A PROGRESS REPORT ON SIMULATED STREAM TESTS OF BLACKFLY LARVICIDES

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The techniques of pesticide application used for routine control of blackflies are almost as numerous as the individual workers (Barnley, 1958; Fairchild and Barreda, 1945; Gjullin *et al.*, 1949; Gjullin *et al.*, 1950; Lea, 1955; and Lebrum, 1954). Because of their unusual susceptibility to pesticides, control procedures invariably have been directed at the larval stages; almost without exception, the results have been spectacular (Hocking, 1950).

The techniques have included the use of sawdust-filled cloth bags saturated with oil solutions of the pesticides suspended in the stream. Plaster of paris blocks made with emulsifiable concentrates have been similarly used. Various drip devices mounted above the water have also been employed. In addition, the pesticides have been spread by sprays delivered both with ground and airplane equipment (Arnason *et al.*, 1949; Collins *et al.*, 1952; Gjullin *et al.*, 1949; Hocking *et al.*, 1949; Kindler and Regan, 1949; and Travis *et al.*, 1951).

Recent developments in pesticides and formulations and new restrictions on the use of pesticides in streams have intensified the need for rapid, naturalistic test methods. Along with the need for improved methods, information is critically needed on the mode of action of the insecticides—whether they behave as contact or as stomach poisons. Also, there are few data published on the relative effectiveness of different formulations of the same chemical.

The results submitted here offer some interesting leads on the mode of entry of the pesticides into the blackfly larvae and on the relative effectiveness of a few insecticides and their formulations. More replications are needed before definite conclusions can be made, but it is hoped that these data will stimulate others to give more attention to the problems of blackfly control.

EQUIPMENT AND MATERIALS. — The equipment as described by Wilton and Travis (1965) consisted of a set of small test troughs through which water flowed to simulate streams. The water and the pesticides that flowed down these troughs were metered in from reservoir tanks. The test larvae, *Simulium pictipes,* were removed from streams in the vicinity of Ithaca, New York, and were placed in the troughs for the calculated exposures. Efforts were made to select large, mature larvae to reduce age and size variations.

The pesticides used were: DDT, methoxychlor, lindane, diazinon, Baytex, malathion, Sevin, DDD and phosdrin. Sevin and DDD were tested only as wettable powders and phosdrin only as emulsions. Baytex was tested as emulsions and as ethanol solutions. All other pesticides were tested as emulsions, as ethanol solutions, and as wettable powders.

The emulsions were made from emulsifiable concentrates. Some of the concentrates were commercial samples and a few were formulated in the laboratory on a weight-volume basis. Those formulated were prepared with 20 grams of pesticide and 10 grams of Triton X-100 made up to 100 ml. with xylene. The alcohol solutions were also prepared on a weight-volume basis; 20 grams of pesticide made up to 100 ml. with 95 percent ethanol. The wettable powders were all commercial samples and were usually made up as one gram of active ingredient in 100 ml. of a 1 percent Triton X-100 solution to help keep the powders in suspension.

METHODS.—Blackfly larvae were removed from the rock on which the colonies were established and were placed in the test troughs. With a little practice,

Table 1.—Percent 48-hour mortality with different exposure times in trough tests with DDT emulsions.

Exposure time, min.	0.25 ppm. (2 tests)	0.50 ppm. (3 tests)
5	32	68
10	25	75
15	50	80
20	57	92

2 controls average mortality 5%

a high percentage of the larvae could be induced to swing down from the fingers by their threads into the moving water of the troughs and attach to the paper lining.

For each test, twenty larvae of the proper size were placed in each of the test troughs. When the larvae were all well established, the spring clamps on the tubes from the pesticide metering bottles were opened for 15 minutes. After the insecticide was shut off, the larvae were left undisturbed in the flowing water of the troughs for a 5-minute wash period. Then the larvae were dislodged with a camel's-hair brush and were flushed into the collecting containers at the lower ends of the troughs. Counts were made of all larvae that had detached during the 15-minute exposure and the 5-minute wash period.

