mined and compared with factors obtained by a different method.

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DIBUTYL O-CRESOL: ITS EFFECTS ON MOSQUITO SURVIVAL AND OVIPOSITION AND ON PLANKTON POPULATIONS

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ABSTRACT. Di-tert-butyl o-cresol at 1.8 ppm reduced larval populations of *Culex peus* Speiser in artificial pools by 75 percent for 6–10 days. This chemical also caused the water to become repellent to ovipositing females for a period of 6–10 days. Marked changes in bacterial, algal, ciliate, and rotifer populations occurred following treatment.

From an ecological point of view, development of mosquito larvicides which do not affect non-target organisms is highly desirable. Aquatic predators such as dragonfly nymphs, predaceous beetles, and notonectids frequently exert considerable control over mosquito populations, but are highly susceptible to insecticides now employed in mosquito control programs (Hurlbert *et al.*, 1971). As a result, the long-run effect of insecticide treatment is sometimes to increase mosquito populations (Mulla and Darwazeh, unpublished data).

In the course of our chemical analysis of larval "overcrowding factors" (Ikeshoji and Mulla 1970a, b), substances having much potential as selective insecticides, we found di-tert-butyl p-cresol, an antioxidant added to certain commercial ether preparations, to be highly toxic to mosquito larvae at ecdysis. Subsequently, we tested the toxicities of its three chemical isomers in the laboratory and found the o-derivative to be the most toxic, having an LC_{50} of 0.1 ppm for 1st instar larvae and 1.0 ppm for 4th instar larvae of *Culex pipiens quinquefasciatus* Say. The present paper describes the toxicity of this compound to *Culex peus* under field conditions, its inhibition of *C. peus* oviposition in treated water, and changes in phytoplankton and zooplankton populations resulting from reduction in numbers of mosquito larvae.

The experiments were conducted in six small artificial pools, each 90 cm x 90 cm x 30 cm, and completely lined with a polyethylene sheet that was replaced at the beginning of each experiment. A 2 cm layer of sandy soil was placed on the bottom of each pool. Water from a nearby reservoir was added and maintained at a depth of 20 cm by means of taps equipped with float valves. To attract ovipositing mosquitoes, 300 g of CSMA fly medium were added to each pool at the time of flooding.

Two experiments were performed during July and August, 1970. In each, 300 mg of di-tert-butyl o-cresol dissolved in 3 ml of pure ether were applied with a pipette to the surface of each of three pools. This produced a concentration of 1.8 ppm, equivalent to the LC_{98} for 1st instar larvae and the LC_{75} for the 4th in-

star larvae of *Culex pipiens quinquefasciatus*. The other three pools were kept as controls. The two experiments differed only in the number of days elapsing between flooding of the pools and introduction of the chemical. These were 5 and 2 days, respectively.

Larvae and pupae of *C. peus* were collected daily or every other day from approximately 100 sq cm of pool surface, 25 sq cm in each corner, with an enamel dipper.

The toxicity of the compound was best demonstrated in the first experiment, where the confounding effect of differential oviposition was eliminated. Every morning during this experiment, all egg rafts laid the previous night were distributed equally among the six pools. Two days after treatment, the total number of larvae (exclusive of 1st instars) and pupae averaged 75 percent fewer in treated than in control pools (Fig. 1). This strong and significant difference persisted for at least 10 days, soon after which the experiment was terminated. The numbers of 1st instar larvae were little different in treated and control pools. This accorded with laboratory observations that newly hatched 1st instars are not especially vulnerable to the chemical and that mortality occurs primarily at times of ecdysis.

When, in the second experiment, the chemical was introduced only 2 days after flooding and introduction of organic matter, toxicity disappeared more rapidly, possibly indicating more rapid decomposition under the more putrid conditions. Mosquito populations in treated pools were significantly lower than those in control pools only over a period of 6 days (Table 1), despite the fact that egg-rafts were not redistributed as in the first experiment.

The chemical caused a marked reduction in the number of mosquito egg-rafts deposited in treated pools. In the first experiment, the numbers of egg-rafts laid increased to a maximum of 120 per control pool on the seventh night after flooding and declined steadily to 2–3 per control pool over the next 10 days (Fig. 1). Oviposition in treated pools was significantly reduced by the second day following treatment and, over this and the following 8 days, averaged 7.3 egg-rafts per night as compared with an average of 62.9 egg-rafts per control pool per night during the same period.

