BioAssay

Received: 21/VII/2006
Accepted: 17/XI/2006
Published: 23/XII/2006
Available online at: www.bioassay.org.br/articles/1.11

SCIENTIFIC NOTES

An Artificial Breeding Site for Larvicide Bioassays and Interaction Studies with Diptera:Simuliidae and Other Macroinvertebrates from Lotic Systems

RONALDO FIGUEIRÓ[1,2], TATIANA N. DOCILE[2] AND ÁRION T. ARANDA[2]

1- Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Ecologia. Av. Brigadeiro Trompowski, s/n. CCS, IB, Laboratório de Ecologia de Insetos, Bloco A, Sala A0111, Ilha do Fundão 21941-590 - Rio de Janeiro, RJ - Brasil - Caixa-Postal: 68020. E-mail: figueiro@ioc.fiocruz.br
2- Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Departamento de Entomologia. Av. Brasil, 4365 - Pav. Carlos Chagas, Laboratório de Referência Nacional em Simulídeos e Oncocercose, 4º andar, Sala 7, Manguinhos 21045-900 - Rio de Janeiro, RJ - Brasil - Caixa-Postal: 926. E-mail: tdocile@ioc.fiocruz.br, arion@ioc.fiocruz.br

BioAssay 1:11 (2006)

Um Criadouro Artificial para Bioensaios com Larvicidas e Estudos de Interação com Diptera: Simuliidae e outros Macroinvertebrados de Sistemas Lóticos

RESUMO – Esta nota apresenta um criadouro artificial para larvas de Diptera: Simuliidae e demais macroinvertebrados de ambientes lóticos, composto de um aquário modificado, contendo um sistema de três canaletas que proporcionam condições simuladas de rio, comportando uma ampla variação controlável da vazão e da velocidade da correnteza. Este criadouro é aplicável para bioensaios com larvas de Diptera: Simuliidae, assim como estudos de interação com outros macroinvertebrados e efeito de larvicidas em organismos não-alvo.

PALAVRAS-CHAVE – Simuliidae, bioensaios, imaturos.

ABSTRACT – This note presents an artifical breeding site, composed of a modified aquarium, which allows a wide range of controllable water discharge and water velocity variation. It is applicable for bioassays with Diptera: Simuliidae and interaction studies with other macroinvertebrates, as well as assessing impact of larvicides on non-target organisms.

KEYWORDS – Simuliidae, bioassays, immatures.

Several artificial breeding systems have been applied in bioassays with blackfly larvae over the years. Every system for evaluating larvicide formulations in the laboratory must generate water current sufficient for stimulating normal feeding behaviour on the blackfly larvae (Barton *et al.* 1991). Essentially, there are two categories of artificial systems available for conducting laboratory bioassays with blackfly larvae. In the closed systems, water circulation is promoted by the water bubbles from the artificial oxygenation (Lacey & Mulla 1977) or magnetic giratory bars (Colbo & Thompson 1978), or through rotating plastic bottles in wax cups (Hembree *et al.* 1980). In the opened systems, the larvae are held on a shallow channel of flowing water (Hartley 1955, Jamnback & Frempong-Boadu 1966, Muirhead-Thomson 1957 & Gaugler *et al.* 1980). The system proposed herein stands in the later category, even though it can also be used as a closed system. It consists of a modified aquarium (Fig. 1), based on the model proposed by Araujo-Coutinho (oral communication), which was applied in Figueiró *et al.* (2002). It is useful not only for larvicide bioassays, but also for carrying out interaction studies, and therefore, it had to be suitable for other macroinvertebrates from lotic systems.

The aquarium itself has 0.45 m lenght, 0.24 m width and is 0.36 m high, and features a small reservoir, located on its top, which has 0.115 m lenght, 0.07 m width and is 0.125 m high. The water is constantly pumped from the bottom to this reservoir, and flows through a channel, which is followed by two other ones. Each channel is placed in a greater slope than the previous one, resulting in three different water current velocities for a given discharge. The three channels are composed of pvc pipes, 0.03 m diameter each, cut in the half, and fixed to the aquarium walls by using silicone.