The larvae were retained in nylon bags in the nearby stream for the 24-and 48-hour mortality counts (Wilton and Travis, 1965). Larvae that could change their position when stimulated with a camel's-hair brush were considered to be alive. If only a rippling of the skin occurred, they were counted as dead.

Comparative tests were made in the laboratory and in the field with larvae contained in the cloth bags and exposed to the pesticides in still water (Jamnback, 1962) in gallon jars (here called jar tests). To determine if significant amounts of the pesticides were absorbed by the paper trough liners, one sample of the pesticide at a given concentration was collected from the mixing funnel before it entered the trough (funnel-jar tests) and another was collected after it had passed through the trough (trough-jar tests).

The control larvae were handled exactly as those exposed to the pesticides except that no chemical was added to the metering bottles. In test runs, ethanol or Triton-X100 was added to the metering jars for the control larvae to determine if these materials in the amounts used were toxic to the larvae.

The pesticides were added to the water in the metering bottles at 1 percent concentrations in the proper amounts to deliver the desired p.p.m. concentrations through the troughs. The water in the metering bottles was rapidly swirled for mixing as the pesticides were added. Agitation during the test was provided by air bubbling up through the contents of the bottle (see Wilton and Travis, 1965) and by hand agitation. The pesticides added in ethanol became suspensions of near colloidal size when the alcohol mixed with the water in the metering bottles, hence these formulations are here called suspensions rather than solutions.

The water temperature range for all reported tests was 51 to 75.5° F. A few tests above 80° were discarded as either the high temperatures or poor circulation of water in the holding cages, or both, caused a very high mortality in the controls. Except for these tests, no measurable differences in results could be detected when the same treatments were compared at the extreme ranges of the temperature conditions.

Results.—In the literature, various exposure times have been reported but there are almost no documentations to justify the period selected (Hocking, 1950). Trough tests with two concentrations of DDT emulsions and with four exposure times indicated that a 15-minute exposure would be acceptable for comparative studies (Table 1). This exposure time was also convenient as the capacity of the metering bottles was just adequate for a 15-minute test without refilling.

Screening tests indicated that, with the exception of malathion, all materials could be compared at concentrations of 0.50 to

0.06 p.p.m. Malathion, for comparable mortality, needed to be increased in concentration by at least eight times. Although there are a few reversals, the mortality data in Tables 2 and 3 show that the most effective pesticides were DDT and Baytex.

Of the three formulations tested (Tables 2 and 3), the emulsions were generally inferior to either suspensions or wettable powders. The differences between the suspensions and the wettable powder formulations appear to vary with the insecticide. The wettable powders were more effective than the suspensions for diazinon and malathion, but the reverse

TABLE 2.—Comparisons of several insecticides, formulations and concentrations (M=avg. percent 48-hour mortality; D=avg. percent larval detachment during tests).