In the second experiment, oviposition rates were not depressed until at least the sixth day after treatment (Table 1). During this and the following 8 days, there were deposited an average of 13.5 egg-rafts per treated pool per night as against an average of 27.5 egg-rafts per control pool per night. On only one of the last five of these nights was the difference between treated and control pools significant (Table 1).

The repulsion of ovipositing females from treated pools appeared not to be an effect of the chemical *per se*. At least females of *C. pipiens quinquefasciatus* in laboratory colonies oviposited in jars containing concentrations of the chemical similar to those used in the pools just as frequently as they did in jars containing distilled water. However, water taken from a treated pool during a period of depressed oviposition rates (August 22–29) in treated pools proved less attractive to ovipositing *C. pipiens quinquefasciatus* females than did distilled water (39 percent vs 61 percent of egg-rafts laid) while water taken from a control pool proved more attractive than distilled water (57 percent vs 43 percent of egg-rafts laid). The difference was significant ($P<.05$) in each case. We conclude that the treated pools contained an oviposition repellent and that this resulted from interaction of the chemical with substances or organisms in the pools.

To determine what organisms might be involved in production of the oviposition repellent(s), we monitored bacterial, phytoplankton, and zooplankton populations during the second experiment. Bacteria and phytoplankton in unconcentrated water samples were counted on a Spencer Brightline hemacytometer. Two

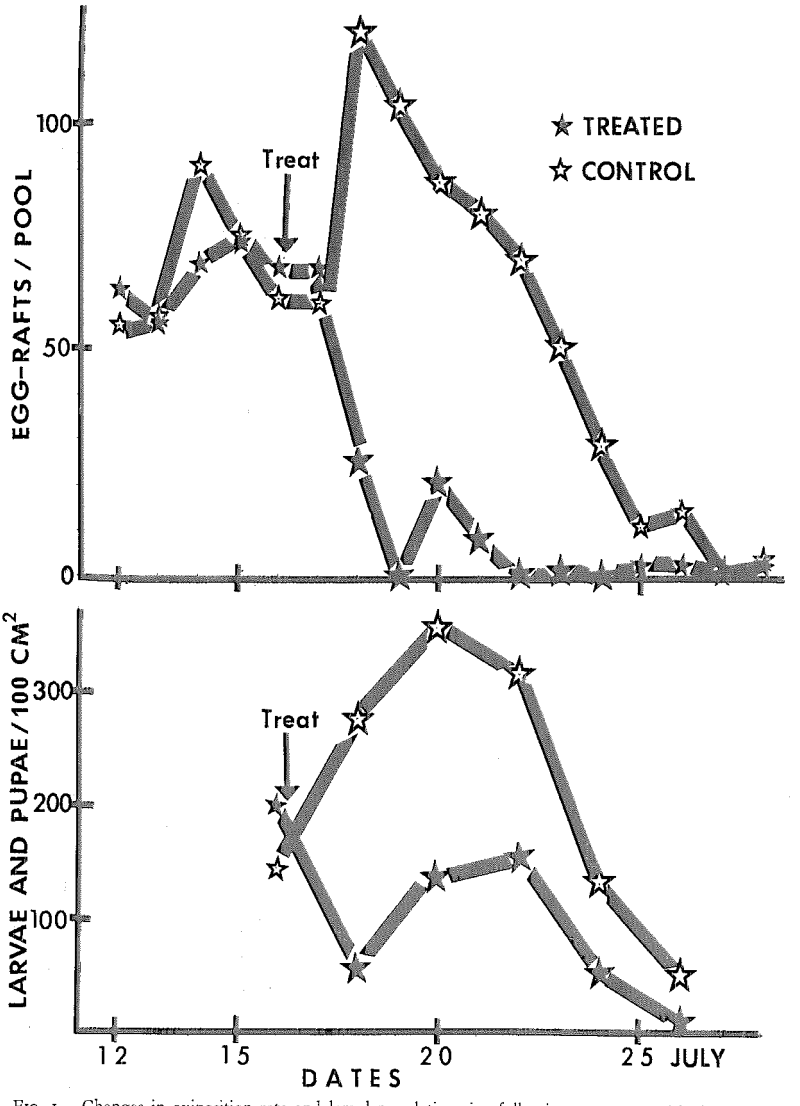

FIG. 1.—Changes in oviposition rate and larval population size following treatment with di-tert-butyl o-cresol at 1.8 ppm. Large stars represent dates on which differences were statistically significant ($P<.0.05$).

liters of water passed through a sieving device with mesh openings 74μ across yielded a zooplankton sample, which was preserved in formaldehyde and examined in a Sedgwick-Rafter counting chamber under a compound microscope.

