

## Breeding of *Aedes aegypti* and *A. simpsoni* under the escarpment of the Tanzanian plateau

MILAN TRPIS<sup>1</sup>

*Villages under the escarpment of the Tanzanian plateau were surveyed for breeding of Aedes aegypti. In some places more than 27% of the water containers outside houses harboured A. aegypti larvae, while there was practically no breeding in containers inside houses. From 2% to 10% of tree holes contained A. aegypti larvae. In places, as many as 47 A. simpsoni larvae were collected from one pineapple plant, and the total mean number of larvae per pineapple was 6.6, while the percentage of plants with larvae was as high as 93.6. The total mean number of larvae per colocasia plant was 2.9, but the number per banana plant was only 0.3. The plant Crinum was discovered to be a breeding site of A. simpsoni. Eggs of A. simpsoni were found in 55-80% of ovitraps placed in four villages. Of 20 traps found to contain eggs of this species 30% were in the village, 60% in gardens, and 10% at the edge of forest. It was observed that A. simpsoni females lay their eggs at all levels up to 5 m, but prefer ground level.*

The breeding of *Aedes aegypti* and *A. simpsoni* was surveyed in 12 villages under the escarpment of the Tanzanian plateau from Kidatu, under Kerenga peak in the Gologolo mountains at the entrance to the Great Ruaha Valley, south-west to Ifakara and Mechenga in the Kilombero Valley. The survey was carried out between 26 April and 5 May 1970.

The object of the survey was to gather information on the distribution and size of local populations of yellow-fever vectors, particularly *A. aegypti* and *A. simpsoni* and possibly other so-called forest *Stegomyia*, and to collect live mosquitos for genetic and other studies. More specifically, information was sought on the density of indoor and outdoor breeding of *A. aegypti*, the extent to which *A. aegypti* and *A. simpsoni* breed in tree holes and *A. simpsoni* in plant axils, the populations of the two species, and the presence of other species.

### METHODS

The breeding of *A. aegypti* was assessed by sampling large numbers of larvae collected from different containers, tree holes, and plant axils; the larvae

were taken live to the laboratory for identification. Soil and debris from dry tree holes were placed in polyethylene bags and flooded with water in the laboratory. The water in plant axils was removed with pipettes. Some plants were taken to the laboratory, where the leaves were stripped off and washed. Each plant was kept in a separate pan and left until next morning, when larvae were counted. Plants from 9 villages were examined, 5-10 plants being sampled in each village (Table 1).

In Ifakara, Sanje, and Mangula, bamboo pots 8-10 cm in diameter and 30 cm high were placed in different surroundings to permit a rapid assessment of mosquito populations. The pots were filled with water to a depth of 70 mm and were lined with a paper towel; a 20×120-mm hardboard "paddle", rough on one side, was placed in each pot. In Kisawasa the ovitraps were black glass jars 70 mm in diameter and 130 mm high with similar paper linings and paddles. Eggs laid on the paper and paddles were counted after 5 days, before being submerged in nutrient broth to hatch; after hatching, the larvae were reared to adulthood for final identification.

### RESULTS

#### *Outdoor and indoor breeding of A. aegypti*

*Containers.* Clay pots, buckets, tins, and drums used for storing water were examined in the villages

<sup>1</sup> Entomologist/ecologist, WHO East Africa *Aedes* Research Unit, Dar es Salaam, Tanzania. Present address: Department of Biology, University of Notre Dame, Indiana 46556, USA.

