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Short Communication

Development of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Larvae Feeding on the Plant Material Contained in the Water

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Keywords

- *Aedes aegypti*
- *Aedes albopictus*
- Plant material
- Log-probit analysis
- Trophic performances

Abstract

Background: In this laboratory study we measured the trophic performances of *Aedes aegypti* and *Aedes albopictus* larvae (Diptera: Culicidae), both being vectors of the dengue fever, chikungunya and zika virus in the world.

Methods: Depending on the quantities of plant material contained in the breeding sites, the bioassays enabled to assess the times it took for 50% of imaginal emergence to occur (IEt_{50}). They also enabled to determine the amounts of plant material needed for 50% of adult emergence (IE_{50}).

Results: Water containing 3.3 g of plant material per liter allowed 50% of *Ae. albopictus* adults to emerge within 8 days (IEt_{50}), against 60 days at 0.5g/liter. As for *Ae. aegypti*, the IEt_{50} took 8 days at 3.3g/l against 29 days at 1.7g/l. The IE_{50} also revealed that 0.61g of plant material were needed for a 50% of adult emergence of *Ae. albopictus*. To reach the same survival rate among *Ae. aegypti*, the larvae must grow in an environment twice as rich in food supply.

Conclusion: This research work has revealed that the *Ae. albopictus* larvae can develop in water collections where the shortage of organic material hinders or compromises the development and survival of *Ae. aegypti*. The outstanding trophic performances of *Ae. albopictus* would thus partly account for the invasive character of *Ae. albopictus*, as well as the dying out of its competitor *Ae. aegypti* in the regions of the world shared by both species.

ABBREVIATIONS

PM: Plant Material; $IE50$: Imaginal Emergence Time 50%; $IE50$: Imaginal Emergence 50%

INTRODUCTION

The distribution of mosquito populations mostly depends on the physico-chemical and biological characteristics of the breeding sites. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) develop in clear domestic and peridomestic water collections, such as earthenware vases, barrels, cisterns, gutters, cans, tires and plant saucers [1-2]. These two mosquitoes are the major vectors of the dengue fever, chikungunya and zika virus in the world [3-10]. It is now clearly stated that the population growth of *Ae. aegypti* and *Ae. albopictus* larvae is closely associated with the nature of the

resources available [11-13]. If the source of food mainly consists in plant detritus (deciduous and coniferous leaves, flowers and grass) it remains quite rich in cellulose and hard to digest for mosquito larvae [14-15]. In this laboratory study, the bioassays consisted in a follow-up of larvae batches until the emergence of adults, whose larvae grew up in environments with different contents of plant material (dry grasses). The follow-up allowed to assess the times taken by the larvae to develop, depending on the availability plant material (IEt_{50}). The quantities of dry grasses needed for each species to reach a 50% adult emergence were also determined (IE_{50}).

MATERIALS AND METHODS

The Bora strain of *Ae. aegypti*, originally from French Polynesia, has been reared for more than 20 years in our laboratory. The

Ae. albopictus strain used in this study comes from the village of Perols located south of the Hérault department (France). This strain of *Ae. albopictus* has been raised in the Institute of Research for Development (IRD) insectarium in Montpellier (France) since September 2014. The plant material (PM) used for the preparation of the larval environment consisted in commercial rodent food (hay) that can be found in pet shops (Zolux®). The quantities of PM assessed on *Ae. aegypti* and *Ae. albopictus* were of 0.5g/l - 1g/l - 2.5g/l and 3.3g/l. A 0.25g/l content was tested on *Ae. albopictus* only and 1.7g/l on *Ae. aegypti* so as to characterize the trophic performances of these two species of mosquitoes more precisely. PM was prepared in 0.0042 m³ plastic trays (length: 0.30m; width: 0.20m; depth: 0.07m), each containing one liter of reverse osmotic water. 24 hr after the preparation of the larval environments, one hundred first instars larvae (L1) of *Ae. aegypti* or *Ae. albopictus* were counted and placed in a tray. Each artificial milieu was evaluated on a total of three replicates. Throughout the duration of the experiment, the trays were maintained at a temperature of 27 ± 2°C in the laboratory.