Concentration ppm	Emulsion No. Tests	Emulsion M	Emulsion D	Suspension No. Tests	Suspension M	Suspension D	Wettable powder No. Tests	Wettable powder M	Wettable powder D
				DDT					
3.00	1	100	50	..	..	..	..	..	..
2.00	2	97	40	..	..	..	..	..	..
1.50	2	100	45	..	..	..	..	..	..
1.00	6	88	21	..	..	..	1	100	0
0.50	9	83	10	2	96	45	4	79	2
0.25	5	68	2	2	96	38	4	76	1
0.12	3	50	0	2	83	10	4	72	1
0.06	1	33	0	..	..	..	2	75	2
				Methoxychlor					
2.00	..	..	..	..	..	..	1	80	85
1.00	2	90	95	2	100	93	4	70	44
0.50	3	68	92	3	93	100	4	70	18
0.25	3	47	72	3	68	92	3	59	0
0.12	1	45	20	2	35	98	2	77	0
0.06	1	65	5	1	20	98	..	..	..
				Lindane					
0.50	2	53	45	2	64	22	3	72	42
0.25	2	40	41	2	70	52	3	55	32
0.12	2	50	38	2	63	50	3	41	27
0.06	1	50	50	2	50	50	2	60	38
				Diazinon					
2.00	2	93	0	1	85	0	..	..	..
1.00	3	98	0	1	100	0	1	95	5
0.50	5	80	2	3	43	0	5	77	1
0.25	5	17	1	3	12	0	5	50	2
0.12	4	20	0	2	18	0	4	35	0
0.06	1	0	0	1	20	0	3	40	0
				Baytex					
0.50	3	98	0	2	100	5	..	..	..
0.25	3	90	0	3	98	2	..	..	..
0.12	3	47	0	3	55	0	..	..	..
0.06	1	10	0	2	47	0	..	..	..
				Malathion					
4.00	1	17	5	..	..	..	2	53	10
2.00	2	20	3	1	20	0	2	62	3
1.00	2	28	5	1	20	0	2	27	0
0.50	3	0	8	1	20	0	2	25	0

32 controls average mortality 9%

Table 3.—Comparisons of several insecticides, formulations and concentrations (M=avg. percent 48-hour mortality; D=avg. percent larval detachment during tests).

Concentration ppm	Sevin wettable powder No. Tests	Sevin wettable powder M	Sevin wettable powder D	DDD wettable powder No. Tests	DDD wettable powder M	DDD wettable powder D	Phosdrin emulsion No. Tests	Phosdrin emulsion M	Phosdrin emulsion D
4.00	..	..	..	1	80	10	..	..	..
2.00	2	36	70	2	53	3	..	..	..
1.00	2	40	58	3	69	2	..	..	..
0.50	3	28	60	3	68	2	1	65	0
0.25	2	18	68	2	85	0	1	75	0
0.12	2	2	33	1	75	0	1	50	0
0.06	2	0	5	..	..	..	1	60	0
0.03	1	0	10	..	..	..	..	..	..
0.01	1	0	10	..	..	..	..	..	..
2 controls average mortality 7%									

was true for DDT. A reversal with methoxychlor at the 0.12 p.p.m. concentration and lindane at two of the four concentrations prevents showing a clear-cut difference between suspensions and wettable powders for all insecticides tested. It appears likely that there is little difference between the suspensions and the wettable powders for lindane.

Comparisons of the percentages of larvae that detached at the 0.50 to 0.06 p.p.m. concentrations (Tables 2 and 3) show that DDT, methoxychlor and DDD wettable powders and all formulations of diazinon, Baytex, malathion, as well as phosdrin emulsions, caused little detachment. Those insecticides causing a moderate detachment were Sevin wettable powder, and all formulations of lindane. It is interesting that the different formulations of lindane show little difference between larval mortality and larval detachment. Methoxychlor emulsions and suspensions caused the greatest percentage of detachment.

In causing detachment, the suspensions of DDT and methoxychlor were more effective than emulsions or wettable powders. Methoxychlor emulsions were better than wettable powders, but for DDT there was no difference between these two formulations. The order of effectiveness for malathion was emulsions >WP> suspensions. There were no distinct differences between the various formulations of lindane, diazinon, and Baytex.

With both lindane and Sevin, larvae started detaching within as short a time as two minutes after the start of an exposure in the troughs. Detachment for all other pesticides was slow, mostly toward the end of the 15-minute exposure time. Within two to five minutes the larvae exposed to lindane and Sevin appeared to be paralyzed and rigid. They often remained attached for some time after all movement ceased. These conditions were true even for the larvae exposed to the low concentrations. The mortalities were moderately low, particularly with lindane, and seemed to have little relationship either to the formulations or to their concentrations.

