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Source: Journal of Medical Entomology, 50(4):791-795. 2013.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/ME12120

Flight Height Preference for Oviposition of Mosquito (Diptera: Culicidae) Vectors of Sylvatic Yellow Fever Virus Near the Hydroelectric Reservoir of Simplício, Minas Gerais, Brazil

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J. Med. Entomol. 50(4): 791–795 (2013); DOI: http://dx.doi.org/10.1603/ME12120

ABSTRACT In this study, the oviposition behavior of mosquito species exhibiting acrodendrophilic habits was investigated. The study was conducted near the Simplício Hydroelectic Reservoir (SHR) located on the border of the states of Minas Gerais and Rio de Janeiro, Brazil. Samples were collected using oviposition traps installed in forest vegetation cover between 1.70 and 4.30 m above ground level during the months of April, June, August, October, and December of 2011. *Haemagogus janthinomys* (Dyar), *Haemagogus leucocelaenus* (Dyar and Shannon), *Aedes albopictus* (Skuse), and *Aedes terrens* (Walker) specimens were present among the collected samples, the first two of which being proven vectors of sylvatic yellow fever (SYF) in Brazil and the latter is a vector of dengue in mainland Asia. As the data set was zero-inflated, a specific Poisson-based model was used for the statistical analysis. When all four species were considered in the model, only heights used for egg laying and months of sampling were explaining the distribution. However, grouping the species under the genera *Haemagogus* Williston and *Aedes* Meigen showed a significant preference for higher traps of the former. Considering the local working population of SHR is very large, fluctuating, and potentially exposed to SYF, and that this virus occurs in almost all Brazilian states, monitoring of Culicidae in Brazil is essential for assessing the risk of transmission of this arbovirus.

KEY WORDS Culicidae, yellow fever vectors, oviposition trap, height preference, acrodendrophily

From an epidemiological point of view, mosquito species (Diptera: Culicidae) of the genera *Haemagogus* and *Sabethes* Robineau-Desvoidy are the most important biological vectors of sylvatic yellow fever (SYF) virus in forested areas of the Americas (Arnell 1973). Although some of these species demonstrate a tendency to inhabit domestic settings, *Haemagogus* species in Brazil are mainly sylvatic with diurnal habits and conduct their activities in the tree canopy (Marcondes and Alencar 2010).

According to Pessanha (2009), with the growing process of deforestation, which has occurred over the past 30 yr, a progressive increase in the number of cases of sylvatic diseases in transitional areas has been observed. Recently, sporadic yellow fever viral circulation has been observed consistently in the state of Minas Gerais and presents a serious risk for geographical expansion throughout Brazil. In this specific case, a strong tendency to migrate to the south and east of Brazil was observed, with human cases having been reported in Minas Gerais and animal cases having been reported in Rio Grande do Sul (Vasconcelos et al. 2003).

Knowledge of the biocenotic community structures of mosquitoes is fundamentally important in areas where the environment has suffered disruption of the natural equilibrium. Such modifications of the environment may alter the dynamics of the mosquito populations when they are impacted by the local activity of human populations (Alencar et al. 2012). Whether during the periods of human activity or during periods of vegetative recovery, knowledge of community biodiversity of mosquitoes in the Atlantic Forest is relevant for assessments of possible changes in behavior and adaptations in the pattern of activities performed by mosquito populations. Until these parameters are known, the behavior will continue to be considered preferentially sylvatic.

Therefore, the objective of this study was to observe the occurrence of mosquito species, which may be

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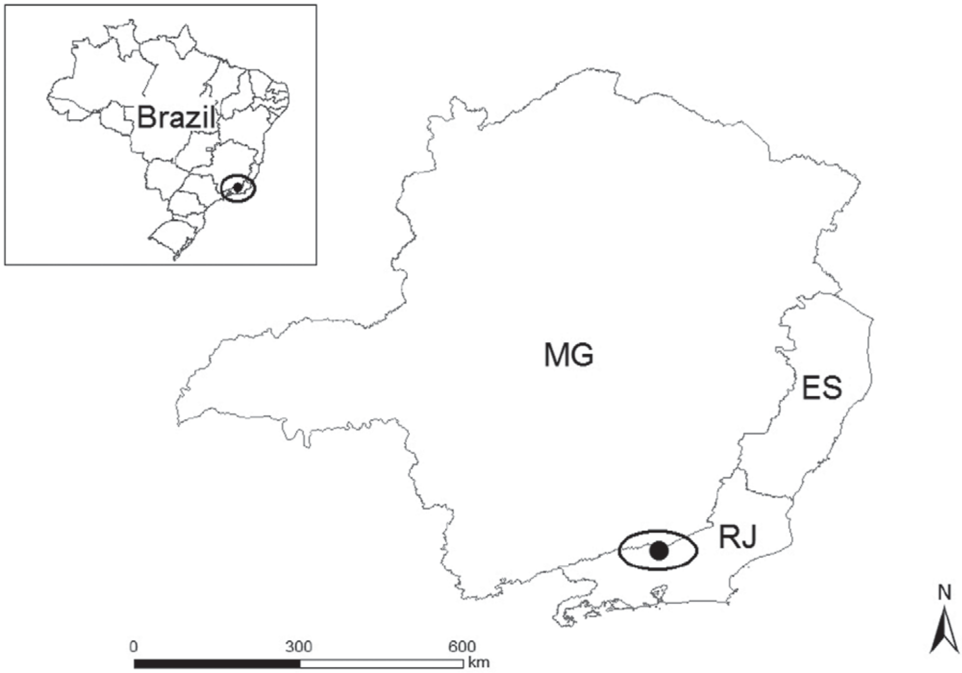

