AN ECLECTIC METHOD OF TESTING THE EFFECTIVENESS OF CHEMICALS IN KILLING BLACKFLY LARVAE (SIMULIIDAE: DIPTERA) [1, 2]

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INTRODUCTION. For many years DDT has been the most widely used insecticide in blackfly larval control programs throughout most of the world (Central America, see Dalmat, 1958; Africa, see Barnley, 1958 and Bertram, 1958; Canada, see Peterson and Wolfe, 1958; U.S.A., see Collins & Jamnback, 1958). It is effective and inexpensive and has a relatively low mammalian toxicity (oral LD_{50} 118, dermal LD_{50} 2510 for female laboratory rats; Gaines, 1960). Partly for these reasons and partly because of the difficulties encountered in testing blackfly larvae under laboratory conditions, relatively little has been done in the way of systematically screening chemicals of potential value as larvicides in the laboratory.

Recent findings that DDT and closely related compounds may persist and accumulate in passage from one biological carrier to another (Barker, 1958—DDT from earthworms to birds; Hunt & Bischoff, 1960—DDD from fish to birds) suggest that one of the less stable insecticides might be used with less possibility of harmful side effects in blackfly larval control programs. In addition, there is also the possibility that the larvae may become resistant to DDT. With these thoughts in mind, methods of testing blackfly larvae in the laboratory were studied. Two different approaches to the problem have been described by Lea and Dalmat (1954) and Muirhead-Thomson (1957) respectively.

In the Lea and Dalmat method th larvae were brought into the laborator still attached to vegetation, then indivi ually removed with forceps and transferre to a pan of shallow water. They wer then transferred, with a syringe, to oper end glass tubes. These were covered wit gauze at the lower end to prevent th larvae from escaping. Twenty-five larva were placed in each tube, then the to was covered with gauze and the tub immersed in test solution for 30 minute The tube was then removed, rinsed i water, and finally held for 36 hours i running water. After 36 hours the mo tality was recorded.

There are two drawbacks to this ver useful technique. Lea and Dalmat, worl ing in a relatively undeveloped are (Guatemala), were able satisfactorily t use untreated river water piped into th laboratory. This is not available in mo laboratories and, when available in mo highly developed areas, the presence contaminants of various kinds, includin insecticides, may be suspected. Lea an Dalmat also reported a considerable larv mortality not related to treatment. Ther was no method of selecting the uninjure larvae and it is difficult to collect ston or vegetation with large numbers of larva without injuring many.

In the Muirhead-Thomson method th larvae were brought into the laborato attached to the vegetation. This was d vided into lots and placed in jars of wate A stream of air bubbles flowed contin ously up one side of the jar. The larva soon migrated to the stream of bubbl and attached themselves to the wall of th container. After a few hours the veget tion was removed and the water decante The larvae, for the most part, remaine

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ttached to the jar which was refilled with resh water. The chemical to be tested was hen added drop by drop so that it was apidly mixed by the current set up by he stream of bubbles. After an exposure eriod of one hour the liquid was decanted nd replaced with fresh water and the nortality determined after 24 hours.

The Muirhead-Thomson technique has he advantage of reducing larval handling o a minimum and eliminates the need for arge quantities of untreated water. How-ever, it has some disadvantages which ecome apparent when it becomes neces-ary to test more than a few chemicals or ilutions at one time. First, it is difficult o divide the vegetation into portions such hat sufficient and comparable numbers of arvae are left in each jar. This becomes ritical when relatively small numbers of arvae are available for testing. Second, nany of the larvae release their holds on he sides of the jar and are lost each time he water is changed. This may con-iderably affect the mortality figures.

MATERIALS AND METHODS. After some preliminary experimentation, a method of testing the susceptibility of blackfly larvae to chemicals in the laboratory was de-veloped that had many of the advantages of the above mentioned techniques and which evaded, to some extent, their dis-advantages.

Small stones or vegetation with blackfly larvae attached were taken from streams and transported to the laboratory in an enamel tray covered with a sheet or poly-ethylene to reduce drying. Larvae were collected fresh each day for that day's testing. In the laboratory they were washed from their attachment sites with a stream of water. Since many of them could not be detached in this manner it was necessary to collect about twice as many as were required for testing to as-sure a sufficient supply. A small water-circulating pump was then set in the enamel pan with the larvae. This set up a current and the uninjured larvae mi-grated to the portion of the pan where the current was swiftest. It was necessary to raise the tube emitting water above the surface to prevent larvae from crawling up into it.

During the period of approximately one-half hour while the larvae were migrating to favorable attachment sites the insecticide exposure jars were prepared. These were battery jars of 3750 ml. capacity filled to the 3000 ml. level with untreated (spring) water to which was added (by pipette) the insecticide to be tested dissolved in 95 percent ethanol. Usually nine exposure jars including the check were prepared for each test series.

