* were obtained. From that number 1,812 (96.4%) were caught with the Shannon traps whereas only 68 (3.6%) with human bait. The species distribution follows:

Species	N	%
<i>Cx. bidens</i>	7	0.4
<i>Cx. chidesterei</i>	19	1.0
<i>Cx. corniger</i>	2	0.1
<i>Cx. coronator</i>	2	0.1
<i>Cx. coronator</i> + <i>Cx. usquatus</i>	190	10.1
<i>Cx. declarator</i>	9	0.5
<i>Cx. dolosus</i>	3	0.2
<i>Cx. dolosus</i> + <i>Cx. eduardoi</i>	2	0.1
<i>Cx. lygrus</i>	11	0.6
<i>Cx. mollis</i>	14	0.7
<i>Cx. nigripalpus</i>	1,464	77.9
<i>Cx. (Culex) sp</i>	157	8.3
Total	1,880	100.0

The results obtained from the ES and GA sites, along with the methods employed, are presented in Table 1. It may be seen that *Cx. nigripalpus* comprised 77.9% and jointly with Coronator Group (*Cx. coronator* and *Cx. usquatus*) reached 88.1% of the total *Cx. (Culex)* specimens collected. The behaviour of *Cx. nigripalpus*, compared to the Coronator Group, will be deserve special attention in this paper. As females of both represented 86.0% of the total adults caught, the following data will relate to them. Specimens determined as *Cx. (Culex) sp*, could not be identified below subgenus because of damages incurred during collection.

Shannon Traps

The monthly distribution of *Cx. nigripalpus* and of the Coronator Group species, are presented in Tables 2 and 3. *Cx. nigripalpus* incidence in the open land (Shan E) of the anthropic environment (ES), that correspond to the rice fields margin, showed a February-April peak. In that agricultural environment of the ES area, 90.5% of the *Cx. nigripalpus* females was caught in the February-April period. Nevertheless, it seems that adult occurrence was explosive at that time, coinciding with higher levels of precipitation and less temperature fluctuations that occurs during that time period (Fig.). The monthly distribution of the Coronator

Table 1- Number of adult *Culex* (*Culex*) mosquitoes collected at the anthropic (ES) and partially disturbed environment (GA). August 1992 through December 1993.

Species	ES remnant forest		ES open land		BC dwellings (hb)				GA		Total				
	Shan 1		Shan 2		Shan E		Outdoor		Indoor		Shan GA		f	m	T
	f	m	f	m	f	m	f	m	f	m	f	m			
<i>Cx. bidens</i>	-	2	-	5	-	-	-	-	-	-	-	-	7	7	
<i>Cx. chidesteri</i>	1	3	4	-	3	2	-	-	-	-	6	-	8	11	19
<i>Cx. corniger</i>	1	-	-	-	-	1	-	-	-	-	-	-	1	1	2
<i>Cx. coronator</i>	-	2	-	-	-	-	-	-	-	-	-	-	-	2	2
<i>Cx. coronator/usquatus</i>	57	-	37	-	33	-	5	-	3	-	55	-	190	-	190
<i>Cx. declarator</i>	-	1	-	8	-	-	-	-	-	-	-	-	-	9	9
<i>Cx. dolosus</i>	1	-	-	2	-	-	-	-	-	-	-	-	1	2	3
<i>Cx. dolosus/eduardoi</i>	2	-	-	-	-	-	-	-	-	-	-	-	2	-	2
<i>Cx. lygrus</i>	-	-	-	11	-	-	-	-	-	-	-	-	-	11	11
<i>Cx. mollis</i>	1	4	-	9	-	-	-	-	-	-	-	-	1	13	14
<i>Cx. nigripalpus</i>	219	17	275	14	827	-	37	-	21	-	48	6	1,427	37	1,464
<i>Cx. (Culex) sp</i>	29	-	68	-	33	-	-	-	2	-	25	-	157	-	157
Total	311	29	384	49	896	3	42	-	26	-	128	12	1,787	93	1,880

BC - "Sítio Barra do Capinzal"

ES - Experimental Station

GA - "Galiléia Farm"

f- female

hb - human bait

m - male

Shan - Shannon trap

Group was more regular, apparently not influenced by environmental factors in any particular period of the year.

