California, who visited the project and offered suggestions pertaining to the technical aspects of the project; and T. E. Hillis, Chemist, Division of Laboratories, California State Department of Public Health.

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LARVICIDE TESTS ON BLACKFLIES IN NEW HAMPSHIRE [1]

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In the summer of 1947 tests were made to evaluate several compounds as larvicides for blackflies breeding in streams in the White Mountains of New Hampshire. A material was sought which would control these insects but would be harmless to the fish population.

Little published information on this subject has been noted. O'Kane (1926) used oils of a soluble or miscible type, and these were effective under some conditions but were injurious to trout at dosages necessary to control larvae. Fairchild and Barreda (1945) found DDT effective against larvae in streams of Guatemala when applied as an emulsion at 0.1 p.p.m. Garnham and McMahon (1947) reported the eradication of simuliid larvae from an area in Kenya Colony, East Africa, with DDT dosages of 1.3 to 35.6 p.p.m.

In New Hampshire pyrethrum, DDT, benzene hexachloride, TDE, chlordan, caustic soda, and chlorinated lime were first tested in flumes at different dosages. The three most effective of these materials —TDE, DDT, and benzene hexachloride —were then tested under natural conditions in streams.

Procedure.—Flumes, or troughs, 6 feet long, lined with metal and water-proofed with shellac, were used for the preliminary tests with measured dosages of larvicides. The ends of the flumes were covered with fine-mesh wire. The flumes were placed in stream rapids so that a constant flow of water was maintained through them. Blackfly larvae attached to blades of grass were placed in the current. The larvicides were introduced into the flumes from a drip funnel gaged to deliver 1,000 cc. over a 10-minute interval. The desired dosages of toxic agent were obtained by varying the concentration of the solution or emulsion. At intervals of 10, 30 and 60 minutes counts were made of the detached larvae and of the normal larvae that remained attached to the blades of grass. Control counts were made of larvae subjected to the same handling in the flumes except that water alone, or acetone solvent alone, was added through the drip funnel.

In the stream tests pretreatment counts of the larval population on marked rocks and blades of grass were made in measured sections of a stream. The larvicides were introduced at the upper end of the test area during a 10-minute period, and posttreatment counts were made 1 or 1½

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[2] The work was carried out under the supervision of C. C. Deonier.

hours afterwards. A 24-hour check was made in two of the tests. The rates of flow of the streams were measured to determine the amount of material to be used.

It has been observed in these and in other tests that the blackfly larvae release themselves from their site of attachment within a short time after they are exposed to larvicides in their natural habitats. Evidently they are swept downstream by the current, but their ultimate fate has not been determined.

Results of Flume Tests.—Data obtained in these tests, on larvae of *Simulium venustum* Say, are shown in Table 1. DDT required a dosage of 1 p.p.m. and benzene

TABLE 1.—Flume tests in which the blackflies *Simulium venustum* were exposed for 10 minutes to larvicides in colloidal suspensions.

Material	Dosage (p.p.m.)	Number of larvae placed in flume	Percentage of larvae detached after 10 minutes	30 minutes	60 minutes
Pyrethrins	0.033	68	35	46 (23) [1]	–
		42	45	62 (10)	–
		69	30	57 (17)	–
		78	27	46 (20)	–
		80	35	61 (39)	–
	.01	31	26	45 (29)	–
	.33	82	26	48 (16)	–
		49	43	67 (33)	–
		37	30	49 (32)	–
	.5	44	34	64 (34)	–
	0 (control)	50	0	2	–
DDT	.05	Small 22	0	14	95
		Large 26	0	0	27
	.1	Small 35	9	48	–
		Large 5	0	0	–
		Small 40	7	25	42
		Large 7	14	14	14
	.2	Small 50	8	44	88
		Large 9	0	0	44
	1.0	Small 56	23	61	100
		Large 23	9	57	96
	0 (control)	48	–	8	0
		47	–	8	8
TDE	.033	Small 39	28	67	82
		Large 27	7	15	19
	.05	Small 48	54	93	–
		Large 2	100	100	–
		Small 28	46	96	100
		Large 8	12	50	87
	.10	Small 27	33	78	81
		Large 11	18	27	36
		Small 47	26	63	85
		Large 10	10	10	20
	.20	Small 40	80	90	100
		Large 3	33	100	100
		Small 35	80	97	100
		Large 10	50	60	100
	.5	Small 47	83	89	98
		Large 7	57	100	100
		Small 41	83	93	100
		Large 9	33	78	100
	1.0	Small 38	92	100	100
		Large 20	60	100	100
	0 (control)	57	–	–	12
		49	–	–	8

