all salt marsh mosquitoes including *Ae. cantator*, and *Ae. taeniorhynchus* although for the purposes of this report, only *Ae. sollicitans* was considered.

It is interesting to note that we located a salt marsh across the Palmer River in Warren, R.I. about ½ to 1 mile away, which breeds heavily and did not appear to significantly affect mosquitoes populations in traps 1 and 4 in Hampden Meadows, suggesting that infestation by *Ae. sollicitans* may be more localized in effect than originally thought.

Over 18,000 feet of ditching have been dug under the supervision of the Army Corps of Engineers, Department of Interior; Fish and Wildlife Service, the Rhode Island State Coastal Resources Management Council, the Rhode Island Fish and Wildlife Service, the local Conservation Commission, and the Town Council. The results of our work have been scrutinized by these agencies and also by the newly formed Rhode Island Mosquito Abatement Board. The reduction of the salt marsh mosquitoes plus the reduction of insecticides used on the salt marsh are considered beneficial by all concerned.

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A BIOASSAY APPARATUS FOR EVALUATING LARVICIDES AGAINST BLACK FLIES[1]

S. C. HEMBREE, R. L. FROMMER AND M. P. REMINGTON

US Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD 21701

Apparatuses for bioassaying candidate microbial or chemical control agents against black fly larvae must provide movement, oxygenation and cleanliness of water and an attachment substrate that is as uniform as possible within and between containers. A means of providing accurately timed exposures is necessary, and the apparatus itself should not be hazardous to the larvae. Troughs of flowing water such as described by Jamnback and Frempong—Boadu (1966) are inappropriate for our laboratory because of space limitations. Preliminary tests with devices that use a stream of air bubbles to provide water movement and oxygenation, such as the apparatus described by Lacey and Mulla (1976), tend to provide an inhomogeneous test environment as reflected by the fact that the black fly larvae congregate as nearly as possible to the air bubbles. Colbo and Thompson (1978) described an apparatus for rearing black fly larvae that was suitable also for bioassay work. Movement of water in their apparatus was provided by a magnetic

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of proprietary names herein does not constitute indorsement.

stirring bar. Larvae attached to the wall of the container but also to the stir bar and thus were subject to physical damage. Additionally, vortexing caused by the rotating bar could have resulted in concentration of particulate agents, such as bacteria, within the vortex. To provide a safe, homogeneous, reproducible test environment and to facilitate timed exposures, the bioassay apparatus for black fly larvae described below (Figure 1) was constructed and has been used in this laboratory for over a year in studies of the dose/time/response relationships between *Bacillus thuringiensis* var. *israelensis* and *Simulium vittatum* larvae. The objective of these studies was to determine LC 50's and LC 90's for durations of exposure varying from 15 min to 24 hr. The results will be the subject of another paper.

The apparatus consisted of a frame holding structural members in which 2 rows of 5 vertical shafts were mounted in sleeve bearings. Four-ounce plastic bottles were attached to the lower ends of the shafts. Pulleys were mounted on the shafts between the 2 bearings that supported each shaft. A variable speed motor with a pulley on the drive shaft was attached at 1 end of the frame and a tensioner pulley near the other end. A continuous belt driven by the motor turned the shafts, rotated the plastic bottles within the disposable waxed paper cups that contained the larvae and test material. The test containers sat on trap doors on a plexiglass shelf.

The frame was 36 in long × 18 in high × 12 in wide and was constructed of 6061 aluminum angle ½ in × ½ in × ⅛ in thick. Machine screws were used to join the members, allowing easy disassembly for transport. The bottom frame members were used to support the ¼ in thick plexiglass shelf about 7 in above the work surface. Hinged doors, held closed by simple lever latches, were designed to open downward to permit easy removal and replacement of individual test containers. Rubber mats were glued to the top surface of the doors to prevent the test containers from moving while the apparatus was in operation. Four structural members supported by the frame held the rotating shafts. These were of 2024 aluminum bar the length of the frame by 1¼ in wide by ⅜