Direct comparisons of trough, trough-jar, and jar tests are made in Table 4. The validity of the single tests for each treatment in Table 4 is quite good as can be observed by comparing the average percent mortality of all trough tests (Table 2) with the mortality for the same treatment in Table 4. With two exceptions, the mortalities are much higher in trough tests than in jar tests. The two exceptions were Baytex emulsions and suspensions at the 0.12 p.p.m. level. As the mortalities in the comparative trough and jar tests are much below the average of all trough tests with these same Baytex

TABLE 4.—Results with comparative parallel tests designed to evaluate testing procedures. One test each treatment (PT=percent 48-hour mortality in comparative parallel tests).

Concentration, ppm	Trough, PT	Trough-jar, PT	Jar, PT
	DDT emulsion		
0.50	100	10	10
0.25	75	0	15
0.12	45	0	0
	DDT suspension		
0.50	100	60	100
0.25	95	50	50
0.12	65	5	40
	Diazinon emulsion		
0.50	25	5	25
0.25	5	5	0
0.12	0	0	0
	Diazinon suspension		
0.50	25	0	5
0.25	10	0	0
0.12	0	0	0
	Baytex emulsion		
0.25	80	60	70
0.12	11	10	25
0.06	..	20	0
	Baytex suspension		
0.25	95	85	100
0.12	20	10	30
0.06	..	0	5
18 controls average mortality 2%			

treatments (47 and 55 percent, respectively), it is likely that more replications would not confirm these reversals. The unusually low mortalities in the comparative parallel trough tests with diazinon emulsion cannot be explained. As the data in Table 4 indicate, the mortality in the trough tests (PT) is far below the overall average for diazinon emulsions. Since the comparative trough tests, the trough-jar, and the jar tests for diazinon emulsions were all run at the same time, they should be comparable.

A bioassay was made on the adsorption of the pesticides on the paper liners of the troughs. Data in Table 4 show that at the higher concentrations there were some lower mortalities with the formulations that had passed through the paper-lined troughs (trough-jar tests) than with formulations that had not (jar tests). Additional data, not tabulated here, indicate that the adsorption probably was not a significant factor in our tests. However, plans have been made to evaluate this problem further with other techniques.

A great saving of time was possible when it was demonstrated that the nylon retainer bag placed at the end of the troughs during the exposure periods did not adsorb enough insecticide to necessitate moving the larvae to clean pieces of cloth. The proof was obtained by dividing the larvae from selected tests into two lots; half were held in the retainer cloth used during the test and half were moved to clean cloths. There was no difference in mortality between the two lots.

DISCUSSION.—All laboratory tests in jars were discarded as the 48-hour mortalities in the controls were much too high for the tests to be useful. When the same type of test was conducted in the field and the larvae held in the stream, the 48-hour mortalities in the control were acceptably low. In fact, in all tests the 48-hour mortalities were so low that no correction was made in the data presented in the tables.

The 24-hour observations are not presented as there were so many moribund larvae that interpretations were difficult. The 24-hour trends paralleled the 48-hour readings. Control mortalities in all field tests averaged only 1.7 percent at 24 hours and 6.4 percent at 48 hours.

One of the interesting features of the trough tests was that the larvae could be seen in a feeding attitude with the mouth brushes well expanded. This same attitude is not common in still or slow-moving water. Also the larvae in the troughs tended to move about but little, indicating that conditions in the troughs were satisfactory.

A comparison of the percentages of the larvae that detached during the exposure and wash periods in the trough tests gives some indication of the potential of these various insecticides as detachment chemicals. From previous experience, it is known that a high percentage of the larvae that are stimulated to detach in streams do not reattach. Thus control can result even though the mortality due to direct toxic action of the insecticides is not high. The detached larvae will be lost to predation and to the unfavorable environments of quiet pools.

Although high mortalities were expected with larvae exposed to lindane and Sevin, the mortalities were surprisingly low. The paralysis of the larvae had disappeared by the 24-hour observation and seldom were there moribund larvae. The recovery from the paralysis observed with lindane and Sevin was indeed miraculous.