Fig. 1. Location of the capture of mosquito eggs in the local area flooded by the Simplício Hydroelectric Reservoir (SHR-Simplício).

current or potential vectors of yellow fever virus, their present arboreal behaviors, and their colonization of oviposition traps installed in the transition zone between forested and deforested areas. Oviposition behaviors of mosquito species, as well as their preferences for traps placed at different heights in relation to ground level, were analyzed.

Materials and Methods

The study was conducted in the Simplício Hydroelectic Reservoir (SHR), located in southeastern Brazil, on the border between the states of Minas Gerais (Além Paraíba and Chiador municipalities) and Rio de Janeiro (Três Rios and Sapucaia municipalities).

Following the methodology used by Silver (2008), monitoring was conducted through the use of oviposition traps that consisted of a no-lid, 1-liter capacity matte black pot with four 2.5 by 14-cm plywood panels (eucatex boards) fastened vertically inside the trap by "clips." To reproduce a more natural ecosystem, natural water and leaf litter were added to the pot. Ovitraps were randomly installed between 1.70 and 4.30 m from ground level and were placed in the trees by throwing a rope with the aid of a fishing sinker ≈4 cm in diameter and hoisting the trap by a nylon rope to the chosen tree. Fifteen heights (1.70, 1.80, 1.98, 2.05, 2.15, 2.17, 2.32, 2.50, 2.51, 2.60, 2.69, 2.76, 2.80, 3.30, and 4.30) in the forest were sampled and monitored biweekly using 17 ovitraps. All panels were sequentially numbered, placed in a humid container and sent to the Diptera Laboratory of the Oswaldo Cruz Institute.

The sampling point was located at 22° 05′37″ S and 43° 05′03″ W at an altitude of 314 m above sea level in the SHR area of influence (Fig. 1). Originally covered by typical Atlantic Forest vegetation, the study area is in a process of advanced regeneration monitored by experts from SHR, and continues to maintain areas with intensive livestock farming in its surroundings. The region presents a mesothermal climate with hot and rainy summers. The average air temperature during the study period was around 22.3°C, similar to the average local temperature of 22.1°C. Total rainfall of 710.6 mm accumulated in the first half of 2011, was close to normal climatic conditions. The heaviest rainfall was concentrated in the first 3 mo of the year, and represented 84% of the total accumulated rainfall in the first half of 2011, conforming with the normal rainfall pattern of the region (Eletrobras–Furnas, Brazil 2011).

In the laboratory, positive panels containing eggs were separated, and eggs were counted and immersed in white, screened 27 by 19 by 7-cm polyethylene trays containing dechlorinated water. Next, the eggs were maintained in controlled experimental setting using an incubator with thermoperiod and photoperiod, set at 28 ± 1°C, 75–90% relative humidity (RH), and a photoperiod of 10 h (day and night). The eggs remained in the incubator for a period of 3 d, with observations performed daily. The pupae were removed from the incubator, placed in beakers, and transferred to 30 by 30 by 30-cm breeding cages for the emergence of adults.