Total numbers of bacteria declined rapidly, possibly as a result of grazing by ciliates and rotifers, and they were more abundant in treated than in control pools on the day following treatment, but otherwise showed no effects (Table 1).

TABLE 1.—Changes in populations of mosquitoes, bacteria, phytoplankton, and zooplankton following treatment of pools with 1.8 ppm of di-tert-butyl o-cresol. Data represent arithmetic means for treated (T) and control (C) pools. Asterisks (*) indicate statistical significance ($P<.05$) as determined by Wilcoxon rank sum test.

	Egg-rafts/pond		Mosquitoes/[1] 25 sq cm		Bacteria (x10^5/ml)		Small algae[2] (x10^4/ml)		*Chlorogonium*[3] (x10/ml)		Ciliates[4] (x1/ml)		Rotifers (x10^{-2}/ml)	
Dates	T	C	T	C	T	C	T	C	T	C	T	C	T	C
Aug. 13					Pools flooded; organic matter introduced									
14	63	92	..	..	438	431	..	..	..	..	..	..	..	..
15	132	107	..	..	297	278	1	1	93	253	..	..	..	..
					Chemical treatment									
16	143	113	..	..	193 *	102	6	7	70	20	..	..	..	..
17	84	79	21 *	151	69	48	136	107	173	68	190	135	..	..
18	73	67	..	..	..	..	376	249	330	40	412 *	103	6	3
19	33	47	309 *	1164	12	13	466	553	88 *	7	133 *	28	55 *	1
20	30	25	..	..	..	..	561	512	77 *	5	178 *	19	188 *	7
21	18	31	389	1118	19	8	396 *	1393	33 *	4	12 *	1	190 *	7
22	6 *	42	..	..	..	..	..	..	..	..	..	..	..	..
23	8 *	25	538	642	28	13	573 *	2121	6 *	1	0	0	2	1
24	20 *	37	..	..	..	..	..	..	..	..	..	..	..	..
25	13	26	583	601	49	33	1426 *	2867	6	1	0	0	8 *	2
26	17	23	..	..	..	..	..	..	..	..	..	..	..	..
27	16	20	270	296	23	17	1491	2400	1	0	0	0	1	0
28	20 *	38	..	..	..	..	..	..	..	..	..	..	..	..
29	8	16	..	..	..	..	..	..	..	..	..	..	..	..

[1] Includes 2d, 3d, and 4th instar larvae, and pupae.
[2] *Ankistrodesmus* sp. (12–15μ x 2–3μ) and an unidentified unicellular alga (5μ diam.).
[3] *Chlorogonium* sp. was a flagellate 35–50μ long.
[4] *Vorticella* and various holotrichous ciliates, 50–150μ long.

The species composition of the bacterial populations may or may not have been affected. Rotifers, represented primarily by *Brachionus calyciflorus* Pallas and *B. bidentata* Anderson, increased dramatically following reduction of larval mosquito populations by the chemical. *Culex peus* larvae were voracious predators of rotifers as evidenced by the abundance of rotifer trophi or "jaws" in larval digestive tracts. Release from mosquito grazing pressure also was the probable cause of increased ciliate and *Chlorogonium* sp. populations in treated pools. On the other hand, small algae of a size utilizable by the rotifers (but probably not by the mosquitoes) decreased in abundance after rotifers increased in the treated pools. Some alterations in phytoplankton populations may have resulted from a selective toxic action of the chemical; in general, phenol compounds are weakly algicidal (Palmer and Maloney, 1955).

The repellent factor in these experiments has not been identified, and many possibilities clearly exist. Previous work (Ikeshoji *et al.*, 1967) has shown bacteria to be capable of producing mosquito oviposition repellents. Some algal metabolites may have similar effects. Changes in the metabolites produced by a mosquito breeding site may be caused by direct effects of the di-tert-butyl o-cresol on the physiology of microorganism populations or by changes in the species composition of microorganism populations as a result of differential susceptibility to the chemical or from changes in grazing pressure. It is also possible that the repellent factor is simply a degradation product of di-tert-butyl o-cresol. Di-tert-butyl p-cresol can react with fatty acids to yield quinones (Reich and Stivala, 1969); and certain quinones are repellent to insects (Eisner, 1970).

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