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Effect of Diet Composition on the Development of the Floodwater Mosquito *Ochlerotatus* (*Ochletotatus*) *albifasciatus* (Macquart) (Diptera: Culicidae)

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Efecto de la Composición de la Dieta sobre el Desarrollo del Mosquito de Inundación *Ochlerotatus* (*Ochletotatus*) *albifasciatus* (Macquart) (Diptera: Culicidae)

RESUMEN - Un paso importante para la colonización de *Ochlerotatus albifasciatus* (Macquart) es conocer las condiciones óptimas para la cría de las larvas, lo cual además posibilita el desarrollo de experimentos en el laboratorio. En este trabajo se estudió el efecto de la dieta sobre el desarrollo de *O. albifasciatus*. Para ello se criaron cohortes de 20 larvas I utilizando cinco tipos de dietas: una mezcla 1:1 de levadura y TetraMin®, una mezcla 1:1 de materia orgánica fina y gruesa, gramíneas cortadas y sumergidas en agua 1h o 24h antes de incorporar las larvas, y una mezcla 1:1:1 de gramíneas cortadas, materia orgánica fina y materia orgánica gruesa. Para cada cohorte se registró la supervivencia, y para cada individuo, el tiempo de desarrollo desde larva I hasta pupa y la longitud del ala de los adultos. El efecto de la dieta sobre estas características se analizó mediante un ANOVA de una vía. Los resultados mostraron que la dieta afecta la supervivencia y la longitud del ala, obteniéndose los mayores valores de estas variables (79-100 % y ~ 4,46 mm) con las dietas consistentes en materia orgánica o materia orgánica más gramíneas. El tiempo de desarrollo no estuvo afectado por la dieta, aunque el tiempo más corto (8,1-8,3 dias) y la menor variación entre réplicas se registraron al criar las larvas con materia orgánica o materia orgánica más gramíneas. Se concluye que las dietas consistentes en materia orgánica o una mezcla de ésta y gramíneas, son las más efectivas para la cría de *O. albifasciatus*.

PALABRAS CLAVE: Supervivencia, tiempo de desarrollo, longitud del ala, alimento, cría de larvas

ABSTRACT - One important step for the colonization of *Ochlerotatus albifasciatus* (Macquart) is to determine the optimal conditions for larval rearing, which makes possible the development of experiments comprising larval rearing in the laboratory. In this research the effect of diet composition on the development of *O. albifasciatus* was studied. For this purpose, cohorts of 20 first instars were reared using five diets: 1:1 mix of yeast and TetraMin®, 1:1 mix of fine and coarse organic matter, grass cuts soaked in water for 1h or 24h before larvae incorporation, and 1:1:1 mix of grass cuts, fine organic matter and coarse organic matter. Survival was recorded for each cohort, while development time from first instar to pupa, and adult wing length were recorded for each individual. The effects of the diet on the observed traits were analyzed by one way ANOVA. Both survival and wing length were affected by diet, being significantly higher (79-100% and ~ 4.46 mm) on diets consisting of organic matter or organic matter plus grass than on those consisting of yeast plus TetraMin® or grass. Development time was not affected by the diet, although the shortest time (8.1 to 8.3 days) and lower variation between replicates were recorded when larvae were reared using organic matter or organic matter plus grass. Thus, it is concluded that the diets consisting of organic matter or a mix of it and grass are the most effective for *O. albifasciatus* rearing.

KEY WORDS: Survival, development time, wing length, food, larval rearing

Ochlerotatus (*Ochlerotatus*) *albifasciatus* (Macquart) is a floodwater mosquito widely distributed in the southern region of South America. It is found from tropical Brazil and Bolivia to Tierra del Fuego in Argentina (Darsie & Mitchell 1985, Forattini *et al*. 1988). The study of this mosquito is important since it is indicated as the main vector of the western equine encephalitis virus in Argentina (Avilés *et al*. 1992). In addition, *O. albifasciatus* is a sinanthropic and rural species which insistently attacks human and animals (Forattini 1965), and as a consequence of its bites, it can reduce caws milk production in some areas of Argentina (Ludueña Almeida & Gorla 1995).

Colonization of this species was not possible yet, and it is an important step for the development of laboratory studies, such as biology (which will provide valuable information for its control), on resistance to insecticides, and on its role in disease transmission. Although the main factor that interferes in *O. albifasciatus* colonization is the obtaining of mating in laboratory conditions, the optimal conditions for larval rearing are still to be determined. Among these conditions, larval nutritional requirements are one of the most important, and it is not only necessary for the maintenance of a colony, but also for the development of experiments comprising larval rearing in the laboratory. For example, it is not possible to evaluate the effect of factors such as competition or temperature during development if larvae are dying or having a suboptimal growth because of the diet. For this type of studies it is important to find a diet that does not affect larval survival and development, so the response could be attributable to the studied factor.