The data obtained in the forest patches (Shan 1 and Shan 2) were statistically compared with those from the open land (Shan E) (Table 2). Through the Mann-Whitney test the *p* values were 0.8918 for *Cx. nigripalpus* and 0.0464 for the Coronator Group, resulting in some significance for this second group of mosquitoes.

In the partially disturbed environment, represented by the GA site, irregular variations were obtained. As a general view, what may be considered a small peak occurred in March for both mosquitoes groups (Table 3).

Domiciliary Environment

Through the use of human bait at the BC dwelling, a total of 66 *Cx. (Culex)* females was caught. From that total 58 (87.9%) belonged to *Cx. nigripalpus* and only 8 (12.1%) to the Coronator Group. According to the sampling sites, the results obtained were as follows (Table 1 and 4):

	Indoor	Outdoor	Total
<i>Cx. nigripalpus</i>	21(87.5%)	37(88.1%)	58(87.9%)
Coronator Group	3(12.5%)	5(11.9%)	8(12.1%)
Total	24	42	66

Comparing the sites sampled, through the Mann-Whitney test, the *p* value was 0.524 for *Cx. nigripalpus*

showing no significant differences between indoor and outdoor collections. Regarding the Coronator Group, the samples obtained were too small and thus insufficient for the statistical test (Table 4).

Synanthropy

Comparison of the three sampled environments was made for both mosquito groups using synanthropic indices (s). The results obtained follow:

	a(%)	b(%)	c(%)	s
<i>Cx. nigripalpus</i>	4.2	60.0	35.8	-1.6
Coronator Group	5.9	24.4	69.6	-51.5

Thus, these data suggest that *Cx. nigripalpus* retained its exophilic preferences, while the Coronator Group species showed a clear sylvatic behaviour.

Considering the synanthropic ratios (sr) at the ES area, comparisons were made, between the total females caught at the open area (Shan E) jointly with that obtained in the domiciliary environment (BC), and the total caught at the remnant forests (Shan 1 and Shan 2). Doing this, the value obtained was 1.53 conjoint for both groups. The specific values were 1.75 for *Cx. nigripalpus* and 0.40 for the Coronator Group. These data suggest that the residual forest patches retain greater numbers of Coronator Group mosquitoes than *Cx. nigripalpus*. Besides, relating the open land (Shan E) data with those of the domiciliary environment (BC), the overall ratio was 13.03 for both mosquito groups considered together, showing greater occurrence

Table 2 - Monthly distribution of *Culex nigripalpus* and Coronator Group females collected in Shannon traps at the anthropic environment area (ES), January through December 1993.

Months	Shan	<i>Cx. nigripalpus</i>		Coronator Group		Total	
		n	%	n	%	n	%
January	1	-	-	-	-	-	-
	2	1	*	3	2.4	4	0.3
	E	-	-	-	-	-	-
February	1	60	4.5	3	2.4	63	4.4
	2	80	6.1	22	17.3	102	7.0
	E	4	0.3	-	-	4	0.3
March	1	140	10.6	2	1.6	142	9.8
	2	166	12.6	-	-	166	11.5
	E	718	54.4	-	-	718	49.6
April	1	12	0.9	7	5.5	19	1.3
	2	5	0.4	1	0.8	6	0.4
	E	10	0.8	6	4.7	16	1.1
May	1	-	-	1	0.8	1	*
	2	-	-	-	-	-	-
	E	1	*	7	5.5	8	0.6
June	1	-	-	1	0.8	1	*
	2	2	0.2	-	-	2	0.1
	E	-	-	1	0.8	1	*
July	1	2	0.2	6	4.7	8	0.6
	2	-	-	1	0.8	1	*
	E	9	0.7	1	0.8	10	0.7
August	1	-	-	12	9.4	12	0.8
	2	8	0.6	1	0.8	9	0.6
	E	-	-	5	3.9	5	0.3
September	1	2	0.2	13	10.2	15	1.0
	2	1	*	1	0.8	2	0.1
	E	15	1.1	7	5.5	22	1.5
October	1	1	*	9	7.1	10	0.7
	2	12	0.9	8	6.3	20	1.4
	E	-	-	-	-	-	-
November	1	-	-	3	2.4	3	0.2
	2	-	-	-	-	-	-
	E	44	3.3	6	4.7	50	3.5
December	1	2	0.2	-	-	2	0.1
	2	-	-	-	-	-	-
	E	26	2.0	-	-	26	1.8
Total	1	219	16.6	57	44.9	276	19.1
	2	275	20.8	37	29.1	312	21.5
	E	827	62.6	33	26.0	860	59.4
T		1,321	100.0	127	100.0	1,448	100.0

