Ovipositional behavior of *Aedes aegypti* (Diptera, Culicidae) in different strata and biological cycle [1]

Comportamento de oviposição de *Aedes aegypti* (Diptera, Culicidae) em diferentes estratos e ciclo biológico [1]

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The seasons that have dry and/or cold weather are not proper for development of *Aedes aegypti*. In those seasons, natural control occurs, reducing the population density, and under their influence, the eggs logical cycle of *A. aegypti*, and is also an important form of passive dispersion and recrudescence of populations of mosquitoes, mainly when related to the present matter of solid non-degradable trash that do not have the proper destiny. This works as breeding environments for mosquitoes, increasing the population density of *A. aegypti* and making easier the transmission of dengue.

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This work had the aim to understand the meaning and the importance of the fact that *A. aegypti* lays eggs in different strata in breeding environments, which will be achieved by the analyses of eclosion rate and development of the biological cycle of the mosquito of each stratum. This will bring up useful information for planning programs of control and biology of the most important vector of dengue and yellow fever, in cosmotropical areas of the world.

MATERIAL AND METHODS

This work begun with the breeding of *A. aegypti*, which has been kept in the Laboratory of Biology and Physiology of Insects, in the Federal University of Goiás. The mosquitoes were bred according to the technique of SILVA *et al.* (1998). The adults were kept in coupling cages, totaling 30 couples. For female feeding, albino mice were used, which were locked inside a nylon net, with the head bent over the belly. Externally, the net was locked by the use of cramps. The males were fed with sugar solution soaked in cotton (female internal absorbent). Sometimes females would feed on this device.

Inside the coupling cage, an amber glass with one third of its capacity filled with water was put and it also contained a part of a paper filter as a layer, where females could lay their eggs. This paper filter was divided, from the part of contact with water, into three parts measuring 1.5 cm each, which were considered egg-laying strata. These parts were marked with circle lines, so as to follow the diameter of the glass. The glass was covered by a cone made out of light cardboard, cut on the top, which made the environment more calm and more appropriated for coupling and laying, fact based in previous experience. Observation took place during longevity of adults.

The experiments occurred in an acclimatized chamber with temperature 28 ± 1°C, moisture 80 ± 5% and 12h photoperiod.

For the study of the biological cycle, using eggs from different strata, one sample of each stratum was taken and observed. The

incubation occurred after immersion of the eggs in water, in white enamelled basins, according to SILVA, SILVA & LIRA (1998). After immersion of the eggs, the basins were examined every 2 hours to check out the eclosion of larvae.

After eclosion, 1st instar larvae were removed using a glass pipette and individualized in plastic tubes. These tubes were numbered and put in plastic trays separated for each extract. To facilitate observation of 1st instar larvae, a light focus from a torch was used on the basin. Larvae were fed immediately after eclosion, with cat food ground in tiny particles (SILVA, SILVA & LIRA, 1998). Using the tip of a plastic spatula, which could collect approximately 1.9mg of cat food, this amount was put inside each tube. In pupa instar, the water of the tube was changed, to which was attached, using an attaching tape, another tube, with the same features, and that remained so until adult emerged.

For comparison of the number of eggs per stratum, the variable used was "changed egg counting". The heterogeneity Qui-square was used to determine sex and Tukey test was used for comparison between cycles and strata.

RESULTS AND DISCUSSION

A. aegypti has the behavior of laying eggs on the wall of the breeding device in three well defined strata, with reference of the level of the water. In 1st stratum, the amount of eggs was significantly higher than on surface and on 2nd and 3rd strata, by Tukey test at 1% level (Fig.1). ROBERTS & HSI (1977) studied five probable attractants for egg deposition and showed that there were significant differences in vertical distribution of eggs, and that the presence of an attractant resulted in a higher proportion of eggs next to water surface. In this work, the results were similar, but no attractant was used, which enforces the idea that the behavior of laying eggs in strata may be related to egg quiescence and mosquito exposure to different periods of weather and rain.

The amount of eggs laid on water surface represented about 8% of the eggs, from which only 2% presented viability, complet

Table 1. Average duration of the biological cycle (in days) of *A. aegypti*, obtained from the eggs from different oviposition strata (n=30).

Strata	Incubation	larvae of: 1st instar	2nd instar	3rd instar	*4th instar*	*Pupa*	*Biol. cycle*
1°	57.8?±2.85a	1.5±0.05a	1.2±0.04a	1.4?±0.7a	5.2±?0.28a	2.0?±0.04a	68.4±?2.92a
2°	49.7?±2.89a	1.5±0.05a	1.0?±0.04a	1.2?± 0.04a	4.7±0.29a	2.0?±0.04a	60.2?±2.96a
3°	54.9?±2.96a	1.5±0.06a	1.2±?0.06a	1.4± 0.07a	5.0±?0.37a	2.0?±0.06a	65.9±3.07a

Means within a column followed by the same letter are not significantly different by the Tukey´s test, (p=0.05).