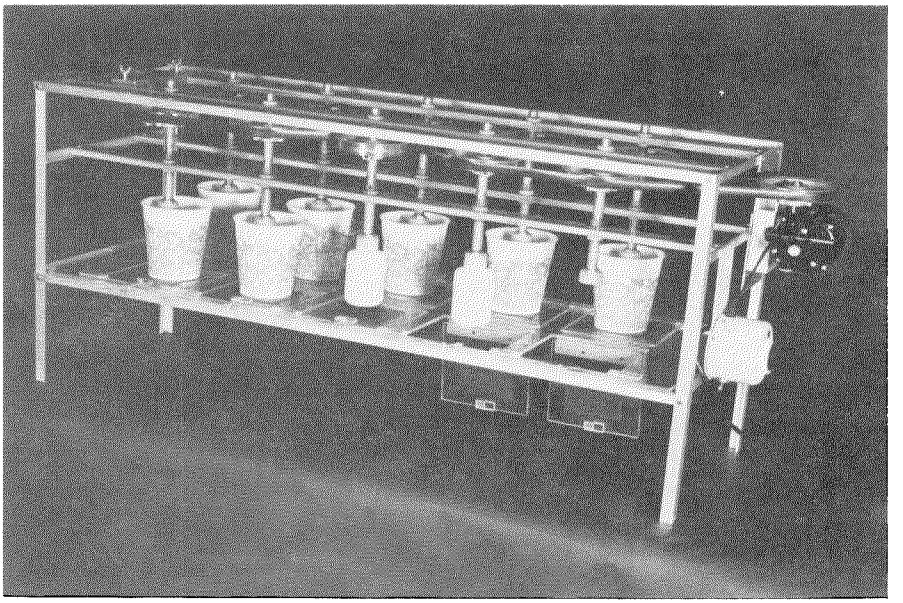

Fig. 1. Apparatus for evaluating larvicides against black flies.

in thick. Two of these were attached to the top of the frame 3¼ in in from each side. The other two were mounted 4 in directly below these. In each of these 4 bars, 5 nylon sleeve bearings (1 in long with inside diameter of ½ in) were press fitted into holes centered at 6 in intervals. The bearings were machined out sufficiently to permit the shafts to rotate freely. The shafts were 6061 aluminum tubing 7¼ in long with ½ in outside diameter. The lower end of the tubing was threaded to facilitate attaching the tops of the 4 oz. plastic bottles with pinch nuts. Brass collars held in place by set screws were placed on the shafts and rode on top of the lower bearings for each shaft. These permitted adjusting the vertical position of the shafts. Single groove "A" pulleys with ½ in bores, outside diameters of 4½ in and pitch diameters of 4¼ in were machined down to provide a ½ in wide flat groove with ⅛ in walls and 3 9/16 in diameter. The pulleys were attached with set screws to the shafts midway between the upper and lower bearings. Identical pulleys were used on the drive motor shaft and as a tensioner. The tensioner pulley was suspended between the upper structural members from an aluminum bar grooved to slide between the structural members and held in place by set screws. Tension on the belt was adjustable by moving the bar back and forth between the upper structural members. The belt was a flat ½ in wide clothes dryer drive belt as used by Colbo and Thompson (1978).

The drive motor was a Talboy® Model 134–2 stirrer motor. It had 3 shafts with reduction gears on 2 shafts. With the accompanying solid state transistorized speed control, speeds of from 10 to 7500 rpm were available. Using the slowest shaft, 10 to 125 rpm were available with a torque of 15 inch-pounds. Since the system was lightly loaded, the motor operated without stress. Usual speed during bioassays with late instar larvae was 120 rpm.

Conventional aeration using airstones was not provided. Instead, this apparatus relied on aeration of the water surface through the rotating action of the plastic bottle. The saturation point of water with oxygen at 22°C, the temperature at which our tests were conducted, is 8.8 ppm. Water which had been in our bioassay apparatus during 24 hr of operation, with larvae and food present held 7.4 ppm oxygen. Either the number of larvae used (10) required very little oxygen or aeration of the water at the surface, facilitated by the rotating bottle, was adequate.