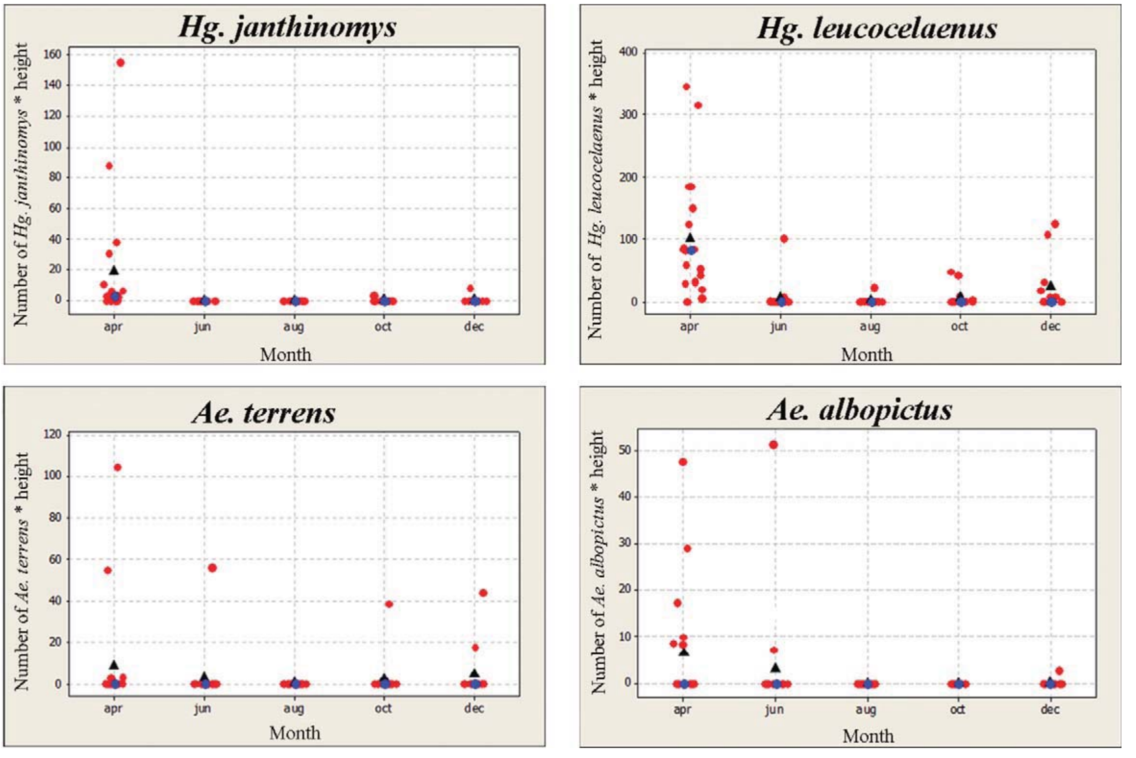

Fig. 2. The number of *Hg. janthinomys*, *Hg. leucocelaenus*, *Ae. terrens*, and *Ae. albopictus* per height and month. The red (circle), black (triangle), and blue (square) dots are the counts, median, and means values, respectively.

Adults were identified by direct observation of morphological characteristics under a stereoscopic microscope and using dichotomous keys prepared by Lane (1953), Consoli and Lourenço-de-Oliveira (1994), and Forattini (2002). Abbreviations for the generic and subgeneric names followed those proposed by Reinert (2001). After species determination, all specimens were incorporated in the Entomological Collection of Instituto Oswaldo Cruz, Fiocruz (Coleção Entomológica do Instituto Oswaldo Cruz, Fiocruz) under the title "Usina Hidrelétrica de Simplício-Minas Gerais/Rio de Janeiro" (UHS-MG/RJ).

For species of the tribe Aedini, recommendations of the *Journal of Medical Entomology* (Editorial 2005), which suggest that *Ochlerotatus* Lynch-Arribalzaga be treated as a subgenus within the genus *Aedes,* were followed. These recommendations are in contrast to the work of Reinert (2000), which raises *Ochlerotatus* to the category of genus, and later superseded by a series of publications (Reinert et al. 2009).

Data were analyzed to compare the flight height preference for the egg-laying behavior of the mosquitoes in trees. Thus, in this sort of count data (number of eggs), absences of eggs or zero values were not only significant but also very numerous in the data set (80%). One of the available methods to analyze such zero-inflated data sets is to use regression models based on zero-inflated Poisson distributions (Ridout et al. 2001). Regressions were done to determine which of the four variables—number of eggs, height, month, and species—significantly explained the distributions. Thus, we used the *zeroinfl* function included in the *pscl* R package (Ihaka and Gentleman, 1996, Zeileis et al. 2008, Jackman 2012, R Core Team, 2012). The R script and the two data sets are joined in Suppl Material [online only]: files "R script.txt," "VOO1.txt," and "VOO2.txt," respectively.