Table 1. Breeding of *Aedes simpsoni* in plant axils

Locality	Plant <sup>a</sup>	No. of plants examined	No. of axils examined	Plants positive		Plants negative		No. of larvae found	Mean no. of larvae per positive plant
				No.	%	No.	%		
Solulu	P	6	186	5	83.3	1	16.7	21	3.5
Sanje	P	11	324	11	100.0	0	0.0	97	8.8
Kiberege	P	7	241	7	100.0	0	0.0	77	11.0
Kisawasawa	P	6	329	6	100.0	0	0.0	31	5.2
Mitalula	P	6	243	6	100.0	0	0.0	21	3.5
Mkula	P	3	173	2	66.7	1	33.3	5	1.7
Kidatu	P	2	60	2	100.0	0	0.0	20	10.0
Naliahe	P	6	314	5	83.3	1	16.7	38	6.3
Sanje	B	18	186	4	22.2	14	77.8	5	0.3
Mangula	C	6	60	5	83.3	1	16.7	9	1.5
Mitalula	C	1	7	1	100.0	0	0.0	3	3.0
Mkula	C	3	22	3	100.0	0	0.0	12	4.0
Kidatu	C	4	24	3	75.0	1	25.0	7	1.8
Kisawasawa	Cr	2	28	3	100.0	0	0.0	39	13.0
	Cr	3	28	3	100.0	0	0.0	39	13.0
	P	47	1 870	44	93.6	93	6.4	310	16.6
	B	18	186	4	22.2	14	77.8	5	0.3
	C	14	81	12	85.7	10	14.3	40	2.9

<sup>a</sup> B, banana; C, colocasia; Cr, *Crinum*; P, pineapple.

on the road from Mechenga near Ifakara to Kidatu. They were grouped according to whether they had been found indoors or outdoors, what they were made of, and the presence or absence of water and of mosquito larvae. In only one village, Mkula, where 30 premises were visited, did a container (1 of 13 examined) provide evidence of indoor breeding of *A. aegypti*. Outdoor breeding of this species was considerable in Ifakara, the container index being 13.0. In Mechenga, a village spread out along the road to Idete west of Ifakara, the outdoor container index was 19.0. *A. simpsoni* and *A. luteocephalus* were also found in man-made receptacles near houses; this is unusual, especially for the latter species, which generally breeds in tree holes.

In Lukamanga, lying in light forest near swamp 20 km north-east of Ifakara, the outdoor container index was the highest (27.2). In Solulu, about 10 km farther, some houses are in woodland and others

near a swamp; the outdoor container index was 12.5. In Kiberege, a large village 10 km farther on along the road, situated in woodland with thick rain forest 5 km to westward, the outdoor container index was 17.6. In Kisawasawa, the next village, also in woodland, it was 24.1. In Sonjo, where the houses are made of sheet metal, only 5 premises were visited and containers were found only indoors. No *A. aegypti* larvae were found, but 57.6% of 220 discarded tires were found to be breeding places of *A. aegypti*. On the previous visit in September 1969 (dry season), positive evidence had been found in only 8% of 300 tires at the same place. The index for the rainy season was thus 7.2 times higher.

Mkula, situated 1 km from Sonjo, had an outdoor container index of 7.1. All containers checked in the next village, Mitalula, were found not to harbour larvae. In the moderate-sized village of Mangula the index was 22.2. In the nearby small village of

Naliahe it was 18.1; *A. aegypti* larvae were found near, but not in, houses. Finally Kidatu, 5 km from the Great Ruaha River, with the Kilombero sugar plantation to the east and Kerenga peak to the west, had an index of 5.3.

**Plant axils.** In Kiberege, Mkula, Mitalula, Naliahe, Kidatu, and Sanje plant axils—most of them of pineapple, banana, or colocasia—were also examined, a pipette being used to draw out the water. Of the 1 215 axils forming the sample, 441 contained water, but in none was *A. aegypti* found to have bred.

**Tree holes.** A total of 318 trees were examined in or near Ifakara, Mangula, and Kidatu, most of them in the villages. Of the 158 trees searched at Ifakara, only 22 had holes, and of these 50% harboured mosquito larvae, but only 9.0% harboured *A. aegypti*. The other species found were *A. simpsoni*, *Toxorhynchites brevivalpis*, *Culex nebulosus*, *Eretmapodites* sp., and *Culex* sp. At Mangula only 10 of the 140 trees searched had holes; 6 of these harboured mosquito larvae and 1 larvae of *A. aegypti*. The other species found here were *T. brevivalpis*, *A. simpsoni*, *Eretmapodites* sp., *C. nebulosus*, and *A. luteocephalus*. In Kidatu, no *A. aegypti* were found to be breeding in the holes discovered in 4 of the 20 trees examined, though 2 of the 4 harboured larvae of *Culex cinereus* and *C. nebulosus*.

**Other breeding places.** *A. aegypti* was also found in banana flower bracts, *Achatina achatina* snail shells, and coconut shells.

