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A BIOASSAY SYSTEM FOR THE EVALUATION OF BLACK FLY (DIPTERA: SIMULIIDAE) CONTROL AGENTS UNDER SIMULATED STREAM CONDITIONS[1]

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Abstract

Can. Ent. 112: 1271–1276 (1980)

A new bioassay unit is described which provides simulated stream conditions for testing control agents against black fly larvae. Attributes include uniform flow, high control survival, and excellent visibility for observing larval behavior. It also possesses the unique advantage of using recycled water, continuously supplied fresh water, or both simultaneously. The unit has performed satisfactorily in assays against black flies using pathogens, parasites, and chemicals. In addition, stream invertebrates from four insect orders have been maintained in the unit.

A simple and inexpensive incubation unit is also described which possesses several of the advantages of the bioassay unit. It lacks the capability for recirculation and is generally less versatile than the bioassay unit; however, it is useful for the maintenance and observation of stream insects.

Introduction

Laboratory apparatus simulating the running water conditions needed for black fly larvae has been based on either an open or closed system of water flow. Most advocates of the open (or flow-through) system have directed the water into inclined troughs (Wood and Davies 1966), although Hartley (1955) used jars which were allowed to overflow. The closed (or recirculation) system uses a limited volume of water which is continuously circulated, usually by compressed air (Lacey and Mulla 1977), stirrers (Colbo and Thompson 1978), or pumps (Brenner and Cupp 1980). Both jar (Muirhead-Thomson 1957) and trough (Wright 1957) techniques have been used in conjunction with this system.

Laboratory evaluations of black fly control agents are best performed in bioassay units which closely simulate the natural larval habitat, because these conditions are most likely to produce results comparable to field trials. Maintenance of black fly larvae in our laboratory was originally accomplished in a simple closed system (i.e., compressed air in a jar of stream water — Jamnback 1962). Later, to simulate natural stream conditions, control agents were tested against black flies in an open system of troughs and running water (Jamnback and Frempong-Boadu 1966). Recently, a versatile new bioassay unit with wider application has been designed. This unit has the capability of being used as either an open or a closed system and incorporates many of the attributes of both systems.

Methods and Materials

Bioassay Unit

The bioassay system is housed in a former fish hatchery near Cambridge, New York. Unfiltered water is gravity-fed from a nearby streampool and into the laboratory, where it enters a 400 l. head tank. The water then flows into a horizontal 10 cm diam. pipe with 36 outlet valves, one supplying each bioassay unit. Water temperature varies seasonally (4°–15°C) but shows little daily fluctuation (< 1.5°C).

Each bioassay unit consists principally of a reservoir tub (A), recirculation pump (B) with hose and valve (C), fresh water supply valve (E), delivery funnel (G), and

[1]Published by permission of the Director, New York State Museum, State Education Department, Journal Series No. 307.

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a tray for larval attachment (I, J) (Fig. 1). In the open, or flow-through, mode, fresh water continuously enters the delivery funnel from the supply valve, passes over the tray with attached larvae, and is allowed to overflow the reservoir tub. The overflowing water spills into a waste trough (L) which directs it to a floor drain. In the recirculation, or closed, mode, the unit is filled with stream water from the supply valve. A pump then lifts the water from the reservoir tub and into the delivery funnel. The water flows down the funnel, over the tray, and is returned to the reservoir tub for recycling. Elevated temperatures may be obtained during recirculation by placing an aquarium heater in the reservoir tub.

The reservoir tub is cut from polyvinyl chloride (PVC) pipe, with PVC plate welded to the bottom. Each tub has a large overflow notch so that when filled to overflowing the unit contains 8 l. The magnetic-driven pump is submerged on the tub bottom and has a vinyl hose fitted over its outlet. Flow rate on recirculation is regulated by the adjustable recirculation valve (C) fitted at the distal end of the hose. Flow rate on flow-through is regulated by the fresh water supply valve (E).