Results

During the sampling period, a total of 1,354 specimens of Culicidae were identified, including the following four species: *Haemagogus* (*Conopostegus*) *leucocelaenus* (Dyar and Shannon 1924): 1,028 specimens; *Haemagogus* (*Haemagogus*) *janthinomys* Dyar, 1921: 133 specimens; *Aedes* (*Stegomyia*) *albopictus* Skuse, 1894: 79 specimens; *Aedes* (*Protomacleaya*) *terrens* (Walker, 1856): 114 specimens. According to these results, the above species demonstrated a flight height preference for laying eggs (Fig. 2). Of the samples analyzed, two of the three species with a clear acrodendrophilic preference are known to be important vectors of yellow fever virus in forest environments: *Hg. janthinomys* and *Hg. leucocelaenus.* These species have the habit of ovipositing in the highest tree strata of the natural environment. The oviposition peak of the collected species occurred in April, with 77.5% of total eggs being laid during this month. *Hg. leucocelaenus* was the species with the highest population density across all studied sampling periods; however, this species showed no preference for any of the months tested for oviposition and was caught predominately between the heights of 1.80 to 2.60 m. Oviposition trap 1 and 12 in April represented the highest species richness, in contrast with traps 13 and 15 in June, which only collected *Ae. albopictus* from heights of $<$3 m. *Ae. terrens* was the only species not found at heights $<$1.80 m and showed a preference for laying eggs in traps located between the heights of 2.50–4.32 m.

The four species laid eggs in the traps located at the highest level of the tree strata, particularly for the samples collected in the reference month of April. However, they exhibited a tendency to lay eggs at the lowest level of the tree strata during the following 2-mo periods. During December, eggs of *Hg. leucocelaenus* and *Ae. terrens* were found primarily in the traps from the highest level of the trees.

Species of the genus *Haemagogus* performed egg laying in higher strata than species from the genus *Aedes*, and *Hg. janthinomys* was the species performing egg laying at highest levels (Fig. 2). *Hg. leucocelaenus* was the species with the highest frequency of females laying eggs in traps located in the highest levels of the trees (Fig. 2). However, the statistical analysis showed that the number of eggs produced may be explained by the height and month of sampling (P values <0.001) but not by the species ($P = 0.94$; $z = 0.068$; see "Method 1 " in Supp. File [online only] "Statistics.doc"). However, if the species were grouped by genus (*Haemagogus* vs. *Aedes*), their contributions to the zero-inflated Poisson model were highly significant ($P < 0.001$), as well as that of the month ($P < 0.001$) and the height ($P < 0.01$) (see "Method 2 " in Suppl. File [online only] "Statistics.doc"). This fact was probably because of comparatively lower occurrences of the *Aedes* species in the total sample. The model obtained may be written as follows: number of eggs = e (4.71789 + (height $\times$ -0.1395) + (month $\times$ -0.0935) + (species $\times$ -0.6895), with height in meter, month = 4; 6; 8; 10; 12, species = 1 (*Haemagogus* spp.); 2 (*Aedes* species).

Discussion

In Brazil, the main research areas that led to studies on the vertical distribution of acrodendrophilic mosquitoes were intended to clarify the transmission of sylvatic yellow fever and simian malaria (Guimarães et al. 1985).

The study of sylvatic mosquito fauna found during the sampling period allowed for the observation that *Hg. janthinomys,* even with a quantitatively smaller population relative to *Hg. leucocelaenus,* frequented traps located at the highest level of the canopy. According to behavioral observations on the vertical distribution of *Haemagogus spegazzinii* Brèthes 1921, a strong tendency for positive phototropism by this species (actually *Hg. janthinomys*) was reported by Bates (1947). According to Consoli and Lourenço–de-Oliveira (1994), *Hg. janthinomys* displays a clear preference for biting at the highest levels of the forest and uses very high and almost unreachable tree holes as breeding sites. However, in the observations made by Trapido and Galindo (1957), a significant increase in the percentage of this species captured in locations near ground level was noted. Based on the observations of Guimarães et al. (1985), *Ae. terrens* accounted for 60% of specimens collected from the tree canopy. These results are in accordance with those found in our study. Davis (1944) and Guimarães (1985) reported the finding of *Haemagogus capricornii* Lutz 1904, a Brazilian species able to efficiently transmit the SYF virus (Waddell 1949) almost exclusively in tree canopy, and which exhibits behavior similar to *Hg. janthinomys.* In contrast, we observed that specimens of the genus *Haemagogus* showed eclectic and adapted behavior in their flight tendencies when searching for a host, as well as in their choice of oviposition trap installed at different levels of stratification (Alencar et al. 2005, 2008).

According to Alencar et al. (2008), *Hg. janthinomys* and *Hg. leucocelaenus* species are opportunistic and eclectic in their food habits, an important factor when considering their mobility between the canopy and the ground in the search for hosts or containers for laying eggs. Although the region studied currently has not presented evidence for recent transmission of the SYF virus, given the strong presence of the main vectors of this virus in Brazil, we believe that special attention should be given to monitoring the emergence of febrile diseases among power plant workers in these communities, as well as, residents in the surrounding areas and in the local population.

Acknowledgments

This work was accomplished with the help of Furnas Centrais Elétricas S.A. (contract. P-1031-00-PJ-/08).

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Received 29 May 2012; accepted 20 March 2013.