After flowing through the channels, the water falls in the bottom of the aquarium, where it will be pumped back to the reservoir, if the artificial breeding site is used as a closed system.

Located on the bottom of the aquarium, it is possible to install a sewer, if one intends to use this as an opened system, allowing a constant substitution of the water used in the system. The angles of inclination of the channels are not fixed, and therefore, they can be customized, as long as they form a sequence of increasingly inclined channels. Therefore, this breeding site can also be used as a closed system.

The pump used within the artificial breeding site was a Sarlo S180 ™, which allows up to 180 liters per hour. This pump features a device which allows controlling the discharge, thus consequently controlling the resulting water currents flowing through all the three channels. This enables a large range of water speed variation. The actual range of speed variation depends mainly on the power of the pump, because it is directly related to the water discharge range. The breeding site proposed by Araújo-Coutinho allowed only one single water current speed for each discharge. However, the architecture of that breeding site did not permit the determination of the velocity itself, so only the discharges applied in the bioassays could be calculated. The architecture of this new artificial breeding site, however, was carefully designed to allow the determination of water current velocity. The water current velocity is an important abiotic factor for blackfly larvae (Eymann 1993). The water depth in the channels allows the use of the float method for determining the water velocity, which was not possible in the breeding site applied in Figueiró *et. al* (2002), since its water layer depth was insufficient for this method.

Another issue that was addressed in this new artificial breeding site is the substrate used in it. Although the glass surface proved to be efficient for blackfly larvae colonization, the same did not apply to other organisms. In order to make the breeding site suitable to other organisms, each of the three channels was covered by a thin layer of sand, providing a substrate that proved to be more suitable for their colonization.

The artificial breeding site presented herein could be used for multiple purposes, aside larvicide bioassays. Its controllable simulated stream conditions allow interaction studies to be performed, as well as to study the influence of abiotic factors on lotic macroinvertebrates and assessing the impact of larvicides on non-target organisms. This feature makes it a valuable tool for ecological studies in the laboratory.

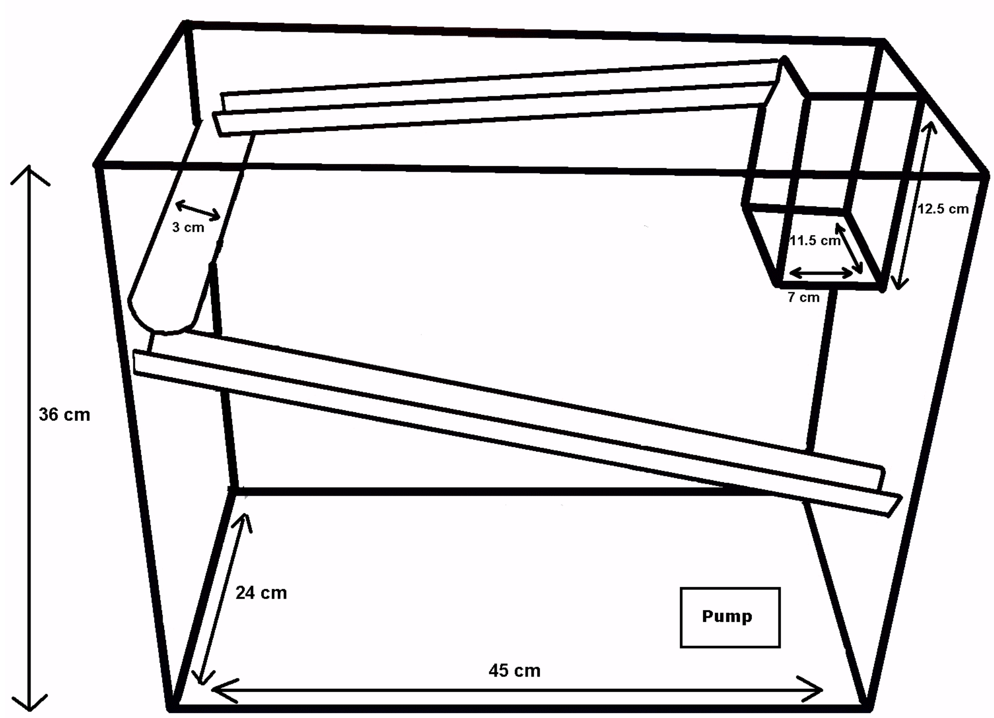

Figure 1. Breeding site for larvicide bioassays with blackfly larvae and other macroinvertebrates.