The recirculation valve is coupled to a PVC T-fitting, which is joined to a standing waste pipe (K). During normal recirculation, water flows into the upper arm of the "T", out of the lower arm, and into the delivery funnel (Fig. 1). When it is necessary to flush out the reservoir tub, either to remove inoculum or wastes, or for cleaning, the bottom arm of the "T" is capped (M). This diverts the water into the waste pipe and down to the waste trough.

The delivery funnel is supported by a horizontal strut (H). A double layer of nylon organdy cloth (F) fits over the funnel mouth and filters large particles ($> 200\ \mu m$) from the incoming water that otherwise clog the tray.

The nucleus of each bioassay unit is the tray, which is composed of two parts: the reservoir (I) and larval attachment (J) sections. A stainless steel screen (300 μm aperture) is positioned between these two sections. Water rushing into the tray reservoir section is extremely turbulent; however, after passing through the screen the flow becomes smooth and laminar, creating an even sheet of swiftly moving water over the larval attachment section. The excellent optics provided by this thin water film allows larval behavior to be closely observed using either a stereomicroscope or the unaided eye. A second screen at the discharge end of the tray prevents the loss of all but the smallest larvae. When an assay is conducted against early instars, an organdy filter is positioned below the tray to catch any detaching larvae. The trays are secured to a horizontal support by Velcro® fasteners.

Although the organdy filter aids in keeping the screens clean, plugging with fine stream particles will occur when the units are used in the flow-through mode. Accordingly, the screens must be cleaned daily.

Bioassay Test Procedure

Field-collected black fly larvae, attached to natural substrates, are transported to the laboratory and washed off into holding receptacles. The bioassay unit flow rate is then restricted to a slow drip and the tray discharge end is propped up, allowing a pool to form at the rear half of the tray. Larvae of the desired size are transferred into this pool with a brush. When sufficient larvae (usually 75–100) have been introduced onto the tray the flow rate is increased moderately. Most larvae are attached within 15 min and the tray is then slowly returned to its usual position and the flow rate accelerated.

Larvae are allowed 24–48 h to acclimate before testing. Shortly before exposure, the tray screens are cleaned of debris, and pupae and patently infected or abnormally behaving larvae are discarded. In tests conducted at stream temperatures (where