ES - Experimental Station

Shan - Shannon trap

* - less than 0.1

outside than inside domiciliary environments. Nevertheless, specifically calculated for each of these mosquito groups were 15.31 for *Cx. nigripalpus* and 4.1 for the Coronator Group, showing greater occurrence outside than inside the dwelling environment. However, as shown in Table 1, the results obtained with the dwelling catches of Coronator Group were quite negligible. Considering the two groups, separately, the calculated ratios were:

Cx. nigripalpus:

$$sr = \frac{Shan E}{Shan 1 + Shan 2} = 1.67$$

$$sr = \frac{BC}{Shan E} = 0.67$$

Coronator Group:

$$sr = \frac{Shan E}{Shan 1 + Shan 2} = 0.34$$

$$sr = \frac{BC}{Shan E} = 0.24$$

Table 3 - Monthly distribution of *Culex nigripalpus* and Coronator Group females collected in Shannon trap in the partially disturbed environment (GA). August 1992 through December 1993.

Year / Months	<i>Cx. nigripalpus</i>		Coronator Group		Total	
	n.	%	n.	%	n.	%
1992						
August	14	29.2	2	3.6	16	15.5
September	6	12.5	2	3.6	8	7.8
October	8	16.7	-	-	8	7.8
November	3	6.2	10	18.2	13	12.6
December	-	-	-	-	-	-
1993						
January	-	-	7	12.7	7	6.8
February	-	-	-	-	-	-
March	10	20.8	11	20.0	21	20.4
April	1	2.1	3	5.5	4	3.9
May	1	2.1	3	5.5	4	3.9
June	-	-	3	5.5	3	2.9
July	-	-	-	-	-	-
August	1	2.1	12	21.8	13	12.6
September	-	-	2	3.6	2	1.9
October	2	4.2	-	-	2	1.9
November	2	4.2	-	-	2	1.9
December	-	-	-	-	-	-
Total:	48	100.1	55	100.0	103	99.9

GA - "Galiléia Farm"

Table 4 - Monthly distribution of *Culex nigripalpus* and Coronator Group females collected through human bait in the domiciliary environment (BC). August 1992 through December 1993.

Months	N	<i>Cx. nigripalpus</i>				Coronator Group				Total		T
		Indoor		Outdoor		Indoor		Outdoor		Indoor	Outdoor	
		n	%	n	%	n	%	n	%	n	%	
1992												
August	2	-	-	16	43.2	-	-	-	-	16	38.1	16
September	2	-	-	-	-	-	-	-	-	-	-	-
October	2	-	-	-	-	1	-	1	4.2	-	-	1
November	2	-	-	-	-	-	-	-	-	-	-	-
December	3	-	-	-	-	-	-	-	-	-	-	-
1993												
January	2	1	4.8	8	21.6	-	-	1	4.2	8	19.0	9
February	2	19	90.4	6	16.2	-	-	19	79.2	6	14.3	25
March	2	1	4.8	6	16.2	1	-	2	8.3	6	14.3	8
April	2	-	-	-	-	-	-	-	-	-	-	-
May	2	-	-	-	-	-	1	-	-	1	2.4	1
June	3	-	-	-	-	-	-	-	-	-	-	-
July	2	-	-	1	2.7	-	1	-	-	2	4.8	2
August	2	-	-	-	-	1	1	1	4.2	1	2.4	2
September	1	-	-	-	-	-	-	-	-	-	-	-
October	2	-	-	-	-	-	-	-	-	-	-	-
November	3	-	-	-	-	-	2	-	-	2	4.8	2
December	2	-	-	-	-	-	-	-	-	-	-	-
Total	34	21	100.0	37	99.9	3	5	24	100.1	42	100.1	66