For many mosquito species, favorable food quantity and quality for larval rearing are known. The diets most commonly used consist of commercial products since they are more homogeneous than natural food, and their acquisition and administration are easier for the maintenance of large colonies. A diet combining commercial products was successful for rearing some species of *Aedes* and *Ochlerotatus* (Gerberg 1970), but it failed in supporting development for other species and/or genera. As a consequence, natural components have been incorporated into the diets (e.g. Chew & Gunstream 1964). Regarding *O. albifasciatus*, there are not specific studies on larval diet, and some studies which included larval rearing led to heterogeneous results. For example, a low mortality was obtained when using powdered rabbit balanced food (Ludueña Almeida & Gorla 1995). On the contrary, a diet containing liver powder led to high mortality (Gleiser *et al*. 2000b, Fava *et al*. 2001). In an attempt to obtain adults in the laboratory, a high mortality was obtained when rearing larvae using yeast as food (Sy, pers. obs.).

The aim of this research is to study the effect of food type on the development of *O. albifasciatus* immatures, in an attempt to determine the most favorable diet for rearing this species for experimental purposes. The hypothesis tested is that diets including natural components would be more efficient for *O. albifasciatus* larval rearing than diets consisting of artificial components. To test this hypothesis, larvae were reared using natural or artificial ingredients, and the efficiency of the diet was assessed by evaluating immature survival, development time and adult size.

Material and Methods

Eggs used for the experiment were obtained from *O. albifasciatus* females collected at Pereyra Iraola Provincial Park (34º5'S, 58º08'W) Buenos Aires Province, Argentina. Females were blood fed with a guinea pig and then transferred into individual tubes conditioned for oviposition with a moist piece of towel paper resting on a cotton pad at the bottom of the tube. After oviposition, towel papers containing the eggs were taken off the tubes and were transferred into nylon bags, where eggs remained for five to six months at 25 ± 2ºC. In order to obtain larvae for the experiments, each towel paper containing eggs was transferred into a petri dish containing a solution of approximately10 mg of yeast in 40 ml of distilled water. After 24h, larvae were pulled and cohorts of 20 individuals were separated for each treatment and replicate.

Diets used for larval rearing were elaborated by combining different food types: (1) commercial powder yeast; (2) TetraMin® "L", a commercial baby fish food; (3) organic matter; and (4) grass cuts. Elements (3) and (4) were obtained from one of the *O. albifasciatus* larval sites at the Pereyra Iraola Provincial Park. This site is enclosed in an area surrounded by trees (*Liquidambar* sp., *Quercus* sp. and *Platanus* sp.) whose leaves cover the soil when the larval site is dry, producing a layer of organic matter among the grass. Organic matter was removed from the surface up to 10 cm deep, and dried in an oven at 60ºC for 48h. The material was then separated into pieces of two different sizes each by passing it through a set of 5, 3 and 0.5 mm mesh sieves, and separated as coarse (between 3 and 5 mm) and fine (between 0.5 and 2.9 mm) organic matter. Grass was manually cut, dried in an oven at 60ºC for 48h, and cut again into pieces of less than 5 cm in length.

These four basic foods were used to elaborate five diets (treatments) that differed in their composition and mode of administration: yeast/TetraMin®: 140 mg of a 1:1 mix of yeast and TetraMin® provided every two days since the day larvae were transferred into the rearing pans. Organic matter: 32 g of a 1:1 mix of fine and coarse organic matter soaked in water 24h ahead the incorporation of the larvae. Grass (24h): 16 g of grass cuts soaked in water 24h ahead the incorporation of the larvae. Grass (1h): 8 g of grass cuts soaked in water 1h ahead the incorporation of the larvae. Organic matter/grass: 24 g of a 1:1:1 mix of grass cuts, fine organic matter and coarse organic matter soaked in water 24h ahead the incorporation of the larvae.

The experiment was conducted in 20 x 30 x 6 cm pans containing the administered food, 2 liters of distilled water and 20 first instars (LI). Water was added daily to the rearing pans to replace the water lost by evaporation. For each treatment four replicates were used. Pans were maintained in a room at variable temperature and 12:12 (light: dark hours) photoperiod. The maximum and minimum air temperatures were daily recorded at midday from the beginning of the experiment until adult emergence, giving an average of 21.0 ± 2.1ºC (n = 22) during the experiment.

Pans were examined every 24h and the day of pupation was recorded. Pupae were transferred into individual tubes (6 cm high x 3 cm in diameter) containing 6 ml of distilled water. Twenty-four hours after adult emergence, mosquitoes were killed with cyanide and males were discarded. Subsequently, one wing was removed from each female and measured to the nearest 0.01 mm using a dissecting microscope fitted with a graduated eye-piece. The measurement was taken from the alular notch to the distal margin excluding the fringe scales (Packer & Corbet 1989).

For each cohort (pan), survival (number of pupae/number of LI), mean female development time from LI (≤ 24h old) to pupae, and mean female wing length were calculated. Data was subjected to one way ANOVA or, alternatively, the equivalent non-parametric Kruskal-Wallis Test, and averages were compared by either the Fisher Test (LSD) or the Student-Newman-Keuls Test (for parametric or non-parametric analysis, respectively). The variable survival was arcsine-transformed before analysis using a modification of the Freeman and Tukey transformation (Zar 1996).