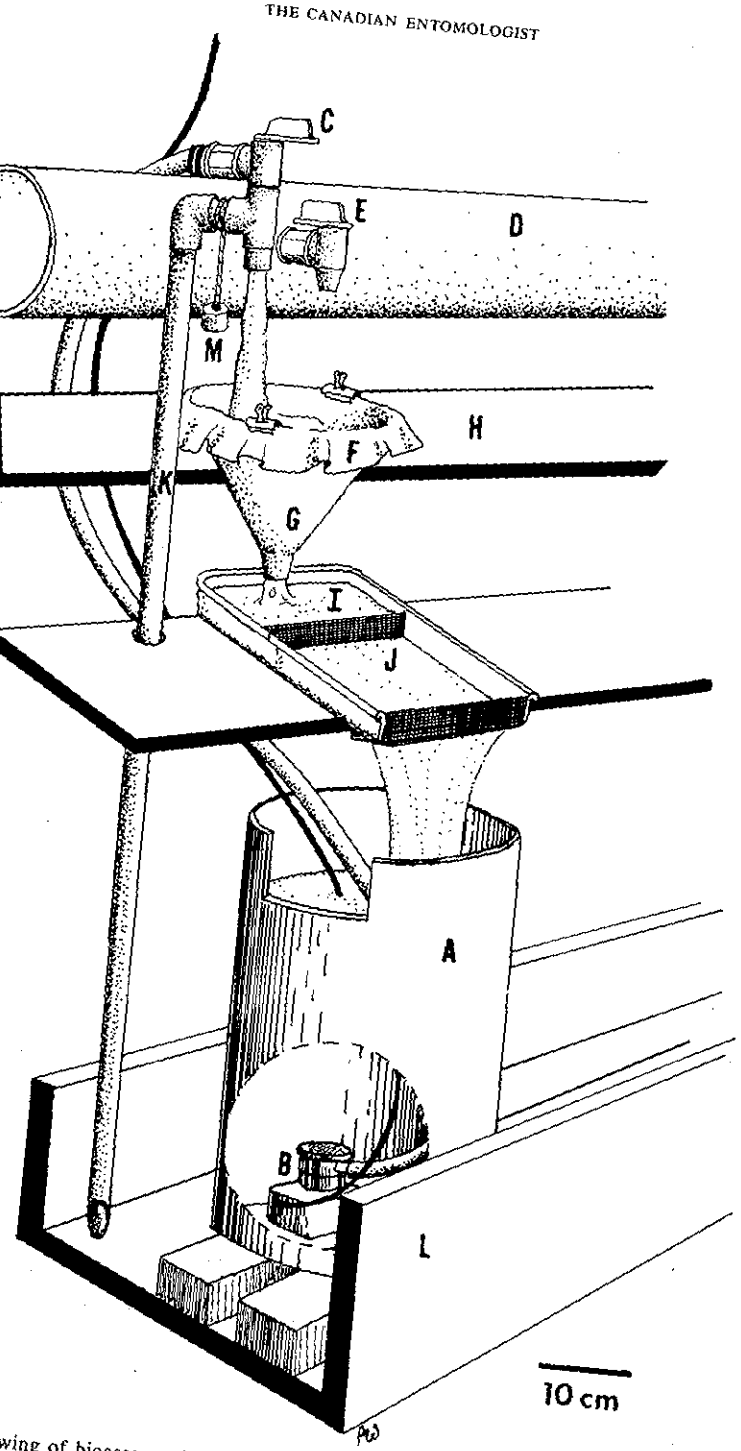

FIG. 1. Schematic drawing of bioassay unit in the recirculation mode. A, reservoir tub; B, recirculation pump; C, recirculation valve; D, water supply pipe; E, supply valve; F, organdy cloth filter; G, delivery funnel; H, funnel support; I, tray reservoir section; J, tray attachment section; K, standing waste pipe; L, waste trough; M, cap.

recirculation is not required), inoculum may simply be dripped into the delivery funnel along with the incoming stream water during the exposure period. Tests requiring elevated temperatures must be conducted entirely in the recirculation mode. These exposures are ended by flushing the unit with the proper temperature stream water pumped from an adjacent holding tank. The tub reservoir is simultaneously drained by capping the T-fitting, thereby diverting the inoculum and flush water into the waste trough. Ending exposures by flushing does not disturb larval feeding. This is important, because interruption of larval feeding after treatment can result in increased mortality (Gaugler and Molloy in press).

Supplementary feeding is not required in the flow-through system, since naturally occurring larval food materials (i.e., diatoms, other algae, detritus) are suspended in the stream water. During recirculation, food materials may either be provided by water exchange or the addition of fish food.

Incubation Unit

A second method used for holding black fly larvae and other stream macroinvertebrates in our laboratory is the incubation unit (Fig. 2). This simple technique consists of a horizontal 10 cm diam. water supply pipe with 72 valves. Each valve continuously delivers fresh stream water to a tray. The discharge flows off the tray and out of the laboratory through a waste pipe. The screens must be cleaned frequently, since no provision is made to prefilter the incoming water.

Results and Discussion

The bioassay unit has performed satisfactorily in tests against black fly larvae using microbial pathogens, entomogenous nematodes, and chemical insecticides. Control mortality in these tests rarely exceeded 4%, despite larvae being held up to 7 days. The unit has also been used successfully to maintain non-target stream macroinvertebrates, including mayflies, stoneflies, elmids, brachycentrids, and hydropsychids. Like black flies, non-target organisms held in the laboratory behaved normally, particularly when natural substrates were provided in the trays.

