

SCIENTIFIC NOTE

A Bioassay Method for Black Flies (Diptera: Simuliidae) Using Larvicides

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Método Para Bioensaios em Simulídeos (Diptera: Simuliidae) com Larvicidas

RESUMO - Esta nota apresenta um método alternativo para bioensaios com larvas de simulídeos, com que se espera proporcionar aos pesquisadores uma ferramenta mais simples, porém igualmente efetiva para a obtenção de dados referentes à eficácia de larvicidas.

PALAVRAS-CHAVE: Controle biológico, eficácia, técnica

ABSTRACT - This note presents an alternative method for bioassays using black fly larvae, with which we intend to provide a simpler but effective tool for accessing data on larvicide efficacy.

KEY WORDS: Biological control, efficacy, technique

The family Simuliidae has a worldwide distribution, extending from the tropics to the arcticcircle, comprising more than 1,750 known species, many of which are hematophagous (Crosskey & Howard 1997). The importance of this family is based on the fact that some species are vectors of *Onchocerca volvulus*, a filarial worm found in the Neotropical region of the Americas and Central and West Africa (Shelley 2002). In North America, black flies affect animal health by causing their death by direct or indirect action, and also by reducing milk yield, in badly infested areas (Anderson & Voskuil 1963).

Black flies are holometabolous insects, whose larvae generally occur in systems showing non-selective filter behavior (Cummins 1973). Most of the known species from the temperate and subarctic regions are univoltine, while the species from the tropics and subtropics are multivoltine (Peckarsky *et al.* 1990).

The first attempt at black fly control in Brazil began in 1957, through the application of organochloride insecticides, performed in a random way (SUCEN 1977).

The residual activity associated with such insecticides cause environmental damage due to low specificity. For this reason they were substituted during the 1970's, by an organophosphate (Temephos), which even though less aggressive to the environment, still resulted in damage to non-target insects, including natural predators of black fly larvae

(Ruas Neto 1984), such as Trichoptera and Ephemeroptera.

The development of insecticide resistance among various vector species encouraged a worldwide effort to find different control methods that are less aggressive to the environment (Zahner 1993). Consequently, a large variety of entomopathogenic microorganisms has been isolated, such as protozoa, fungi, nematodes, rickettsia, bacteria and viruses (Cavados *et al.* 2001).

During the 1980's, the substitution of chemical control by biological control based on *Bacillus thuringiensis* serovar *israelensis* (BTI), a highly specific insecticide with lower activity on non-target organisms in lotic systems, began to take place (Colbo & Undeen 1980, Merritt *et al.* 1989). From then on, BTI formulations were further developed for improvements in potency, stability, ease of application and residual activity (Couch 2000).

The improvements on the formulations were partly due to the results of laboratory bioassays, which allowed the evaluation of their efficacy. However, the applicability of these results depended intimately on the accurate reproduction of field conditions in the laboratory.

The exposure time is a factor that differentiates bioassays using black fly larvae from those using mosquito larvae. Often, the time during which BTI formulations are in contact with black fly larvae under field conditions is brief, since the water flow rapidly carries the material downstream. In field

conditions, application times can be short, as in aerial application, or as long as 10-30 min, when carried out at ground level (Lacey 1997). Shorter application times at ground level can also be adopted, as is the case in the black fly control program in São Paulo state, in which applications of 15 ppm / 1 min were made (Araújo-Coutinho 1995).

There are basically two systems available for laboratory bioassays using black fly larvae: closed systems in which water circulation is enhanced by bubbles (Lacey & Mulla 1977) or magnetic giratory bars (Colbo & Thompson 1978), or through rotating plastic bottles in wax cups (Hembree *et al.* 1980) and open systems, where larvae are held in a shallow channel of flowing water (Hartley 1955, Muirhead-Thomson 1957, Jamnback & Frempong-Boadu 1966, Gaugler *et al.* 1980).

In both systems, the elimination of the larvicide after the desired exposure time implies a greater complexity in the architecture of the apparatus used in the bioassays. In open systems, Lacey & Mulla (1977) developed an apparatus that consisted of a Pyrex® funnel fused to a Pyrex® glass tubing, with an overflow tube and a hole that allowed the gradual substitution of the larvicide impregnated water for pure water. However, construction of this apparatus is difficult. In the case of the open systems, these generally require a large amount of space, like the apparatus used by Jamnback & Frempong-Boadu (1966).

In this paper we describe a method for the evaluation of the efficacy of black fly larvicides in the laboratory, derived from existing closed systems, of easy maintenance and low space requirements, as an alternative for the existing methods.

The healthy larvae selected for the experiment must be separated in groups equal to or bigger than 50 individuals, and then each group must be placed in a 300 ml plastic recipient filled with 200 ml of water. The water must be from the natural breeding site, to eliminate the need of adding food like Purina®, used by Lacey & Mulla (1977). Each recipient must have a pump for circulation of water to the larvae to allow them to filter feed.

Each plastic recipient filled with larvae must be accompanied by another recipient, filled with the same water volume, but without larvae. Since the recipients are closed systems, the larvae must be removed from the original recipient after the desired exposure time to larvicide and be placed in the corresponding recipient without larvicida (Fig. 1).

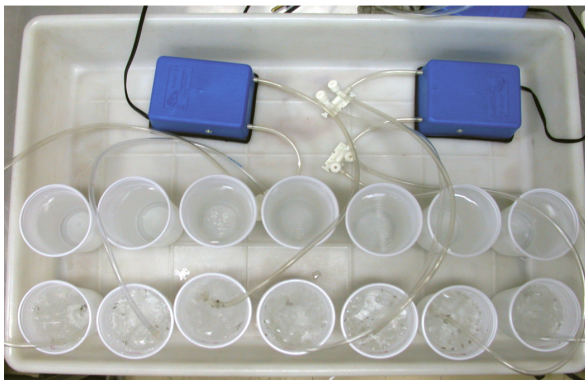


Figure 1. System apparatus used for bioassays using black fly larvae.

The same procedure must be applied to the control group, to standardize any side effects that the transference may have on the larvae. Therefore, this method allows the adjustment of the desired exposure time in the laboratory, thereby presenting a simpler methodology for the reproduction of field conditions.

The results to notice on bioassays conducted with black flies larvae in the laboratory are satisfactory, but further testing of the method is being held, in order to statistically establish the exact applicability of this method.

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