tant, black decoy and CO_2, to show an additive effect may simply indicate a lack of sensitivity in the sampling techniques. However, another valid assumption is that the sphere of influence of the 2 types of attractants is the same. Thus, all the flies in an area would respond, and there would not be 2 separate populations, one reacting only to the visual attractant and the other only to the chemical attractant. The host-seeking female thus seems to be responding to both types of attractant at the same time.

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COMPARATIVE TOLERANCE LEVELS OF BLACK FLY (*SIMULIUM*) LARVAE TO PERMETHRIN (NRDC 143) AND TEMEPHOS

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INTRODUCTION. The screening and evaluation of new and more selective larvicides as replacements of DDT are playing an increasingly important part in black fly (*Simulium*) control programs (Chance 1970, Jamnback 1973, Le Berre et al. 1976, WHO, unpublished, Wallace et al. 1973). Despite a wide selection of candidate insecticides available, evaluation of their effect on *Simulium* larvae, as well as on associated non-target invertebrates, has been hampered by the paucity of base-line data of the type which can be provided only by strictly controlled and reproducible laboratory experiments. The great value of a logically phased evaluation scheme, progressing from laboratory through semi-natural to natural field trial, which has characterized the classic studies on fish toxicants (Lennon et al. 1971, Lennon 1973) is very far from being realized in the field of *Simulium* control. Some of the long recognized difficulties in the way of establishing *Simulium* lar-

vae in artificial or simulated streams, in which they could be exposed to exact time/concentrations of different larvicides in flowing water rather than in static water, are gradually being dealt with, and the advantages and limitations of these, particularly the laboratory type "trough" test, have been reviewed by Jamnback (1973).

Of these continuous flow evaluation techniques, the simple experimental channel designed by the author (Muirhead-Thomson 1969, 1970) appears to have many advantages in the way of simplicity and compactness, requiring the minimum of laboratory space, and well adapted for design of replicated tests. It has continued to give consistent results with both European and African species tested, and is the standard method used in this laboratory for continuing evaluation of both new and established *Simulium* larvicides.

This technique has been used in this investigation to reveal any gross and consistent differences between the effect of the new candidate *Simulium* larvicide, Permethrin, which has not yet been field-tested, and for the longer established Abate, which has been available for 10 years or more (Quéllenec 1967, WHO, unpublished) and about which there is a great deal of information (Jamnback 1973, Le Berre et al. 1976, WHO, unpublished, Escaffre et al. 1976, WHO, unpublished).

METHODS. Improvements which have been made to the original design of experimental channel are briefly described.

The basic test unit is a 10-liter aspirator bottle the top of which has been cut off to produce a straight-sided test vessel (Fig 1 A,B) (this can best be done by the manufacturers at the time of ordering). The advantage of this glass aspirator over the glass battery jars originally used is that the aspirator bottle is already provided with an outlet tube through which glass tubes carrying water and air supply can be introduced into the vessel.

The conversion of this vessel into an experimental channel with *Simulium* larvae firmly established takes place in the following stages.

The vessel is placed upright and filled with water. Air from an air pump is conducted through a rubber bung inserted in the basal outlet tube of the vessel, and—by means of a disposable hypodermic tube—produces a jet of fine air bubbles impinging against the lower part of the inner wall of the vessel (Fig 1B). Aquatic vegetation with attached *Simulium* larvae collected from natural habitats is brought