BC - "Sítio Barra do Capinzal"

N - number of catches

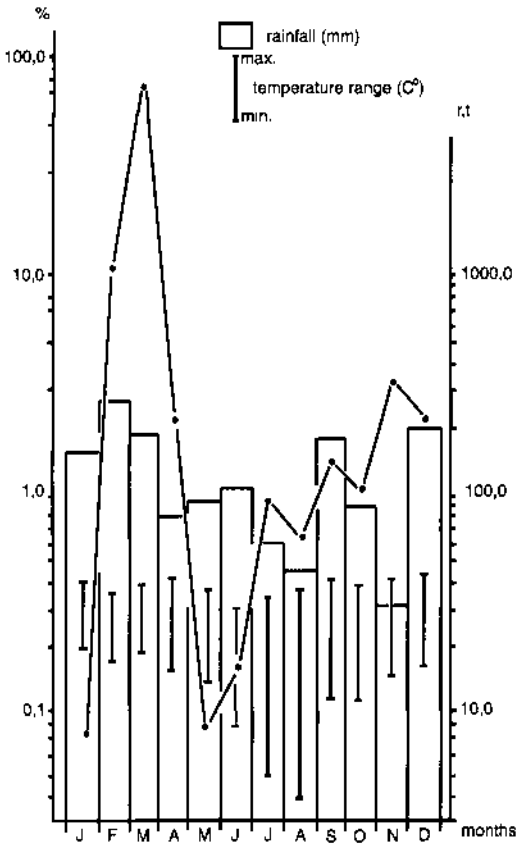


Figure- Monthly incidence of females *Cx. nigripalpus* in the anthropic environment of the Experimental Station (ES), caught through Shannon traps during January-December 1993 period.

r,t - total mms monthly rainfalls (r) and monthly °C temperature range (t).

% - percentage from the total females caught.

In the domiciliary environment (BC) both *Cx. nigripalpus* and the Coronator Group species showed a lower synanthropy level. A clear tendency to reach dwellings, in a significant manner, was not detected.

Behavioural succession, estimated through the index of change (IC), compares phase 1 (Shan 1 +

Shan 2) with the phase 2 (Shan E + BC) (Table 5). These indices were calculated as 0.9853 for *Cx. nigripalpus* and 0.3433 for the Coronator Group, indicating changes for both these mosquitoes groups. Estimating the changes from phase 1 to phase 2, that mosquito increased 126.31 times while the others increased only 2.04 times.

Discussion

Among the mosquitoes sampled in the collections reported above, *Cx. nigripalpus* predominated among the *Cx. (Culex)* species. The Coronator Group species were second in frequency. In previous studies in the same Ribeira Valley region *Cx. nigripalpus* was constantly poorly represented in collections taken inside the primitive forest environment but readily found in the modified areas represented by remnant and secondary forests (Forattini et al.^{7,9,13}, 1986, 1989, 1993). Probably this pattern may be related to sampling performed at the forest ground, as this mosquito seems to have a good degree of acrodendrophily (Guimarães et al.¹⁹, 1985). Nonetheless, at least in southern Brazil, *Cx. nigripalpus* may frequent anthropic modified environments, in some localities (Lourenço-de-Oliveira²², 1984). Besides, in the results reported here, no difference was observed between the remnant forest and the open cultivated land of the anthropic environment, regarding collections of that mosquito. The Coronator Group showed a slight preference for the remnant forest environment. An insufficient number of females caught limited the meaning of the statistical analysis. Regarding the data obtained at the partially disturbed environment of the GA site, no difference was found between those two mosquito groups.