Female and male adults were counted in each environment to establish the averages of imaginal emergence with a 95% confidence interval [16]. The larval environments allowing more than 50% of adult emergences were analyzed using the log-probit software [17] so as to determine the duration of preimaginal developments leading to 50% of imaginal emergence (*IE*₅₀). Based on this same log-probit analysis, the quantity of plant material needed for 50% adult emergence was assessed as well (*IE*₅₀).

RESULTS

Figure 1 shows the averages of male and female emergences in the different larval environment. The imaginal emergences of *Ae. albopictus* were above 50% with only 0.5g of PM per liter. As much as 1.7g/l of PM are needed to reach the same survival rate of *Ae. aegypti*. Except for the 2.5g/l quantity of PM, for which the averages of male and female *Ae. albopictus* proved statistically different (*P* = 0.03), the other quantities tested on both strains did not display significant differences (0.094 < *P* < 0.89). The times needed for 50% of adult *Ae. albopictus* and *Ae. aegypti* to emerge (*IE*₅₀) (Table 1) were 8 and 11 days respectively, with 3.3g and 2.5g of PM per liter. *IE*₅₀ exceeded a period of one month for 1g/l and equaled two months for 0.5g/l. At the concentration of 1.7g/l, 50% of *Ae. aegypti* adults emerged after 29 days of study. Whatever the species studied, it is worth noticing the regression line slopes steepened as the quantities of PM increased. This observation simply shows that the more food in the environment, the faster the larval growth.

A quantity of 0.61g of PM per liter is enough for 50% (*IE*₅₀) of the *Ae. albopictus* larvae to develop all the way to imaginal stage (Table 2). For a similar survivorship with *Ae. aegypti* larvae, twice as much organic material is needed in the water. The difference of trophic performances translates in log-probit regression lines whose slopes almost double depending on the species. *Ae. albopictus* larvae's ability to grow in environments that can be either poor or rich in organic matter (Figure 1) will produce a more gradual response in adult with a gentler slope of the regression line.

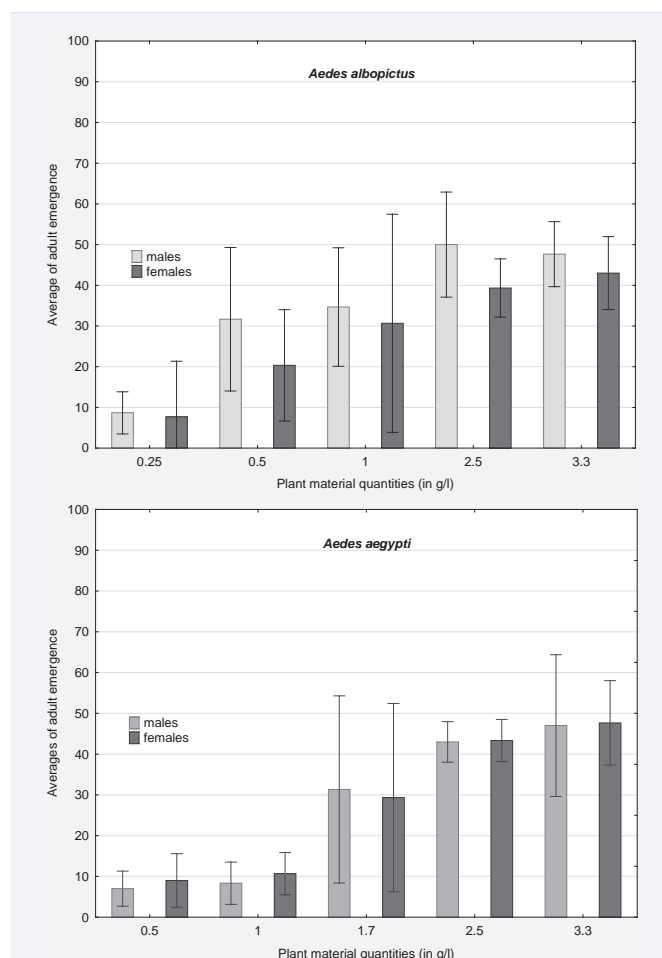


Figure 1 Averages (95% CI) of males and females mosquitoes whose larvae lived in environments containing different quantities of plant material.