#### *Breeding of A. simpsoni in plant axils*

*A. simpsoni* is a predominating species in the Kilombero Valley and particularly in the survey area below the escarpment. The plants of pineapple (*Ananas comosus*), colocasia (*Xanthosoma sagittifolium*), and banana (*Musa sapientum*)—both of the tall "Malindi" variety, which can grow 10 m high and 30 cm in diameter, and of the short "Kiguruwe" variety (1–2 m)—are the commonest breeding places of the species in the area, where breeding in tree holes is not very common and breeding in containers is very rare.

In samples of 5–10 plants from each of the 9 places covered the average number of *A. simpsoni* larvae per pineapple plant varied from 1.7 to 11.0. The overall average was 6.6, but as many as 47 larvae were collected from one plant. The average percentage of pineapple plants with larvae of *A. simpsoni* was 93.6%.

For the total of 14 colocasia plants cut and examined, the average number of larvae per plant was 2.9, and 85.7% of the plants harboured larvae. The average number of larvae per banana plant was 0.3 for the 18 plants examined.

The crinum plant, which belongs to the Amaryllidaceae, was identified by Dr B. Harris of the Botany Department, University College, Dar es Salaam. Three plants of *Crinum* sp. found in Kisawasawa; all harboured larvae of *A. simpsoni*, and the index was 13.0 per plant. This is the first record of *A. simpsoni* breeding in *Crinum*. No other species of mosquito was found to breed in the axils of the plant along the escarpment.

#### *Oviposition of A. simpsoni and A. aegypti*

Rows of ovitraps were set out in Sanje, Mangula, Kisawasawa, and Ifakara. In Sanje, 25 bamboo ovitraps were set in vegetation in a line from the village through gardens up to the edge of thick rain forest. In Mangula 28 bamboo ovitraps were set in a line southwards from close under the hill (550 m) on which the village is situated to a large depression, across a river, and up to a swamp. In Kisawasawa, 24 black glass traps were laid through the village and about 100 m into thick forest. In Ifakara, 29 bamboo traps were set in a garden where pineapple and grapefruit grow.

After 5 days 80% of the traps in Sanje were found to contain *A. simpsoni* eggs; 2 084 eggs were found, giving an average of 104.2 per positive trap. In Mangula the percentage was over 64%, with 1 392 *A. simpsoni* eggs in 18 traps (average per positive trap, 77.3). In Ifakara, the percentage was 55.1%, the total 1 318, and the average 82.4.

Entirely different results were obtained in Kisawasawa, where the glass jars were found to contain eggs of *A. simpsoni* or *A. aegypti*. In all, 18 of the 24 traps (75%) contained eggs of one species or the other, 5 of them (27.8%) harbouring *A. aegypti* and 13 (72.2%) *A. simpsoni*. The total numbers of eggs were 391 and 1 143, respectively, giving averages of 63.8 and 87.9 per positive trap.

**Distribution of ovitraps by biotope.** In Sanje eggs of *A. simpsoni* were found in 6 of the 8 traps set in the village, 12 of the 15 set in gardens, and in both those set at the edge of the forest (30%, 60%, and 10% of the total of 20 positive traps, respectively). The average numbers of eggs per positive trap were 41.6, 134.9, and 102.5, respectively. In Mangula, 3 of the 5 traps placed near houses in the village and 15 of the 23 traps placed in gardens were found to

Table 2. Oviposition of *Aedes simpsoni* at ground level and at 100, 200, and 300 cm

Height above ground level (cm)	No. of traps examined	Traps positive		Traps negative		Total no. of eggs	Mean no. of eggs per trap	Mean no. of eggs per positive trap
		No.	%	No.	%			
0	46	33	71.7	13	28.3	3 567	77.5	108.0
100	17	10	58.8	7	41.2	610	35.9	61.5
200	14	8	57.1	6	42.9	458	32.7	57.2
300	5	3	60.0	2	40.0	159	31.8	53.0

contain eggs of *A. simpsoni*, giving 16.7% and 83.3%, respectively, of the total of 18 positive traps. The average numbers of eggs per positive trap were 42.0 and 84.3, respectively. Gardens were thus shown to be the preferred site of oviposition for *A. simpsoni*.

