Comunicação Científica

Notes on Artificial Substrates for Black Fly (Diptera: Simuliidae) Larvae and Microsporidian Infection in Central Amazonia, Brazil

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Notas sobre Substratos Artificiais para Larvas de Pium (Diptera: Simuliidae) e Infecção por Microsporídeo na Amazônia Central, Brasil

RESUMO - Comparou-se três tipos de substratos (filamento plástico branco; ladrilho, blocos de ardósia cinza; e fita plástica, cor amarela) na captura de larvas de simulídeo em 5 períodos de exposição (1, 5, 7, 10 e 14 dias). O estudo foi realizado a 51 km a SE de Manaus, AM, onde foram coletadas 3 espécies: *Simulium quadrifidum* Lutz (81%), *Simulium* sp. (17,3%) e *Simulium perflavum* Roubaud (1,7%). Através da análise de variância dos parâmetros estudados verificou-se diferenças significativas entre os substratos utilizados ($P < 0,001$), mas não entre os períodos de colonização. A fita plástica foi o substrato mais eficiente para capturar imaturos de simulideos ($P < 0,05$), não havendo diferença significativa entre os substratos ladrilho e filamento plástico. Durante o período de estudo foi observado infecção por microsporídio (*Amblyospora bracteata* (Strickland)) apenas em *S. quadrifidum*.

PALAVRAS-CHAVE: Insecta, Microsporidia, parasitismo, *Simulium*, substrato artificial.

Ecological studies, such as investigations of population dynamics, are best conducted with quantitative samples in standardized conditions (Moreira *et al*. 1994). Use of artificial substrates is one way to attain this objective. However, there are some limitations on using this technique: substrates can differ in their attractivity to each species (chemical components, size, color texture, shape, etc.) and their quality can change during the season. There are several reports on use of artificial substrates by aquatic insects, including black fly (Diptera: Simuliidae) immatures (Zahar 1951, Lewis & Bennett 1974, Fredeen & Spurr 1978, Gersabeck & Merritt 1979, Elouard 1984, Das *et al.* 1989). However, this subject has not been studied in the Amazon Region. Time of exposure needs to be evaluated because it can affect colonization by organisms and the quality (=attractivity) of the substrate (Zahar 1951, Fredeen & Spurr 1978, Gersabeck & Merritt 1979). This note reports results on artificial substrates for black fly immatures in the Amazon Region, comparing the capture efficiency of three artificial substrates for five periods of exposure to these

insects. In addition, observations are reported on pathogens associated with black flies in the study area.

This experiment was conducted from September 22 to October 6 and from November 14-25, 1993 (dry season) in a stream located in an area preserved by the Brazilian Army, on Highway AM 010, 51 km SE of Manaus, Amazonas. The stream is an artificial dam outlet with a width of 1.5 m and a maximum depth of 45 cm; temperature at the time of collection varied from 26 to 28°C and pH varied from 4.9 to 5.1. The evaluations were made in 15 sites (10 m apart), in the first 200 m of the stream. Two wooden sticks (one on each side of the stream) with a nylon filament tied between them were placed at each site. The substrates were hung from another nylon filament tied to the one connecting the sticks. At each point, four units of each substrate were randomly arranged, totalling 180 units for all sites. The substrates were: white plastic filaments (15.0 x 0.15 cm); tiles (= gray slate, 7.0 x 3.0 x 0.3 cm); and yellow plastic tape (15.0 x 2.8 cm). All substrates remained under the water surface. The tiles were kept suspended (not on the bottom) due to the water current. Five different exposure times for each substrate were evaluated: 1, 5, 7, 10 and 14 days. For each period, 12 units of each substrate were randomly retrieved, placed in individual plastic containers with water and transported in an ice box to the laboratory. The immatures were preserved in 80% ethanol, except for larvae with visible microsporidian infection. Smears of abdominal fat tissue from infected larvae were air dried, fixed in absolute ethanol and stained with giemsa solution (3g giemsa, 260ml methanol, 140ml glycerol). The carcass of each larva was individually preserved in 80% ethanol in order to associate the microsporidia with the black fly species. From 14 to 25 November, additional samplings were made using 21 units of yellow plastic tape (15.0 x 2.8 cm), exposed for 11 days, to verify if the frequency of occurrence of each species was the same as in the previous period. All data were transformed [log(Y + 1)] before statistical analysis, as the data distribution was not normal, which is common in aquatic insects (Elliott 1983). Two-way ANOVA and the Tukey test were used to compare the mean number of black fly larvae among substrates and exposure times.

Three species of black flies were collected: *Simulium quadrifidum* Lutz (81.0%), *Simulium* sp. (17.3%) and *Simulium perflavum* Roubaud (1.7%), based on pupae that colonized the substrates in the Sept.-Oct. period. In the second period evaluated (Nov. 14-25) the species proportions, based on pupae colonizing plastic tapes, were 84.5%, 14.0% and 1.5%, respectively. Qualitative observations on natural substrates confirmed these results, demonstrating that the artificial substrates extracted a representative sample of black fly species in the stream. *Simulium quadrifidum* larvae (n=28) were found infected by *Amblyospora bracteata* (Strickland) (Meiodihaplophasida, Amblyosporidae), a microsporidian which is known to infect several species of black flies (Garcia *et al*. 1989, Garcia 1992). This is the first record of microsporidian infection in black fly larvae and in *S. quadrifidum* in the Brazilian Amazon Region, a fact which reflects the paucity of surveys on black flies diseases rather than rarity of microsporidian incidence on these insects in the Amazon Region. For pathogens to be used as control agents, their life cycle and the way they are transmitted need to be known, but this information is still lacking for black fly microsporidians (Branco & Andrade 1993, Ledin 1994).