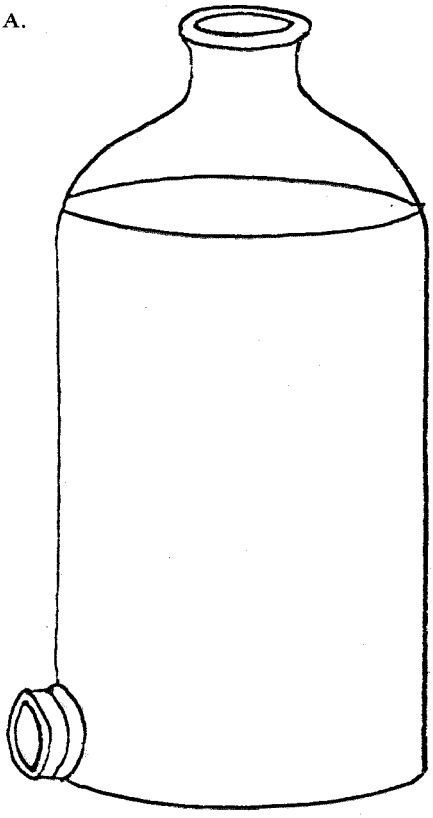
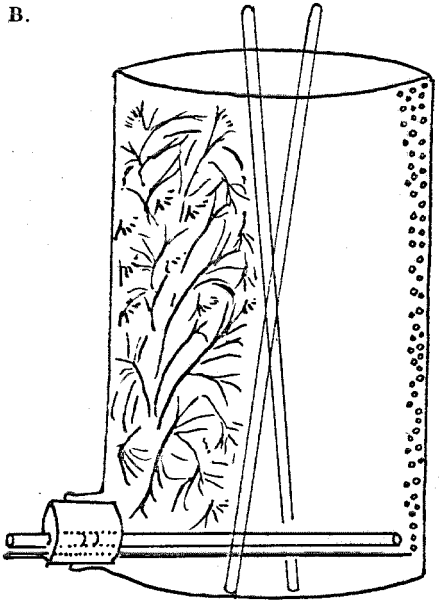
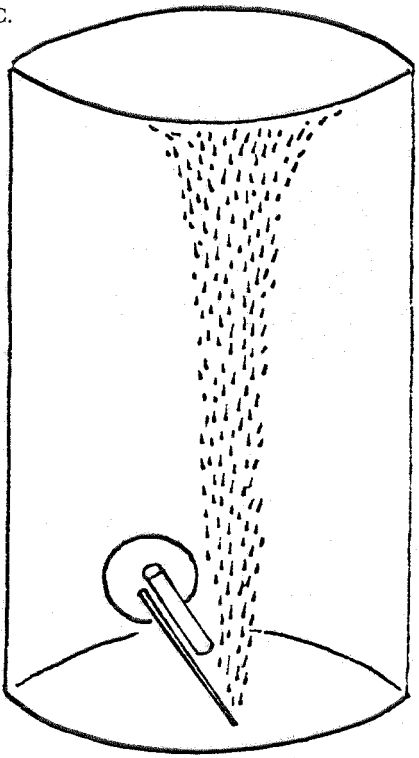


Fig. 1. First stage in establishing *Simulium* larvae in experimental vessel, B and C, formed by removing top of 10-litre aspirator bottle, A.

B.



C.



into the laboratory in a plastic bag (as described previously), damp without water, and immersed in the water in the vessel. One or two glass rods restrict the vegetation to the rear half of the vessel. In the course of a few hours, in practice overnight, larvae move spontaneously from the vegetation to the vertical zone of maximum aeration and agitation caused by the rising stream of air bubbles. The vegetation is then removed and, after a settling down period of an hour or two, the next stage in the test is carried out. (Fig 1, C).

A water flow from a series of 40-gallon (182 liters) fiber glass tanks containing de-chlorinated water is introduced into the vessel through the basal opening. At the same time the contents of the vessel are decanted by rotating the vessel through 90° into a horizontal position (Fig 2, A & B). The great majority of *Simulium* larvae remain firmly attached throughout this rotation, and now form a dense band of larvae exposed to a continuous and controlled flow of water.

To facilitate this conversion from vertical to horizontal position a temporary extension of the vessel is fitted in the form of a plastic bucket from which half the bottom has been cut out (Fig 3, A). This is fixed in position by tape and enables the decanting contents of the vessel to flow out clear of the funnel trap (Fig 3B, 2B). Immediately the vessel is in the horizontal position the temporary extension is quickly removed (Fig 3C). A simple wooden frame is designed to support the vessel in both the vertical and horizontal position (Fig 2B).

Larvae which have been stranded outside the central stream of water can be induced to move by squirts of water directed from a plastic wash bottle.

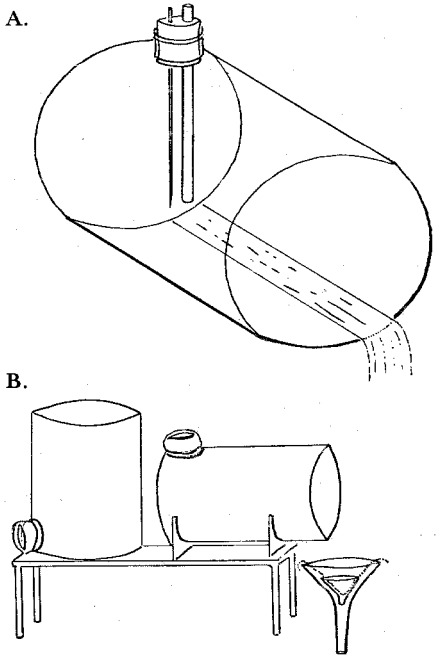


Fig 2. Second stage in establishing *Simulium* larvae in experimental channel A, by converting upright test vessel into horizontal one, B.