Tests were conducted using tap water that had been aerated for 24 hrs. Ten late instar *S. vittatum* larvae, reared from field collected eggs, were transferred with 180 ml water to each 16 oz test container on the bioassay apparatus. The larvae attached quickly to either the plastic bottles or to the walls of the test containers and were allowed to acclimatize for several hours. Their distribution appeared to be random, indicating a relatively homogeneous environment. A small amount of food Tetramin® was provided as food during testing. The agent being tested was added in sufficient volume of water to give a final volume of 200 ml and the desired concentration of the agent. One container on each apparatus was used as an untreated control during each test. Duration of exposure was varied from 15 min to 24 hr. Exposures were terminated by removing the test containers through the doors in the plexiglass shelf and simply pouring out the test medium. The larvae remained attached, and the container was immediately refilled with fresh water. Although trace amounts of the test medium undoubtedly remained, this would have been reduced in concentration by more than 2 logs and would have been inconsequential at the test concentrations at which we were working. The same method was used to change rearing water daily, when larvae were held for several days. Accurate control of duration of exposure, in experiments in which the duration was less than 1 hr, was effected by individually timing the addition of the bacteria to each test container. Following each test, the test containers were discarded and the plastic bottles were removed and thoroughly cleaned before being reused. A detailed description of test procedures will be provided elsewhere (Frommer et al. 1980).

Excellent control survival through a large number of experiments indicated that the test environment was suitable for the larvae and that the handling techniques used were innocuous. The bottle rotating within a cup provided a more homogeneous test environment than in any test apparatus yet described and should have permitted a more uniform distribution of particulate material than given by a rotating stir bar. Removing test containers individually permitted accurate timing of duration of exposure, and pouring off the test medium permitted more abrupt and complete elimination of the test medium than a flow-through flushing system. The number of manual operations necessary with this apparatus limits the number of tests that can be conducted daily by one technician, but this has not been a problem at our level of effort.

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ACTIVITY BY *AEDES TRISERIATUS* IN OPEN TERRAIN[1]

GENE R. DEFOLIART AND MARSHA A. LISITZA

Department of Entomology, University of Wisconsin, Madison, Wisconsin

Most research on the epidemiology of LaCrosse (LAC) virus has aimed at elucidating the mechanisms whereby the virus survives in its natural endemic foci—forests and large woodlots with water-containing treeholes that are suitable for larval development of the vector, *Aedes triseriatus* Say. It has become increasingly apparent, however, that the majority of human clinical cases of LaCrosse encephalitis are contracted from mosquitoes bred in man-made water containers in the vicinity of human habitations. One of us (DeFoliart 1980) stated that "quantitative documentation is lacking, but the available evidence suggests that a high proportion of LaCrosse encephalitis cases in Wisconsin could be prevented through simple sanitation—the removal of old tires and other man-made containers from the vicinity of human habitations. Such containers around farm and suburban hillside homes and in shaded residential neighborhoods are readily colonized by female mosquitoes emigrating from their woodland haunts, and, with transovarial transmission of virus to progeny, they serve as long-term foci of infection that are in intimate contact with humans."

Documentation of the preceding is now becoming available. A followup of 34 clinical cases in Minnesota during 1979 (C. W. Hedberg and J. W. Washburn, Minn. Dept. of Health, pers. com.) revealed that man-made water containers, including old tires, were near the residences of 32 of the cases. Of 18 cases with no reported history of travel to other sites of possible infection, *Ae. triseriatus* was found breeding in artificial containers near 15 cases. A similar followup of 22 cases during 1979 in Ohio (Peterson 1980, personal communication) revealed man-made containers near the probable site of infection of 15 of the cases. Earlier, Barton (1978) conducted a survey in western Hennepin County, Minnesota, following hospitalization of two LaCrosse encephalitis cases and reported that "The most common breeding site found was the discarded tire."

Incidental to an endeavor during 1979 to identify woodlots that would be suitable as future experimental plots, we made several observations that bear upon the activity of *Ae. triseriatus* in open areas and its utilization of discarded tires and other man-made water containers as breeding sites. During early May, before spring vegetative growth was well underway, we entered 15 wooded areas in three southwestern Wisconsin counties and one south-central county (Dane) to determine whether ground-level treeholes were present. A return visit was made to 8 of the sites in the southwestern counties during late August in order to obtain biting counts (Table 1). Thirteen of the 15 areas were more or less isolated from surrounding woods, two areas were relatively narrow extensions from large forested areas (plots C and D, Table 1). The search for breeding sites, with one exception (plot C), was not thorough, only sufficient to establish that breeding sites were or were not present and to gain a rough estimate of their density.

Biting counts were taken by capturing individually, in test tubes, mosquitoes attracted to the observer. Exposure continued for 20 min

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