The design of most previous systems made it difficult to closely observe larval behavior because of the generally poor visibility inherent in units using compressed air or stirrers to create current. A major feature of the present unit is the excellent visibility of the test insects, allowing assessment of larval response to a stimulus. This assures that only normally feeding larvae are treated, an important consideration in tests with ingested control agents. It also permits quick recognition of dead and moribund larvae (running a thumbnail along the tray screen induces healthy larvae to recoil), which may then be easily removed without interrupting the flow.

A second important feature of the design is the uniform rate of flow provided. It is essential that all attachment sites available within a bioassay unit receive similar flow, otherwise larvae will be exposed to unequal dosages of a control agent. Fredeen (1964) has indicated that compressed air systems provide heterogeneous flow.

Larvae on recirculation were occasionally found with small air bubbles adhering to the mouthparts. The reasons for bubble formation are unclear; however, such larvae should be removed when assaying ingested control agents since this condition hinders normal feeding.

The incubation unit provides the same excellent visibility and high survival as the bioassay unit. However, it has only a flow-through capability, so important parameters such as temperature can not be manipulated. It has, nevertheless, proved useful for maintaining larvae in the laboratory. For example, when temperature is

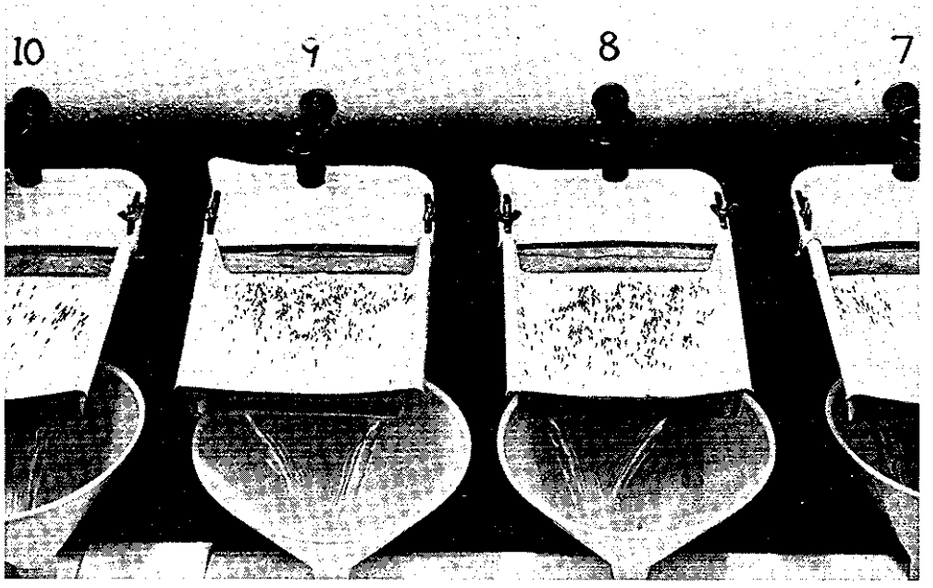

FIG. 2. Incubation units showing *Simulium vittatum* larvae attached to trays. Note the excellent visibility for observing behavior.

not being studied, previously treated trays may be transferred from the bioassay units to the incubation units, freeing the bioassay units for additional testing. This is critical in tests with insect-growth regulators, where posttreatment observation periods may exceed 1 month. Larvae may also be transported into the laboratory from treated streams and held for observation under more controlled conditions. The mortality rates among larvae transferred to the incubation units have been found to be similar to the rates reported from the field (unpub. data).

This bioassay unit was developed for use with a continuous supply of natural water, a resource unavailable at most laboratories. Nevertheless, it may be used with minor modification in laboratories with limited water, although only the recirculation mode could be utilized. Although primarily designed for evaluating black fly larvicides, the unit is suitable for numerous other purposes where excellent visibility, uniform flow, and closely simulated stream conditions are desired.

Acknowledgments

The technical assistance of B. Kaplan and P. Wills is gratefully acknowledged. This work was supported in part by National Institutes of Health Grant 1R01AI15605-01.

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(Received 6 June 1980)