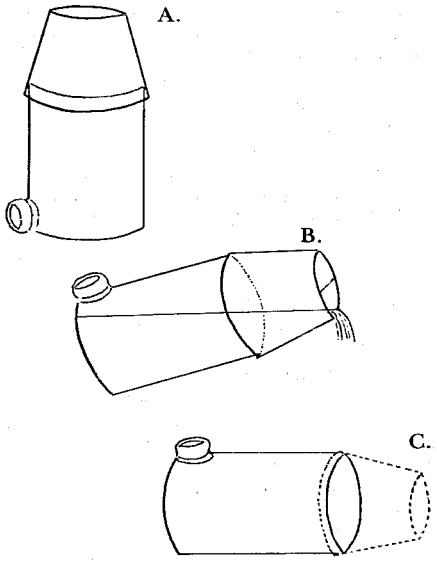


Fig. 3. Diagram showing use of temporary plastic-bucket fitment to facilitate conversion of the vertical test vessel into a horizontal channel.

The end result of this operation, in which there is no actual handling of larvae, is to establish a dense band of larvae, up to several hundreds if required or available, in an area of rapidly flowing water 25 cm long by 4 cm wide and 5 mm deep. Water is normally allowed to flow through at the rate of 2–3 liters per minute, sufficient to produce a flow of approximately 1 ft per sec. This rate of flow could be increased both by increasing the inflow or by slightly tilting the channel. Such an increase might be necessary to deal with the special requirements of certain cytotypes of *Simulium damnosum* (Quélennec, pers. comm). In the channel larvae normally remain attached and healthy for at least 3–4 days, long enough to carry out valid tests, and feeding movements continue throughout this period even in the absence of an obvious food supply. In short term experiments, completed within 24 hr, fragments of aquatic vegetation (e.g. *Ranunculus* plants) are introduced to the inner end of the channel in order to provide sufficient algae, bacteria and detritus.

For longer term experiments an additional flow of enriched natural water is provided by gravity flow from a model ecosystem containing components from the original habitat. A more critical approach to the question of standardizing the nature and quantity of larval food supply in such experiments is indicated, and work on those lines continues. When larvae have settled down, usually within 1–2 hours of establishing the artificial stream, exact dilutions (0.1 ppm, 0.05 ppm, 0.01 ppm etc) of the formulated larvicide are made up fresh in chlorine-free

water* in a series of 20-liter aspirator bottles, and monitored through the test vessel by gravity flow. The introduction of the larvicide dilution and the simultaneous cutting-off of the normal flow of clean water takes place smoothly so that there is no alteration in the rate of flow to which the larvae are exposed. At the end of the exposure period the resumption of the clean water flow is carried out in the same way.

Exposure periods are normally 1 hr, 30 min and 15 min, but the system would allow for much shorter period of exposure, say 5 min, to be equally accurately assessed.

As the chlorine-free water in the 40-gallon (182 liter) reservoirs is itself being constantly replaced from large outdoor tanks, a measure of temperature control is achieved automatically. Most tests in this series were carried out at 17.5°C–1°C. Unlike static tests, temperature control in running water systems requires further elaboration, and so far no attempt has been made to repeat these experiments at a range of controlled constant temperatures.

Larvae which detach or die during or following exposure to the larvicide, and are washed downstream, are trapped in a netting-lined funnel so constructed that it always contains a small central reservoir of water in which larvae have a chance of surviving when there is a continuous flow of clean water through the system (Fig 2B). This system is designed, not only to observe the lethal effects of different treatments, but also to reveal any irritant or activating effect which might lead to detachment and drift. Detachment becomes particularly significant if it takes place to a marked degree during the actual short exposure period, or if it operates independently of the lethal effects.

SPECIES TESTED. Larvae used in the present series of tests were those of *Simulium equinum* L, *S.erythrocephalum* De Geer, and *S.ornatum* Meigen which were present in varying proportions in the field samples collected. Previous comparisons between the reactions of pure samples of *S.ornatum* larvae and those of mixed *S.equinum* and *S.erythrocephalum* failed to reveal any appreciable difference between those two groups at least, and it was concluded that if specific differences do exist they are too small to influence the main objective of this study, viz., to reveal any gross and consistent differences to different larvicides on the part of *Simulium* larvae in general.