*Distribution of ovitraps by height above ground.* For the total of 82 bamboo traps in Sanje, Mangula, and Ifakara, the average number of *A. simpsoni* eggs per positive trap was 108.0 at ground level, 61.5 at 100 cm, 57.2 at 200 cm, and 53.0 at 300 cm (Table 2); 98 *A. simpsoni* eggs were collected from one trap that was hung on a palm tree at a height of 5 m. *A. simpsoni* is thus seen to prefer to lay its eggs at ground level, but the species flies as high as the tops of banana plants and trees and can feed on monkeys and birds—a fact of great ecological importance in the cycle of transmission of the yellow-fever virus.

#### Laboratory studies

The larvae from the different containers and breeding sites were taken live to the laboratory for further study. Some were preserved in 70% ethanol and the rest were kept for identification at adulthood.

The *A. aegypti* larvae from Kisawasawa, from the tire dump near Sonjo, and from Mechenga were used to establish laboratory colonies for genetic analysis, as were male and female *A. aegypti* from Kisawasawa and Kiberege.

The several thousand eggs of *A. simpsoni* and *A. aegypti* collected from ovitraps were also brought to the laboratory for further studies. Those of *A. aegypti* from Kisawasawa were hatched in the laboratory and when the larvae reached adulthood they were passed to the geneticist for analysis. The *A. simpsoni* eggs from Ifakara, Mangula, and Sanje were flooded with water in the laboratory, and when

the larvae reached adulthood some were used for an olfactometric host-preference study and the others to establish a laboratory colony.

#### Distribution of mosquito species in the survey area

Ten species of mosquito were found in the survey area: *A. aegypti* (in water pots, buckets, tins, drums, tires, tree holes, bamboo stumps, and flower bracts); *A. simpsoni* (in water pots, plants axils, and tree holes); *A. luteocephalus* (in water pots and tree holes); *A. dendrophilus* (in tree holes); *Anopheles gambiae* (in houses and a ground pool); *Toxorhynchites brevipalpis* (in tree holes and bamboo stumps); *Culex cinereus* (in tins, drums, and tree holes); *C. nebulosus* (in tins and tree holes); *C. duttoni* (in tins); and *C. tigripes* (in tins). Some species of the genus *Eretmapodites*, as well as the *Culex* from tree holes and *Aedes* from tree holes and bamboo stumps, were sent to Mrs van Someren for identification.

All the species in the *Stegomyia* group found during this survey (*A. aegypti*, *A. simpsoni*, *A. dendrophilus* and *A. luteocephalus*) are important as potential vectors of yellow-fever. It is interesting that *C. fatigans* was not found in tins, drums, or other typical breeding places, though it may be present as a scarce species. The incidence of elephantiasis is however quite high in the villages covered by the survey, and it is probable that mosquitos other than culicines—e.g., anophelines, which are quite numerous—are responsible for the transmission of microfilariae.

#### CONCLUSIONS

Generally speaking no evidence was found of the breeding of *A. aegypti* in containers inside houses, the only exception being the larvae discovered in a single water pot indoors in Mkula. The main reason

for the absence of this species is the custom of storing water in containers of only 5–20 litres. These are washed and used to fetch fresh water daily, and any larvae present are thus unable to develop. There is, however, much breeding of *A. aegypti* in outdoor containers near houses—in some places in as many as 27% of those with water in them. It was observed that the female *A. aegypti* feed on man in the area, and it is therefore interesting that the average Breteau index (i.e., the number of positive containers per 100 houses) was 12.3.

There is little breeding of *A. aegypti* in tree holes; larvae of the species were found in only about 10% of the positive tree holes in or near villages. This situation should be compared with that in local rain forest, but to make such a comparative survey for *A. aegypti* and other *Stegomyia* mosquitos would require special equipment and personnel. *A. aegypti* does not breed in plant axils in the area covered by the survey. Eggs of *A. aegypti* appeared in ovitraps only in Kisawasawa, where the black glass jars were used. It would be interesting to investigate whether the material of the jars or the behaviour of the local species is responsible for this phenomenon.