Material	Dosage (p.p.m.)	Number of larvae placed in flume	Percentage of larvae detached after 10 minutes	30 minutes	60 minutes
Benzene hexachloride (12 percent gamma isomer)	0.2	Small 34	6	59	–
		Large 17	0	0	–
	0.5	Small 89	15	69	85
		Large 6	17	33	33
	1.0	Small 48	15	54	92
		Large 1	0	0	0
	2.0	Small 38	53	100	100
		Large 2	60	100	100
	0 (control)	27	–	0	4
		45	–	4	9
Chlordan	1	Small 29	7	14	–
		Large 13	0	0	–
	2	Small 23	17	26	65
		Large 8	0	0	12
	4	Small 33	55	67	91
		Large 5	20	60	80
	0 (control)	47	–	6	11
		35	–	6	11
Acetone alone	100	47	0	0	–
	100	57	4	4	–
	1000	51	0	0	–
	5000	64	45	45	–

[1] Percentages of larvae noticeably affected but not detached are shown in parentheses.

hexachloride 2 p.p.m. to cause the detachment of all larvae within 60 minutes. Chlordan did not cause 100 per cent detachment at 4 p.p.m. The 60-minute observation period was probably too short to show accurately the full effect of these compounds. TDE caused all the larvae to detach in 60 minutes at only 0.2 p.p.m., and therefore appeared to be about 5 times as effective as DDT and 10 times as toxic as benzene hexachloride. Large larvae were more resistant to the materials than the small ones.

Pyrethrum caused some of the larvae to detach, but others, although visibly affected, remained on the grass blades and assumed a characteristic looped position. They appeared to attach themselves by silk secretion, and whether or not they subsequently recovered was not determined. For this reason the effectiveness of pyrethrum was difficult to determine. The percentages of detached larvae were about the same at all dosages. In two additional tests (not shown in the table) piperonyl cyclonene was added at the rate of 10 p.p.m., but it did not increase the effect of the pyrethrum noticeably.

Sodium hydroxide and chlorinated lime were also tested to determine whether their irritating effect might be sufficient to cause the larvae to detach. They were not completely effective at high dosages.

Results of Stream Tests.—DDT, TDE, and technical benzene hexachloride (12 per cent gamma isomer) were tested in 200-foot sections of streams heavily infested with larvae of *Simulium venustum* and smaller numbers of *Prosimulium hirtipes* Fries. The materials were applied in emulsions containing kerosene and *Triton X-100* (an aralkyl polyether alcohol). In two tests with TDE at 0.2 p.p.m. all the larvae were eliminated, and in one test at 0.05 p.p.m. less than 1 per cent of the larvae remained after treatment. The other two compounds gave almost complete control in one test each at 0.2 p.p.m.

The data are summarized in Table 2.

In another test TDE was similarly applied at intervals of 1 mile over a 5-mile section of one stream. Larval counts were made over a distance of 200 feet near the end of each mile section. About 1½ hours at 1 p.p.m.; at 2 p.p.m. all the small fish were severely affected, and at 3 p.p.m. the larger fish were also affected. All of these fish recovered within a few hours. At 5 p.p.m. 4 fish were killed and 12 others were severely affected but recovered later.

TABLE 2.—Stream tests on blackfly larvae with three compounds.