While no differences between species tested were apparent, there are considerable differences between the earliest instars and the late instars (full grown larvae) in any one species, with regard to susceptibility to insecticides. For this reason test mortality data are concerned mainly with late instar larvae which, at critical levels, may survive at exposures which are sufficient to produce high mortality in the earliest instars.

LARVICIDES TESTED. The two larvicides evaluated in this first series of continuing laboratory tests are the organophosphorus insecticide Temephos (Abate®) 20% EC, (Procida), and the synthetic pyrethroid, Permethrin (NRDC 143) 25% EC (Elliott et al. 1975).

RESULTS. The results are shown in Tables 1 & 2.

(1) Lethal effects. Under these experimental conditions there is a wide difference between the acute toxic effects of Abate and of Permethrin, the difference being large enough and consistent enough to rule out experimental error. In the case of Abate the minimum effective concentration producing a mortality of 90% or more in late instars, 24 hr after a 30 min exposure, is in the range of 0.2–0.5 ppm. With Permethrin the same effect is produced at a concentration range of 0.005–0.01 ppm.

The results with Procida Abate are somewhat at variance with field experience in that the recommended field dosage of 0.1 ppm for 15 min (Jamnback 1973, Wallace 1973, Philippon et al. 1976, WHO, unpublished) would be only partly

* Composition of water. Total hardness (as $CaCO_3$) 225 mg/l Conductivity 550 mhos at 18°C pH 7.4–7.6.

Table 1. Acute toxicity of Abate 20% E.C. to late instar larvae of *Simulium (S.equinum., S.erythrocephalum* and *S.ornatum)* 24 hours after exposures of 1 hour, 30 minutes and 15 mins. at 17.5° C ± 1.0

Concentration	Exposure time	Total late instars	Number dead after 24-hours	% mortality
1.0 ppm	30 mins	66	65	99%
	15 mins	531	398	75%
0.5 ppm	1 hour	115	115	100%
	30 mins	67	67	100%
0.2 ppm	1 hour	241	217	90%
0.1 ppm	1 hour	56	34	60%
0.01 ppm	1 hour	140	4	3%
Control		102	0	0%

effective against late instars in the laboratory tests, judging from the 1 hr exposure at that concentration. In contrast, similar laboratory tests with chlorpyrifos (Methyl dursban) EC formulation consistently produced 100% mortality at the recommended field dosage of that larvicide, viz 0.1 ppm for 30 min. These apparent discrepancies with regard to Abate could be attributed to one or other of several factors. Field trials with Abate have shown that some formulations are very much less effective than others (Escaffre et al. 1976, WHO, unpublished), and it is possible that the Procida formulation used in the present test series differed significantly to the batches provided for field trials. Another possible reason for the enhanced efficacy under field trial conditions is that in short intense aerial application—Lee (1973)—there might be a tendency to overdose, so that the *Simulium* larvae under attack are actually exposed to higher concentrations than calculated.

(2) Behavioral effects. With regard to differences in behavioral impact—Tables 3,4—it is seen that with Abate, as with several other organophosphorus larvicides previously tested by the same technique (Muirhead-Thomson 1970), signs of irritation and detachment on the part of late instar larvae do not begin to appear until about 1 hr after first introduction of the insecticide, at least in the time/concentration range used in these tests. In the case of the more susceptible early instars, significant detachment (17% to 27%) is evident 30 min after first introduction of the larvicide, increasing up to about 34% at the end of 1 hr.

Table 2. Acute toxicity of Permethrin (NRDC 143) 25% E.C. to late instar larvae of *Simulium (S.equinum, S.erythrocephalum* & *S.ornatum)* 24 hours after exposure of 1 hour, 30 minutes and 15 minutes, at 17.5° C ±1.0

Concentration	Exposure time	Total late instars	Number dead after 24-hours	% mortality
0.01 ppm	30 mins	1045	1025	98%
	15 mins	874	750	86%
0.005 ppm	1 hour	143	136	95%
	30 mins	559	525	94%
	30 mins	492	437	89%
0.001 ppm	1 hour	104	62	60%
0.0005 ppm	1 hour	80	0	0%
0.0002 ppm	1 hour	464	7	1.5%