Considerable circumstantial evidence has been accumulating to indicate that the larvae screen out insecticides with their mouth brushes. Several bits of evidence from the present work seem to support this thought. First, the larvae that were exposed to lindane and Sevin were quickly paralyzed yet a high percentage recovered in the holding cages. Perhaps the early paralysis prevented a continuation of feeding during the exposure period so that toxins sufficient to cause death were not consumed. One of the first symptoms of this paralysis was seen in the immobilized mouth brushes. The second but perhaps less defendable evidence on the role of the feeding mechanism is that the solids were more effective than the emulsions. Whether actual feeding is the mechanism or whether this is merely a passive screening out of the materials on the surfaces of the mouth brushes needs to be confirmed. A third thought presented here as possible evidence to implicate the feeding mechanism is the high mortalities obtained in control operations with oil solutions even one-half to one and a half miles downstream from the point of application. Since much of the oil is trapped along the sides of the stream and on rocks and vegetation, the concentrations of the insecticide downstream must soon reach a level too low to justify support of a contact mechanism explanation. The efficiency of the mouth brushes could permit a high mortality at very low concentrations of the toxins if they were to pass down the stream as particulate matter or as oil droplets.

Finally, future tests are indicated with the trough equipment to provide for more replications. The equipment must be modified to provide more uniform mixing of the wettable powders in the metering bottles. At any rate, the system here described does provide a reasonably good naturalistic method of testing insecticides against blackfly larvae. There seems little doubt that the results from tests in simulated streams will be more applicable to routine control than will the results from tests conducted in quiet water.

Summary.—Tests were made against *Simulium pictipes* larvae in the vicinity of Ithaca, New York, with several insecticides and several formulations in small troughs to simulate streams. Comparisons were made by exposing larvae in the troughs for 15 minutes to various concentrations of the insecticides. Of the three formulations tested, emulsions caused less mortality than either suspensions or wettable powders. Wettable powders of diazinon, methoxychlor and malathion were more effective than suspensions, but the reverse was true for DDT. There were no clear differences between the effectiveness of lindane suspensions and wettable powders. Little detachment of larvae occurred during the exposure period either to DDT, methoxychlor or DDD wettable powders or to all formulations of diazinon, Baytex, malathion or to phosdrin emulsions. Moderate detachment was caused by Sevin wettable powders, and all formulations of lindane. Methoxychlor emulsions and suspensions caused the greatest detachment. The mortalities in trough tests were with only two exceptions higher than those in jar tests. Explanations of the superiority of particulate formulations over emulsions and of exposure in fast-moving versus quiet water are discussed.

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AN IMPROVED METHOD FOR SIMULATED STREAM TESTS OF BLACKFLY LARVICIDES

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The problem of testing chemicals for use as blackfly larvicides has been approached in several principal ways; by means of stream sector tests and by large-scale trough tests conducted beside the stream (Gjullin *et al.* 1949), or by container tests in which the larvae are brought into the laboratory and exposed to the chemicals in battery jars or similar containers (Muirhead-Thompson, 1957; Jamnback, 1962), or in glass tubes (Lea and Dalmat, 1954; W.H.O., 1954). Each of these procedures has both advantages and disadvantages.

Stream sector work has certain inherent advantages simply because the tests are made entirely within the natural environment of the larvae. The disadvantages of stream tests outweigh their advantages, however, especially when large numbers of chemicals are to be tested. Such tests are very time-consuming since an accurate determination of stream flow must be made at each test location. In addition, serious limitations on the number of tests which can be run may be caused by difficulty in locating comparable stream situation where larvae occur throughout an adequate length of stream. This factor may be critical since a given test site can be used only once unless rocks or other objects with larvae attached can be moved in. One of the least desirable features of stream sector testing is the direct addition