The insecticide was then thoroughly mixed in the exposure jars. Uninjured larvae were selected from the enameled pan and transferred with a medicine dropper to a piece of wetted white organdy about six inches square. The organdy with a mesh of 70 x 78 per inch was well suited to retaining the larvae while allowing reasonably free interchange of liquid. About 30 larvae were deposited in the middle of each cloth, then the corners were brought together forming a bag. The bag was lowered into the insecticide suspension. It was stiff enough so that when the corners were brought together the sides of the bag did not press against the larvae. The bag was dipped three times in the suspension, then a glass plate was set across the top of the jar so that it held the bag in position with the larvae immersed in the insecticide.

After preliminary experimentation 20 minutes was selected as a convenient ex-posure period. At the end of the 20-minute period the bag was removed and, after the excess insecticide drained off, it was dipped three times in 3000 ml. of untreated water in a holding jar. Then the bag was fixed in position with the larvae immersed in the untreated water. It was assumed that the small amounts of chemical transferred with the larvae would be so diluted as to have no appreciable effect on the larval mortalities. The jars were arranged in pairs so that there was a holding jar be-hind each exposure jar making transfer from one to another a simple matter. The holding jars (but not the exposure jars) were equipped with small porous stone

bubblers through which compressed air entered setting up currents of water. The air was supplied by an aquarium air pump capable of delivering air to five jars simultaneously.

The chemicals to be tested were added to water as one-half to one percent stock solutions (depending on their solubility in ethanol). The technical DDT, *pp'*DDT, lindane and methoxychlor were Entomological Society of America insecticide reference standards purchased from Nutritional Biochemicals Corp., 21010 Miles Ave., Cleveland 28, Ohio. Other chemicals were obtained from the manufacturers concerned.[3] Usually two ml. or less of the solution were added to each jar. Five ml. of ethanol were routinely added to the check jar.

After a nominal 24 hours (actually 22–23 hours) mortality counts were made. Larvae were counted as dead if they did not respond when touched repeatedly with forceps. Larvae that pupated during the holding period were counted as living larvae. In routine testing at the Blue Mountain Lake laboratory the check mortality averaged 2.5 percent after 24 hours (1814 larvae tested in 50 checks). The average initial water temperature was 56.8° F. and the average final water temperature 58.2° F. for tests carried out between June 4 and July 18, 1962. The jars were kept on shelves on a covered porch outside of the laboratory accounting for the relatively low water temperatures for this time of year.

[3] In Table 1 only the common names of the insecticides are given. In most cases the chemical names and sources are given by Kenaga (1960). He does not give these data for the insecticides given below: Imidan, phthalimidomethyl-O, O-dimethyl phosphorodithioate supplied by Stauffer Chemical Co., 380 Madison Ave., New York 17, New York; Sumithion (47300), O, O-dimethyl O- (3-methyl-4-nitrophenyl) phosphorothioate supplied by American Cyanamid Co., Box 400, Princeton, N. J.; Chinch Rid (PPC#3), O-isopropylphenyl N-methylcarbamate supplied by Plant Products Corp., Kennedy Ave., Blue Pt., New York; and 39007, O-isopropylphenyl methylcarbamate supplied by Chemagro Corp., Box 4913, Hawthorn Rd., Kansas City 20, Missouri.

The exposure and holding jars were scrubbed with detergent and water followed by a thorough water rinse after each test. About once each week they were soaked in dilute acetone-water solution. Since the jars were randomly selected and no increases in check mortality were noted during the testing period, it was concluded that there was not appreciable contamination from tests on previous days. The pipettes which were exposed to concentrations of one-half to one percent of the toxicants were washed in acetone.

Larvae were not routinely identified, but representative collections were kept. Later examination indicated *Simulium venustum* Say and *S. verecundum* Stone and Jamnback were present in approximately equal numbers and *S. tuberosum* (Lundstroem) in smaller but substantial numbers. No attempt was made to distinguish possible differences in mortalities between species. Only medium to large larvae were used in the tests. All specimens for the regular test series were taken from streams near Blue Mountain Lake. These streams had a history of prior exposure to DDT (see discussion).

RESULTS. In preliminary tests with *pp'*DDT, carried out at the State Health Laboratory in Guilderland, near Albany, New York during the period from April 27 to May 15 with larvae from presumably untreated streams, about 95 percent of the larvae exposed to a concentration of 3.3 ppm. died, while at lower concentrations proportionately fewer died (Fig. 1). The species involved in these tests included species in the *Prosimulium hirtipes* group, *P. magnum* D. and S. and a few *C. mutata* (Malloch) and *S. venustum* Say.