Table 3. Detachment of early and late instar larvae of *Simulium (S.equinum, S.erythrocephalum* and *S.ornatum)* during exposures to Abate 20% E.C. at lethal levels

Concentration	Exposure period	Total larvae	Numbers detached at intervals after first exposure		
			0–30 min	0–60 min	0–2 hrs
1.0 ppm	30 mins	66 late	0	15	34
		130 early	35	45	122
0.5 ppm	30 mins	66 late	0	5	34
	1 hour	115 late	0	8	103
		130 early	22	45	126
0.2 ppm	1 hour	241 late	0	0	0
		272 early	0	6	60

In contrast, it is seen that Permethrin, like other synthetic pyrethroids as well as pyrethrin (Muirhead-Thomson 1970), produces a rather rapid irritant effect on late instar larvae, even at sub-lethal levels. At the concentrations tested approximately 50% (45%–53%) of the exposed late instars become detached and drift downstream by the end of the 30 min exposure period, and a high degree of detachment can be attained within 15 min of first exposure. This reaction is so strong and consistent under these controlled and reproducible laboratory conditions that it seems very likely that it also manifests itself in natural habitats under treatment. If this is indeed confirmed in the field, then the fraction detached during the actual short exposure period may be further exposed to the effects of the larvicide as they drift downstream with the "slug" or "bolt" of insecticide.

DISCUSSION. The experimental methods described in this report, and the first results with Abate and Permethrin are intended to assist in the overall evaluation of *Simulium* larvicides, and also to aid in the accurate interpretation of field trial data. Investigations at field and at laboratory level should be complementary, not necessarily antagonistic. The fact remains that there are certain facets in the reactions of *Simulium* larvae (and non-target invertebrates as well) to insecticides, which simply cannot be analyzed except by the controlled experimental approach.

The basic technique, in its earlier torm, proved to be equally effective for African *Simulium* as well as with the European species dealt with in this report. The equipment is simple and movable and could well be a useful adjunct to the extensive field trials on *S.damnosum* in Africa. The system is also by no means completely dependent on electricity, and the comparatively few hours of aeration necessary to induce larvae to attach to the wall of the vessel in the first stage of the test, could

Table 4. Detachment of late instar larvae of *Simulium (S.equinum, S.erythrocephalum* and *S.ornatum)* during exposures to Permethrin (NRDC 143) 25% E.C. approaching lethal levels.

Concentration	Exposure period	Total larvae	Numbers detached at intervals after first exposure	
			0–15 mins	0–30 mins
0.01 ppm	30 mins	1045	—	550 (53%)
	15 mins	874	420 (48%)	—
0.005 ppm	30 mins	559	—	265 (47%)
	30 mins	492	—	222 (45%)

be provided in an emergency by battery-operated aerators, or by displacement of air caused by water slowly flowing into a large sealed drum.

Clearly, the main contribution of this present report is to establish principles of laboratory investigation, and to reveal gross and consistent differences in reaction to different larvicides and formulations on the part of *Simulium* larvae in general. Their relevance to the Volta Basin project will have to await a repeat of these studies on *Simulium damnosum* itself, as well as on work in progress on the impact of these critical *Simulium* larvicidal concentrations on non-target stream biota.

SUMMARY. The acute toxicity, and behavioral impact, of Abate (Temephos) and Permethrin (NRDC 143) on *Simulium* larvae have been studied in an experimental channel or simulated stream. Insecticides were tested in the EC formulations used in field practice, at standard exposure periods of 1 hr. 30 min. and 15 min. The established laboratory technique has been redescribed to include modifications and improvements.

At standard 1-hr. and 30 min. exposures at 17.5 ± 1°C the minimum effective concentration sufficient to produce at least 90% mortality of late instar larvae (of *S. equinum, S. erythrocephalum, S. ornatum*) after a 24-hr. holding period in clean water was in the range 0.2 - 0.5 ppm for Abate, and 0.005 - 0.01 ppm for Permethrin. Under these conditions Permethrin is approximately 40 times more toxic than Abate.

At levels at and approaching lethal thresholds of Abate, there is very little detachment of late instar *Simulium* larvae during exposures of 30 min. and 1 hr. In contrast, roughly from 45%–53% of late stage larvae exposed to minimum lethal dosages of Permethrin become detached at the end of the short exposure of 15 min. and 30 min. The significance of these differences in behavioral impact of the two insecticides is discussed.

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