Table 1: Determination for each mosquito species of the value of imaginal emergence time 50% (*IE*₅₀), estimated from the log-probit analysis.

Species	Plant material quantities (g/l)	<i>IE</i> ₅₀ (in day) (95% CI)	Slope (± SE)
<i>Aedes albopictus</i>	0.5	60.2 (56.7-64.6)	2.01 (± 0.13)
	1	35.7 (34.7-36.8)	2.6 (± 0.10)
	2.5	10.7 (10.3-11.1)	4.9 (± 0.3)
	3.3	7.7 (6.4-9.1)	5.9 (± 0.6)
<i>Aedes aegypti</i>	1.7	28.8 (27.3-30.6)	1.9 (± 0.14)
	2.5	11.1 (10.7-11.4)	5.4 (± 0.3)
	3.3	8.2 (7.9-8.4)	5.9 (± 0.3)

DISCUSSION

Mosquito larvae breed in water collections whose physico-chemical and biological characteristics differ a lot from one

Table 2: Determination for each mosquito species of the value of imaginal emergence 50% (IE_{50}), estimated from the log-probit analysis.

Species	IE_{50} (in g/l of plant material) (95% CI)	Slope (\pm SE)
<i>Aedes albopictus</i>	0.61 (0.49-0.76)	1.98 (\pm 0.23)
<i>Aedes aegypti</i>	1.3 (1.0-1.7)	3.4 (\pm 0.7)

site to another [18,11,19]. The bioassays carried out on *Ae. albopictus* showed the larvae needed 0.61g of plant material per liter of water to ensure a 50% adult emergence (IE_{50}). To reach the same percentages, twice as much organic matter (1.3g/l) is needed for *Ae. aegypti* larvae. As for the development time of the preimaginal stages more specifically, the bioassays carried out on *Ae. albopictus* showed that water containing 3.3g of plant material per liter allowed a 50% adult emergence within 8 days (IEt_{50}). At 0.5g/l, 60 days are eventually needed to get the same percentage. *Ae. aegypti* IEt_{50} will take 8 days at a concentration of 3.3g/l as opposed to 29 days at 1.7g/l. The slowing down of the larvae growth in environments lacking in food may have impacted the range geographic expansion of these two mosquitoes. The colonization of Americas by *Ae. aegypti* from its native African forests took place during the 17th century thanks to the ships sailing the Atlantic ocean. The eggs of *Ae. aegypti* are known for their resistance to desiccation which allows them to be transported over long distances and extended stretches of time. At a time when travelling between Africa and the Americas took several months, drinkable water containers were the perfect shelter for the mosquito larvae. The emerging females would feed on the crew members and were therefore able to keep their biological cycle going all along the ocean crossing [15,20]. As for *Ae. albopictus* it must have been disseminated by the Indonesians from south-east Asia to Madagascar and the surrounding islands two thousand years ago [20]. However the more recent international trade of tires between Asia, the United States and Europe has played a predominant part in its propagation. The eggs of *Ae. aegypti* and *Ae. albopictus* resisting desiccation greatly contributed to their scattering worldwide, along with the larvae surviving the transoceanic crossings. Whether natural or artificial, the water collections where the mosquito larvae grow keep being replenished in organic detritus thanks to animal carcasses, grasses, leaves, flower and so on. Many fungi and cellulolytic bacteria contribute to the cellulose degradation [21]. The cellulose that represents 35 to 50% of the dry matter of plant is hard to digest by the mosquito larvae [14] even if the bacteria living in the guts of the mosquito larvae play an important role in the food assimilation (Minard et al., 2013) [22].

This research work undoubtedly shows that *albopictus* develops in water collections where the shortage of organic material hinders or compromises the development and survival of *Ae. aegypti*. The outstanding trophic performances would therefore partly account for the invasive character of *Ae. albopictus*, as well as the dying out of its competitor *Ae. aegypti* in the regions of the world shared by both species [23-25].

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