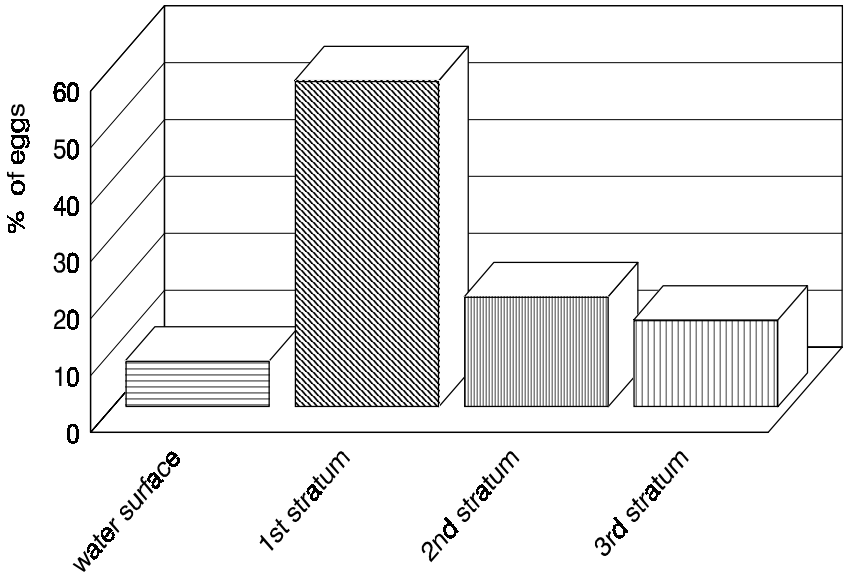

Fig. 1. Percentage of eggs of *A. aegypti according to oviposition strata.*

ing the cycle with not enough number of mosquitoes, which made not possible the expression of results for the lack of statistical significance. Those results drive to the necessity that *A. aegypti* have to lay eggs in a substratum above water surface, so that the eggs become quiescent, increasing its viability, as shown by Fig. 2. These results agreed with SILVA & SILVA'S (1999).

The eclosion rate was the same in the three strata, at 5% level (Fig. 2). The eclosion rate of the eggs put on water was significantly lower than that of the strata.

Fig. 2. Oviposition of *Aedes aegypti* in strata obtained at laboratory. The glass with paper filter for oviposition. B - Distribution of the eggs in the paper filter.

It was shown that evolution cycles beginning from the strata were statistically equal, by Analyses of Variance, at 5% level (Table 1).

There was no influence of the stratum in sex determination, at 5% level. The strata were homogenous in proportion of emergence of 1:1.

This habit of the mosquito *A. aegypti* of laying eggs on the walls of the breeding device could mean a mechanism to increase dispersion and density. This behavior is important to control this mosquito, and that is because the eggs attached to recipient walls resist to desiccation and hatch later when the level of water rises. There comes the importance of sending non-degradable trash, such as plastic, glass, rubber and others, to proper destiny.

After eclosion, a group of each stratum was observed in simultaneous period and the duration of larval and pupa period was statistically the same. In similar climatic conditions, SILVA *et al.* (1995) observed the same behavior. Experiments to verify the selection of laying sites of *A. aegypti* showed that it preferred liquid surface to humid and rough (O'GOWER, 1947). In this work, after following

daily laying, an opposite behavior was seen, in which females would rather lay eggs on humid and rough surface, which contained three well defined strata above water surface (Fig.1).

It sounds reasonable to presume that *A. aegypti* lays eggs in strata because of the possibility that eggs can get into quiescence, considering that this situation facilitated eclosion (SILVA, 1999) and that quiescence did not occur with eggs laid on water surface. Another probable reason is that the mosquito use this mechanism to increase population of adults in nature, in periods that have more chances of survival. Beyond that, strata would permit emergence of populations, according to rain periods. That would be a form of permitting incubation of eggs on three strata, according to gradual rise on water level in breeding devices, as it happens in rainy seasons or; resisting to unfavourable conditions, such as in dry seasons.

SUMMARY

Ovipositional behavior, biological cycle, fertility and eclosion rate of the larvae of *Aedes aegypti* were studied in laboratory, all data related to three different laying strata, situated 1.5, 3.0 and 4.5 cm above water surface. The experiments were realized in a biological chamber, kept at 28 ±? 1°C temperature, with 80 ±? 5% of relative humidity and 12 hours of photophase. The biological cycle of *A. aegypti* studied from eggs which came from the 1st, 2nd and 3rd laying strata had average duration of 68, 60 and 65 days, respectively. There was no significant differences between the cycles neither between eclosion rate of larvae. Among three laying strata, the 1st had an amount of egg significantly higher, at 1% level. The average fertility was the same in three strata, but was significantly lower in eggs laid on water surface, at 5% level.