Shortly thereafter the laboratory was moved to Blue Mountain Lake in the Adirondacks where a summer-long supply of blackfly larvae was assured. The insecticides selected for testing were, for the most part, those with relatively low oral and dermal mammalian toxicities. As a first step in screening the selected chemicals their effectiveness was compared with *pp'*DDT and technical DDT at a concen-

TABLE 1.—Laboratory tests of blackfly larvicides at a concentration of 3.33 ppm. toxicant (ethanol solvent) in water.

Insecticide	Percent mortality [1]	Approximate Mammalian [2] LD_{50} (mg./kg.)	
		Oral	Dermal
Baytex (p) [3]	100.0 (3)	310	330
Diazinon (p)	100.0 (4)	100	900
UC 8305 (p)	100.0 (5)	120	360
Lindane	99.2 (5)	91	900
Sumithion (p)	95.5 (5)	870	3000
Tech. DDT	94.2 (6)	118	2510
*pp'*DDT	93.7 (5)	118	2510
Imidan (p)	93.2 (5)	147	3160+
Vapona (p)	90.6 (5)	56	75
Dibrom (p)	89.2 (3)	430	1100
Methoxychlor	86.8 (7)	6000	6000+
Sevin (c)	52.8 (5)	540	5000
Ronnel (p)	49.6 (5)	1700	2000
Malathion (p)	34.9 (10)	1000	4444+
Chinch Rid (c)	23.2 (6)	400	2000
Paris green	16.8 (5)	100	2400+
Dylox (p)	14.6 (5)	500	1250
Dimethoate (p)	7.2 (5)	215	1000+
39007 (c)	5.4 (5)	104	1000+
Pyramat (c)	2.9 (5)	220	..

[1] Mortality after 24 hours, following 20 minutes exposure, corrected for check mortality by Abbott's formula. Figures in parentheses indicate number of replicates.
[2] Data supplied by manufacturer or from Gaines (1960).
[3] (p) = A phosphorous compound; (c) = a carbamate.

ration of 3.33 ppm. Usually five replicates were run, each on a different day with a different collection of larvae. These initial tests, summarized in Table 1, indicate that Baytex, Diazinon, and UC 8305 killed all larvae tested at 3.33 ppm. Lindane, Sumithion, technical DDT, *pp'*DDT, Imidan, Vapona, Dibrom, and methoxychlor were slightly less effective and Sevin, Ronnel, malathion, Chinch Rid, paris green, Dylox, dimethoate, 39007, and Pyramat were much less effective. Those in the latter group were dropped from further consideration.

As a second step the most promising chemicals were tested at one-tenth the first concentration or 0.33 ppm. In this series Baytex proved to be outstanding (Table 2).

As a third step Baytex and Diazinon were tested over a range of dosages to determine the concentration-mortality relationship. Technical and *pp'*DDT were

TABLE 2.—Laboratory tests of blackfly larvicides at a concentration of 0.33 ppm. toxicant (ethanol solvent) in water.

Insecticide	Percent mortality [1]
Baytex (p) [2]	85.5 (11)
Diazinon (p)	57.3 (11)
UC 8305 (p)	43.2 (5)
Lindane	30.3 (6)
Methoxychlor	19.9 (9)
Imidan (p)	19.3 (5)
Sumithion (p)	9.9 (5)
Vapona (p)	7.1 (5)

[1] Mortality after 24 hours, following 20 minute exposure, corrected for check mortality by Abbott's formula. Figure in parentheses indicates number of replicates tested.
[2] (p) = A phosphorous compound.

TABLE 3.—Median lethal concentration derived from concentration-mortality lines.

Chemical	Concentration (ppm.)
Baytex	0.20
Diazinon	0.26
*pp'*DDT	1.20
Prelim. test *pp'*DDT	1.34
Tech. DDT	1.54