Results and Discussion

Survival and wing length of *O. albifasciatus* varied according to the rearing diet (Survival: F = 8.31; d.f. = 4, 15; P < 0.001; Wing length: H = 14.32; d.f. = 4; N = 20; P < 0.01), but no difference was detected for the development (Development time: F = 1.49; d.f. = 4, 15; P = 0.25).

Survival was the most affected trait, with diets yielding from 20% to 100% survivorship. The highest survival was observed in the organic matter treatment, where all individuals pupated. This treatment showed significant differences with all but with the organic matter/grass treatment. The mean survival was also high in the organic matter/grass and the yeast/TetraMin® treatments, but with the yeast/TetraMin® diet, a high variation between replicates was found. The low survival in some replicates of this treatment could be associated with the formation of scum, which was also observed when rearing other species with artificial foods (Gerberg 1970). On the other hand, the survival in the grass (24h) and grass (1h) treatments was very low, possibly as a result of the fast and foul decomposition of the rearing medium. This effect was attenuated by reducing the proportion of grass in the medium as observed in the organic matter / grass treatment. Therefore, diets consisting of organic matter or organic matter plus grass seem to be the most effective for *O. albifasciatus* rearing, as they allowed the highest survival with the least variation among replicates. Survival in these treatments was similar to that reported by Ludueña Almeida & Gorla (1995) when rearing larvae using powdered rabbit balanced food. However, the survival obtained by them might have been favored by the fact that they used water from the larval sites and initiated the rearing by using field-collected second instars.

Wing length was also affected by the diet, and two significantly different groups were found: (1) females emerging from the organic matter (n = 32) and the organic matter/grass (n = 27) treatments, which had a mean wing length of 4.5 mm and a low variation between replicates; and (2) females emerging from the remaining treatments (yeast/TetraMin®: n = 32; grass (24h): n = 16; grass (1h): n = 7) which had a mean wing length ranging from 4.0 mm to 4.3 mm and a high variation among replicates (Table 1). Among the females from group (1), the smallest wings measured 4.10 mm and 4.25 mm in the organic matter and the organic matter/grass treatments, respectively; while the largest measured 4.70 mm in both treatments. Among the females from group (2), the smallest individuals had a wing length of 3.00 mm in the grass (24h) treatment, 3.25 mm in the yeast/TetraMin® treatment, and 3.7 mm in the grass (1h) treatment; while the biggest had wings ranging between 4.40 mm and 4.55 mm length. The range of wing length found with all diets (3.0 to 4.7 mm) is similar to that reported by Maciá (1996) for adults collected in the field (2.8 to 4.6 mm). Although diet composition used in laboratory rearing was different from that in the field, it is possible that variation in wing length in nature occurs, at least partially, as a consequence of diet variation. Other factors that may influence adult size are temperature (Maciá 1996) as well as density-dependent factors (Gleiser *et al*. 2000a).

Table 1. Survival, development time and wing length (x+ sd, n = 4) of *O. albifasciatus* cohorts of 20 individuals each reared with different diets.

Diet (treatment)	Survival (proportion)	Development time[1] (days)	Wing length[1] (mm)
Yeast/ TetraMin®	0.7 ± 0.35bc	9.3 ± 1.60	3.98 ± 0.50b
Organic matter	1.0 ± 0.00a	8.2 ± 0.49	4.46 ± 0.05a
Grass (24h)[2]	0.4 ± 0.32cd	9.1 ± 0.90	4.26 ± 0.18b
Grass (1h)[3]	0.2 ± 0.13d	8.1 ± 0.83	4.21 ± 0.11b
Organic matter/grass	0.8 ± 0.13ab	8.3 ± 0.38	4.47 ± 0.05a

Means within a column followed by the same letter do not significantly differ (P < 0.05) when tested with either the Fisher Test (for survival) or the Student-Newman-Keuls Test (for wing length).

[1] Based on female data.

[2] Grass soaked 24h before the incorporation of the larvae.

[3] Grass soaked 1h before the incorporation of the larvae.

The first pupae were recorded between the 7th and 8th days in all treatments showing that the period of development to initiate pupation was very similar regardless of the diet. However, the time required for most larvae pupate differed among treatments. While the last pupae were recorded on the 9th day in the grass (1h) and the organic matter/grass treatments, in the yeast/TetraMin® treatment they were observed only on the 13th day. Although the development time was not affected by the diet, the organic matter and the organic matter/grass treatments seem to be the most effective diets, as larval development is homogenous (Table 1).

Our data do not support the hypothesis that diets including natural components are more efficient for *O. albifasciatus* larval rearing than diets consisting of artificial components. However, diet composition affected the development of *O. albifasciatus*, and natural diets consisting of organic matter or a mix of both organic matter and grass cuts were the most effective for *O. albifasciatus* rearing. These diets have the disadvantage of being not practical for mass rearing, since it is necessary to process a huge amount of material. However, they are suitable for specific experiments comprising larval rearing, as they yield high survival and optimal growth, as evidenced by the wing length. In addition, the low variation between replicates increases our confidence in these diets reported in here for use in further research on this insect species, and the use of a diet similar to that found in nature makes interpretation of observations in nature more real, when they are made from experiments in laboratory.

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