The breeding site was submitted to 5 series of trials, consisting of 5 days each, on which the survival of Blackfly larvae, in the absence of the larvicide, was evaluated. In each of these series, a number of 20 blackfly larvae were placed in the breeding site, and submitted to an intermediate regime of water current velocity, what was previously determined empirically as the optimal conditions for feeding (Lacey & Mulla, 1977, Braimah, 1987 and Figueiró *et al.* 2002). In these trials, the mean survival observed for blackflies after 5 days being reared in the breeding site varied from 47% to 60%, with 4-6% of them getting to pupae.

In order to further testing the breeding site, the experiment of Figueiró *et al.* (2002), on which the optimal discharge for blackfly larvae feeding was determined, was duplicated with the presented artificial breeding site, achieving the same pattern observed in the forementioned study. This proves it is appropriate for bioassays with biological larvicides, because these kind of agents require that larvae ingest the active ingredient. Therefore, an equipment which permits the adjustment of the water current to a speed where the feeding of the target organism is optimal reducts the risk of miscalculating the efficacy of these agents. The preliminary results on bioassays conducted with blackfly larvae in the laboratory were satisfactory, but further tests of the artificial breeding site for this purpose were held, and interaction studies are currently being conducted.

Acknowledgments

We thank Dr. Marilza Maia-Herzog, for all the support given, as well as the rest of the staff of the Laboratório de Referência Nacional em Simulídeos e Oncocercose.

Cited Literature

Barton, W. E., R. Noblet & D.C. Kurtak. 1991. A Simple Technique for determining relative toxicities of *Bacillus thuringiensis* var. *israelensis* formulations against larval blackflies (Diptera: Simuliidae). J. Am. Mosq. Control Assoc. 7(2): 313-315.

Braimah, S.A. 1987. The influence of water velocity on particle capture by the labral fans of larvae of *Simulium* Malloch (Diptera: Simuliidae). Can. J. Zool. 65: 2395-2399.

Colbo, M.H. & B.H. Thompson. 1978. An efficient technique for laboratory rearing of *Simulium verecundum* S. & J. (Diptera: Simuliidae). Can, J. Zool. 56:507-510.

Eymann, M. 1993. Some boundary layer characteristics of microhabitats occupied by larval black flies (Diptera: Simuliidae). Hydrobiol. 259: 57-67.

Figueiró, R., E.S. Nascimento & C. J. P. C. Araújo-Coutinho. 2002. Avaliação da influência da vazão da coluna d'água do criadouro artificial sobre a captação de partículas por larvas de *Simulium pertinax* (Diptera: Simuliidae). Entomologia y Vectores. 9(2): 251-261.

Gaugler, R., D. Molloy, T. Haskins & G. Rider. 1980. A bioassay system for the evaluation of blackfly (Diptera: Simuliidae) control agents under simulated stream conditions. Can. Entomol. 112: 1271-1276.

Hartley, C.F. 1955. Rearing simuliids in the laboratory from eggs to adults. Proc. Helminthol. Soc. Wash. 22: 93-95.

Hembree, S.C., R.L. Frommer & M.P. Remington. 1980. A bioassay apparatus for evaluating larvicides against blackflies. Mosq. News 40: 647-650.

Jamnback, H. & J. Frempong-Boadu 1966. Testing black fly larvicides in the laboratory and in streams.Bull. Wld Hlth Org. 34: 405-421.

Lacey, L.A. & M.S. Mulla. 1977. A new bioassay unit for evaluating larvicides against blackflies. J. Econ. Entomol. 70: 453-456.

Muirhead-Thomson, R.C., 1957. Laboratory studies on the reactions of *Simulium* larvae to insecticides. Am. J. Trop. Med. Hyg. 6: 920-925.