The monthly distribution of *Cx. nigripalpus* in the ES cultivated environment shown a major peak in February-April. This period coincided with the beginning of the fallow uncultivated phase of the rice paddies cycle. Thus, it followed the crop harvesting phase where the empty rice fields and in-

Table 5 - Index of change (IC) and abundance (a) ratios of *Cx. nigripalpus* and Coronator Group females in two environment developmental phases.

Species	Phase 1 (Shan 1 + Shan 2)			Phase 2 (Shan E + BC)			IC	a ₂ /a ₁
	d	o	a ₁	d	o	a ₂		
<i>Cx. nigripalpus</i>	0.0012	0.023	2.85x10 ⁻⁵	0.021	0.017	0.0036	0.9853	126.31
Coronator Group	0.002	0.0043	8.8x10 ⁻⁶	0.023	0.00077	1.8x10 ⁻⁵	0.3433	2.04

a₁ - overall abundance in the phase 1.

a₂ - overall abundance in the phase 2.

d - relative density.

o - percentage occurrence.

Shan 1 + Shan 2 - remnant forests

Shan E + BC - open cultivated land.

creased rainfall favoured larval breeding. However, that association seems quite variable and remains unclear (Forattini et al.^{12,15}, 1993, 1994). Anyway, the results seem to correlate with an increase in rainfall and higher temperature levels (Fig.), as was observed in others regions of southern Brazil (Guimarães and Arlé¹⁸, 1984; Lourenço-de-Oliveira et al.²³, 1985).

Considering the domiciliary environment, the data obtained were insufficient to estimate the Williams' means. For *Cx. nigripalpus*, no significant difference was evident between indoor and outdoor catches. About its synanthropic level, the estimation based on the *s* indices, gave lower values, around the independence degree (0.0). No clear tendency to enter dwellings was detected, even through the *sr* ratios.

Finally, the changing pattern of the succession, estimated by the indices of change (IC), indicate that changes occurred for *Cx. nigripalpus*. These results agree with what was said at the beginning of this discussion. Certainly that mosquito, at least in this southern region of Brazil, is favoured by the man-made environment, even though not reaching, in a significant manner, human habitations.

These data obtained in the Ribeira Valley region support the hypothesis that *Cx. nigripalpus* may fulfill a role in the local arbovirus cycle. Its biting habits were reported elsewhere and, if not definitive, they suggested that this and other *Cx. (Culex)* mosquitoes, some having even a great degree of ornithophily, may develop a suitable level of anthropophily (Lourenço-de-Oliveira and Heyden²⁴, 1986; Guimarães et al.²⁰, 1987; Forattini et al.⁹, 1989). So, in southern Brazil, *Cx. nigripalpus* at least deserves attention as a possible vector of arboviruses. Among these viruses, the St. Louis encephalitis maybe transmitted outside the domiciliary environment.

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Resumo

São relatados os resultados obtidos mediante coletas regulares de adultos de *Culex* (*Culex*) em ambientes antrópico do Vale do Ribeira, SP, Brasil, no período de agosto de 1992 a dezembro de 1993. Pôde-se evidenciar a dominância de *Culex nigripalpus* nas várias coletas efetuadas. Revelou-se claramente a preferência por parte desse mosquito em aumentar sua densidade no ambiente antrópico. Todavia, sua frequência ao domicílio mostrou-se baixa, revelando fraco grau de sinantropia. Assim sendo, seu papel vetor de arbovirus parece restringir-se à participação no ciclo natural desses agentes infecciosos. Contudo, pode-se considerá-lo como vetor potencial no meio extradomiciliar. Nesse particular, seu papel pode não ser negligenciável, especialmente no que tange à possibilidade de transmissão de encefalite de S. Luís, cujo agente já foi assinalado na região.

Culex. Ecologia de vetores. Encefalite de St. Louis, transmission.