The results for the number of black fly larvae collected on the three substrates in five periods of exposure were based on total number of larvae, as numbers of last-instar larvae (usually the one that can be identified to species) were too low to apply statistical tests. Because *S. quadrifidum* was the most abundant species, overall results might have been affected by its substrate preferences. We found differences in colonization among the substrates ($P<0.001$), but not among exposure periods ($P > 0.05$). Plastic tape was the most effective substrate ($P < 0.05$) for capturing

Table 1. ANOVA and Tukey multiple comparison analysis of black fly (Diptera: Simuliidae) larvae on three artificial substrates in Manaus, Amazonas, 1993.

Substrate	Mean number of larvae	
Plastic Tape (n=59)	0.557 a	(6.6 ± 1.3)
Tile (n=58)	0.224 b	(1.3 ± 0.36)
Plastic filament (n=59)	0.136 b	(0.6 ± 0.18)
$F_{2,176}$	20.77***	

Note: means (log_{y+1}) followed by same letters were not significantly different (P>0.05); values in parentheses are mean ± SE.
*** P<0.001

black fly immatures, and there were no differences (P > 0.05) between the tile and plastic filament substrates (Table 1). Although the periods of exposure were not significantly different (P > 0.05), an increasing trend was observed in the number of larvae on the plastic tape substrate from 1 to 10 days of exposure.

Gersabeck & Merritt (1979) in their study of the *Prosimulium mixtum/fuscum* Syme & Davies complex in the U.S.A., observed a peak density at an exposure time of 5-7 days using ceramic tile and clear plastic tape. They concluded that depth and exposure time were the major factors affecting the number of black

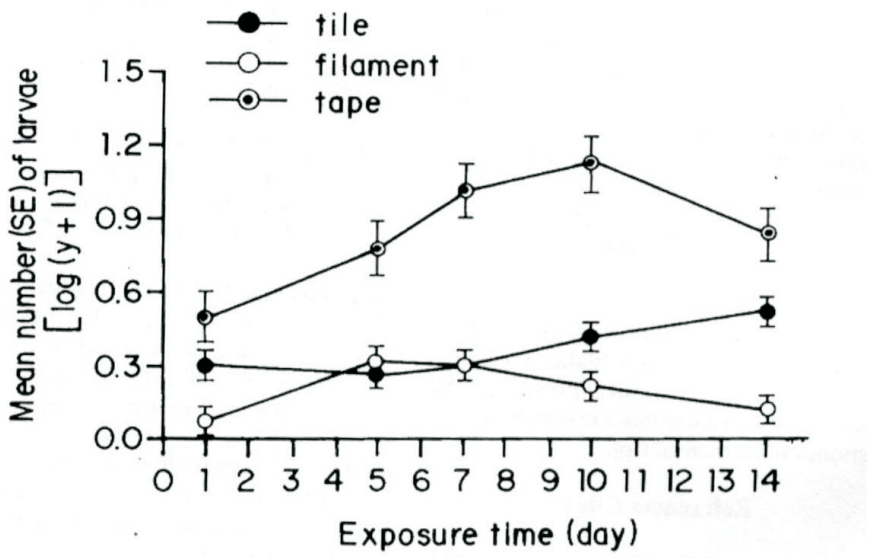

Figure 1. Means and standard errors of the numbers (log_{y+1}) of black fly larvae on three artificial substrates at different exposure periods in Manaus, Amazonas, 1993.

fly larvae colonizing a substrate, and that the decline of larvae on substrates placed on the stream bottom would be due to the substrate fouling by material moving along the streambed. The decline on substrates above the streambed appeared to be due to territorial behavior among the older larvae. They also found that ceramic tile was a better substrate than clear plastic tape, since the tapes became coated with slime after a few hours of exposure and thus offered a poor surface for larval attachment. Lewis & Bennett (1974) also found that ceramic tile (slate) was the best substrate to sample immature black flies in Canada, and suggest that an identical sampling methodology should be used in different geographic locations to compare population density data for the same or different black fly species, electing the tile as the ideal sampling substrate. However, in our study tile was not an effective substrate (Fig. 1) for black fly larvae. The plastic filament we used as a substrate was successfully used by Ledin (1994) for populations of *Simulium vittatum* IIIL-1 in the U.S.A. in the same kind of habitat studied by us. However, this type of substrate was very poorly used by our black fly species (Fig. 1). We suggest that there is no ideal substrate for sampling black fly immatures world-wide, and that, before beginning any study where artificial substrate would be used, a test would be necessary to determine the substrate that best suits the conditions of each region.

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