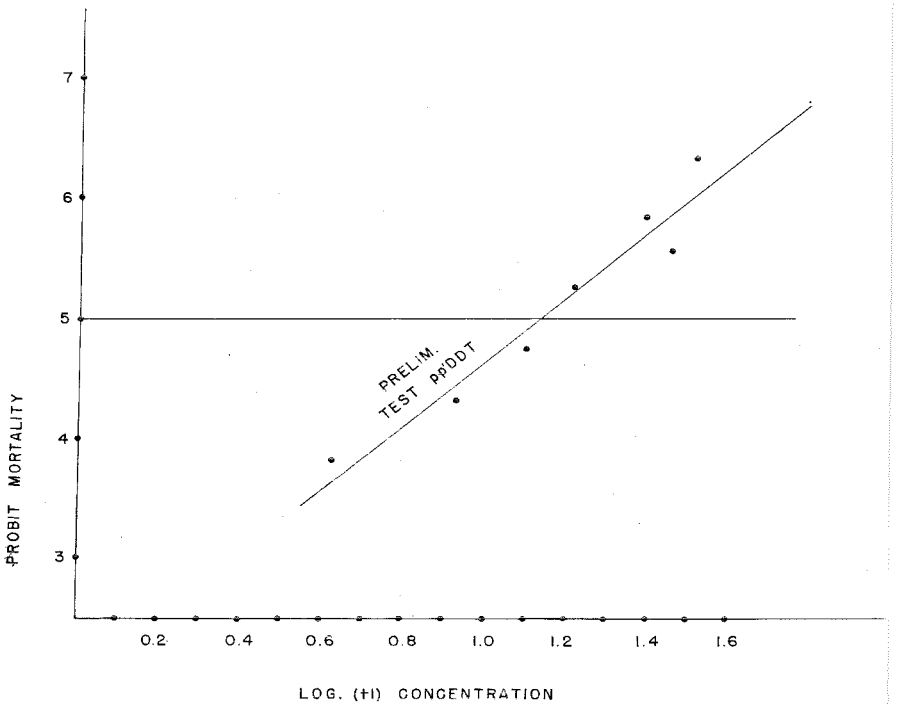

FIG. 1.—Concentration-mortality relationship of *pp'*DDT tested against blackfly larvae in the laboratory (preliminary tests).

also tested over a range of concentrations for comparison.

Five or six replicates were usually run at each dosage with an average of 31.1 larvae per test. The concentration-mortality lines were fitted by eye and the LC_{50} values derived from these lines (Fig. 2 and Table 3).

DISCUSSION. Concentration-mortality lines shown in Figure 2 were reasonably consistent considering that the larvae tested were collected in the field and were not of uniform species composition or age. The results compare favorably with those obtained by Muirhead-Thomson (1957) for *S. damnosum* Theobald although the concentration-mortality levels are quite different. This is probably due to differences in testing methods (e.g. his exposure period was one hour, ours 20 minutes), climatic differences (Liberia *vs.* upper New York State) and species differences (*S. damnosum vs.* a mixture of New York State species). No attempt was made to compare effective field and laboratory concentrations of the chemicals since the two may be quite dissimilar. Under laboratory conditions, Baytex was the most effective larvicide tested.

The testing method was also useful in determining whether or not the larvae were becoming DDT-resistant in areas that had been treated regularly over a long period. Larvae from streams that had been exposed to two blackfly larval control treatments per year for a period of about ten years were as susceptible to *pp'*DDT (Fig. 2) as larvae from streams that had never been treated with DDT (Fig. 1).

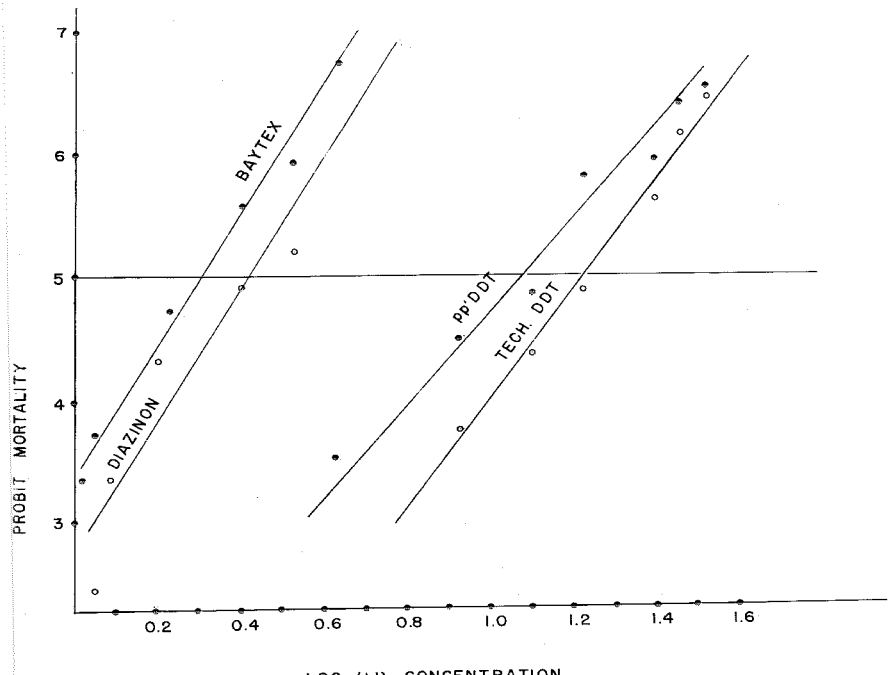

FIG. 2.—Concentration-mortality relationships of four chemicals tested against blackfly larvae in the laboratory.

SUMMARY. A simple method of testing the effectiveness of chemicals in killing blackfly larvae in the laboratory is described. Baytex was the most efficient of 9 chemicals tested. There was no indication of resistance to DDT in streams that had been treated for blackfly larval control twice annually over a ten year period.

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