Thick vegetation, high humidity, relatively low temperature, and the proximity of gardens to forest are ecological factors determining the size of the population of *A. simpsoni*, a predominating species in the area, where it bites man. Breeding sites are the abundant pineapple plants growing wild and in gardens, and colocasia and banana plants, though the latter contribute little to the numbers. *Crinum* was also found to be a breeding site of this mosquito. The high density of *A. simpsoni* is confirmed by the count of larvae from the plants mentioned and by the assessment of oviposition, which also reveals that they prefer garden and forest to house and garden biotopes, and choose sites from ground level up to 5 m. From an epidemiological viewpoint this is of considerable importance.

*A. luteocephalus*, *A. dendrophilus*, and *A. africanus* are present near the villages and it is assumed that they are more common towards the rain forest. The presence of these forest *Stegomyia* mosquitos, together with the high density of *A. simpsoni* and the considerable breeding of *A. aegypti*, means that there is a definite risk that yellow fever could circulate if the virus were introduced.

## ACKNOWLEDGEMENTS

The author thanks Professor D. R. Geigy of the Ifakara Rural Aid Centre of the Swiss Tropical Institute, Basle, for providing accommodation, laboratory space, and the use of equipment at the Centre and Dr and Mrs V. Schuppler for their hospitality and their help with laboratory work. He also thanks the mosquito crew, Lucas

Mahikwano, Seifu Ungando, and Charles Peter, and the driver, Musa Salehe, for their skilful work. This study was supported by Public Health Service research grant No. CC 00261 from the Center for Disease Control, Atlanta, Ga., USA.

## RÉSUMÉ

### REPRODUCTION D'*Aedes aegypti* ET D'*A. simpsoni* DANS UNE RÉGION SITUÉE SOUS L'ESCARPEMENT DU PLATEAU TANZANIE

On a enquêté sur le nombre et la répartition des populations d'*Aedes aegypti* et d'*A. simpsoni*, ainsi que sur la présence d'autres *Stegomyia* vecteurs potentiels de fièvre jaune et d'autres espèces de moustiques, dans 12 villages situés au pied de l'escarpement du plateau tanzanien, entre Kilombero et la vallée de Great Ruaha.

*A. aegypti* se reproduit très activement dans les gîtes artificiels situés à l'extérieur des habitations; dans certains endroits plus de 27% d'entre eux étaient occupés par des larves de cette espèce. Par contre, on n'a pratiquement pas décelé sa présence dans les récipients disposés à l'intérieur des maisons. On a trouvé des larves d'*A.*

*aegypti* dans 2 à 10% des creux d'arbre examinés, mais une prospection très poussée n'a fait découvrir aucune larve dans l'eau accumulée à l'aisselle des feuilles de végétaux.

*A. simpsoni*, espèce dominante dans la vallée de Kilombero, installe généralement ses gîtes à l'aisselle des feuilles d'ananas, de colocases et de bananiers. Le développement a rarement lieu dans les creux d'arbre et il est exceptionnel dans les récipients disposés à l'extérieur des habitations. Aucune larve n'a été récoltée à l'intérieur des maisons. Les ananas fournissent un très grand nombre de gîtes à *A. simpsoni*. Sur l'un d'eux, on a

recueilli 47 larves. Le nombre moyen de larves récoltées par plante était de 6,6 et la proportion des ananas hébergeant des larves de 93,6%. Sur 14 colocases examinées, 85,7% hébergeaient des larves avec un nombre moyen de 2,9 larves par plante. Très peu de larves ont été trouvées sur les bananiers (0,3 larve en moyenne). Enfin, on a découvert pour la première fois des gîtes d'*A. simpsoni* sur *Crinum* sp., plante de la famille des amaryllidacées.

On a réparti une centaine de pondoirs pièges dans 4 villages. Après 5 jours, 55 à 80% d'entre eux contenaient

des œufs d'*A. simpsoni*. Des œufs d'*A. aegypti* n'ont été découverts que dans les pondoirs installés dans le village de Kisawasawa, où près de 28% d'entre eux étaient positifs.

La proportion des pondoirs renfermant des œufs d'*A. simpsoni* variait suivant le biotope: 30% dans les villages, 60% dans les jardins et 10% à l'orée de la forêt. L'examen de certains d'entre eux, diversement étagés, a montré que les femelles d'*A. simpsoni* effectuaient leur ponte jusqu'à 5 m de hauteur, mais préféraient déposer leurs œufs au niveau du sol.