Material	Dosage (p.p.m.)		Larvae counted before treatment	Interval (hours)	Per cent reduction
		200-foot stream section			
TDE	0.05		6490	1	99.6
				24	99.5
	.2		369	1	100
			5000	1½	100 [1]
DDT	.2		2002	1	97.6
				24	99.8
Benzene hexachloride (12 per cent gamma isomer)	.2		3500	1 to 1½	99.1
		Five-mile stream section			
TDE	0.2	1st mile	2304	About 1½	98.8
		2nd mile	1376	1½	95.8
		3rd mile	1566	1½	99.3
		4th mile	1304	1½	100
		5th mile	27	1½	100
		Total	6577		98.6

[1] One large larva found a quarter mile below the point of application.

after treatment (Table 2) no larvae were found in the lower 2 miles of the area, and the control over the entire 5-mile section was 98.6 per cent.

Tests on Fish.—Preliminary tests were conducted to determine the relative toxicity of TDE and DDT to brook trout, *Salvelinus fontinalis,* and rainbow trout, *Salmo* sp., furnished by the State Game and Fish Commission from rearing ponds at Whitefield, N. H. A pen for holding the fish was constructed of quarter-inch mesh wire in the outlet stream from the rearing ponds. The compounds in emulsion form were applied above the pen in the same manner as in the larvicide tests.

TDE at 1, 1.5 and 5 p.p.m. had no noticeable effect on the fish. With DDT one of the small fish was slightly affected

Summary.—In flume tests on larvae of the blackfly *Simulium venustum* Say, a suspension of TDE caused the detachment from blades of grass of all larvae within 60 minutes at a dosage of 0.2 p.p.m. At this strength it was indicated to be about 5 times more toxic than DDT and 10 times more toxic than benzene hexachloride. Chlordan did not cause 100 per cent detachment at 4 p.p.m. Pyrethrum at low dosages affected the larvae, but caused only a part of them to detach.

In stream tests the larvae were almost completely cleared from 200-foot sections of the streams within 1½ hours after 10-minute applications of emulsions of TDE were applied at 0.05 p.p.m. and DDT and benzene hexachloride at 0.2 p.p.m. TDE was completely effective at 0.2 p.p.m. At

this dosage also, applications of TDE at intervals of 1 mile over a 5-mile section of one stream gave 98.6 per cent control.

TDE was apparently much less toxic than DDT to rainbow and brook trout, having no noticeable effect on those exposed for short periods to applications in emulsion form up to 5 p.p.m. With DDT at this dosage, 4 of 16 trout were killed and the others were severely affected. Severe temporary effects on fish exposed to dosages of 2 and 3 p.p.m. were also observed.

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REARING OF *ANOPHELES QUADRIMACULATUS* SAY AND *AEDES AEGYPTI* (L.) IN THE LABORATORY[1]

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The importance of *Anopheles quadrimaculatus* Say as a common vector of malaria has caused many workers to try to rear this species. The purpose of this paper is to give a simple practical method of rearing this species under laboratory conditions. Directions are also given for rearing *Aedes aegypti* (L.).

Anopheles Quadrimaculatus

The original culture of *Anopheles quadrimaculatus* maintained at the Bureau of Entomology and Plant Quarantine laboratory, Agricultural Research Center, Beltsville, Md., was obtained through the courtesy of Walter Reed Medical Center, Washington, D. C. This culture was producing a maximum of 100 adults a day, but as more work was to be done with this species it was necessary to increase the culture to produce a maximum of 5,000 adults a day. Several shipments of eggs were made from the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine. Three shipments were made from the Tennessee Valley Authority, one lot was received from the United States Public Health Service, and numerous shipments extending over a period of three months were received from a commercial concern. As a result of this increase there was always a surplus of eggs for experimental use.

More than a hundred methods and techniques[2] using various foods and media were tested in this laboratory. The method that was evolved as a result of these tests produces from 3,000 to 5,000 pupae a day. This method has been used for 3 years and has proved very efficient.

TREATMENT OF EGGS

White-enamel pans 8 inches in diameter are used for oviposition. They are filled two-thirds full of pond water,[3] and on the

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[3] The pond is an abandoned iron mine that is fed by springs and has a slight run-off. It has about 1 acre of surface.