KEY WORDS: Insecta, mosquitoes, yellow-fever.

RESUMO

Estudou-se o comportamento de oviposição, ciclo evolutivo, fertilidade e taxa de eclosão das larvas de *Aedes aegypti* em laboratório, relacionados aos três diferentes estratos de postura, situados a 1,5; 3,0 e 4,5 cm acima da superfície da água. Os experimentos foram realizados numa câmara biológica, climatizada a 28 ± 1°C, com umidade relativa de 80 ± 5% e fotofase aproximadamente de 12 horas. O ciclo biológico de A.

aegypti a partir de ovos provenientes do 1°, 2° e 3° estratos de postura teve duração média de 68, 60 e 65 dias, respectivamente. Não houve diferenças significativas entre os ciclos e nem entre a taxa de eclosão das larvas. Entre os três estratos de postura, o 1º teve uma quantidade de ovos significativamente maior, ao nível de 1%. A fertilidade média foi igual nos três estratos, mas foi significativamente menor nos ovos colocados na superfície da água, ao nível de 5%.

PALAVRAS-CHAVE: Insecta, Diptera, febre-amarela.

RÉSUMÉ

Le comportement pendant l' oviposition, le cycle biologique, la fertilité et le taux d'éclosion de l' *Aedes aegypti* ont été étudiés en conditions de laboratoire, relatifs a 3 sphères de l' oviposition, 1.5, 3.0 et 4.5 cm au-dessus de la surface de l' eau. Les essais ont été réalisés dans une chambre climatisée, régulée à 28 ± 1°C, 80% humidité relative et une photophase de 12 h. Le cycle biologique de l' *A. aegypti* étudié à partir des oeufs dans les 3 sphères avait une durée moyenne de 68, 60 et 65 jours, respectivement. Des différences significatives n'ont pas été observées, ni entre les cycles, ni entre les taux d' éclosion des larves. Parmi les 3 sphères de l' oviposition, la première avait un nombre d' oeufs significativement élevé, au niveau de 1%. La fertilité moyenne fût la même dans les trois sphères, mais le numéro d'oeufs, déposés sur la surface de l' eau fût significativement inférieur, au niveau de 5%.

MOTS CLÉS: Insecta, Diptera, fièvre-jaune

BIBLIOGRAFIA

LE VAN, J. H. 1940. Viability of *Aedes aegypti* eggs. *V. S. Pub. Hlth. Repts. 55*: 900.

O'GOWER, A. K. 1947. The influence of the surface on oviposition by *Aedes aegytpi* (L.) (Diptera, Culicidae). *Proc. Lin. Soc. N. S. Wales 82*: 240-244.

ROBERTS, D.R. & B. P. A. HSI. 1977. A method of evaluating ovipositional attractants of *Aedes aegypti* (Diptera: Culicidae), with preliminary results. *J. Med. Entomol. 14*: 129-131.

SHANNON, R.C. & P. PUTNAM. 1934. The biology of *Stegomyia* under laboratory conditions. I. The analysis of factors which influences larval development. *Proc. Ent. Soc. Wash. 36*: 185-216.

SILVA, H. H. G. & I. G. SILVA. 1999. Influência do período de quiescência dos ovos sobre o ciclo de vida de *Aedes aegypti* (Linnaeus, 1762) (Diptera, Culicidae) em condições de laboratório. *Rev. Soc. Bras. Med. Trop. 32*: 349-355.

SILVA, H. H. G.; I. G. SILVA; C. N. ELIAS; S. P. S. LEMOS; A. P. ROCHA. 1995. Idade fisiológica de *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera, Culicidae). *Rev. Pat. Trop. 24*: 269-273.

SILVA, H. H. G.; I. G. SILVA; K. S. LIRA. 1998. Metodologia de criação, manutenção de adultos e estocagem de ovos de *Aedes aegypti* (Linnaeus, 1762) em laboratório. *Rev.Pat.Trop. 27*: 53-63.

SILVEIRA-NETO, S.; S. O. NAKANO; N. BARBIN; N. A. VILLA-NOVA. 1976. Fatores ecológicos, p.34-76. In: *Manual de Ecologia dos Insetos.* Ed. Agronômica Ceres Ltda. 419pp.

WIGGLESWORTH, V. B. *Insect Physiology*. 7th ed., Tokyo, Toppan Co. Ltd, 1974. 166 pp.

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