A MOSQUITO LARVICIDE WITH FAVORABLE ENVIRONMENTAL PROPERTIES

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ABSTRACT. One part of 2,6-di-*t*-butyl-4-(α,α-dimethylbenzyl) phenol in 10,000,000 parts of water blocks the metamorphosis of all instars of mosquito larvae at the prepupal stage. Ninety-two percent of fourth stage larvae were killed as pre-pupae after 1 hour exposure to the chemical at 1.0 ppm. The compound is highly selective to mosquito larvae; it is non-toxic to fish and mammals and is biodegradable.

Several groups of chemicals have been reported to mimic juvenile hormone activity in insects (Bower *et al.*, 1966; Bower, 1968; Wigglesworth, 1969). For the most part, these chemicals are analogs of the authentic hormone and act in a similar manner (Spielman and Williams, 1966; Srivastava and Gilbert, 1968). Unfortunately, only few of these analogs were evaluated against economically important species in tests that would indicate potential utility as insecticides rather than mere hormonal effect. This paper presents a new chemical with a juvenile hormone-type activity which is chemically unrelated to the insect hormone. This chemical 2,6-di-*t*-butyl-4-(α,α-dimethylbenzyl) phenol, is being tested under the code designation MON-0585. The experiments reported here indicate that MON-0585 may well have practical importance as a "third generation insecticide" (Williams, 1967).

MATERIALS AND METHODS. For testing, one-tenth ml of the test solutions containing MON-0585 in acetone were pipetted into 50 ml of water in large, 3 cm ID, test tubes. Approximately 25 second instar *Aedes aegypti* larvae were introduced into each tube and kept in a constant temperature (26° C.) and humidity (RH 60 percent) box. The tubes were checked daily and 1.0 ml food (2 percent liver powder, Nutritional Biochemical Corporation) was added. Shortly after pupation the tubes were fitted with a half-pint ice cream carton, having a screen top; thus the emerging adults could be collected, counted and used for further studies. Variations of the above test included the use of third and fourth instar larvae and short-term exposure to the chemical followed by transfer of the larvae to distilled water.

Each test was replicated on at least 3 different days. For LD_{50} determinations the average corrected percent mortalities were plotted on log-probit paper and the dosage mortality lines fitted by eye.

RESULTS AND DISCUSSION. Activity Against Mosquito Larvae. Figure 1 shows the morphogenetic effects of MON-0585 on *Aedes aegypti* larvae. All stages of mosquito larvae, when exposed to the chemical, seem to develop normally through their larval instars. However, metamorphosis is blocked in the early stages of pupation and the resulting pre-pupae die in a characteristic compact, stalky and unmelanized form. It is interesting to note that even at the high dose levels, little or no mortality occurred at the larval stages. Similar effects were also demonstrated with *Anopheles albimanus* and *Culex restuans*.

Table 1 summarizes the activity of MON-0585 against three larval instars of *A. aegypti* under continuous exposure. Re-

TABLE 1.—Toxicity of MON-0585 to three larval instars of *Aedes aegypti*, continuous exposure.

Instar	LD_{50} (ppm)	LD_{50} (ppm)
2nd	0.065	0.12
3rd	0.05	0.1
4th	0.04	0.1

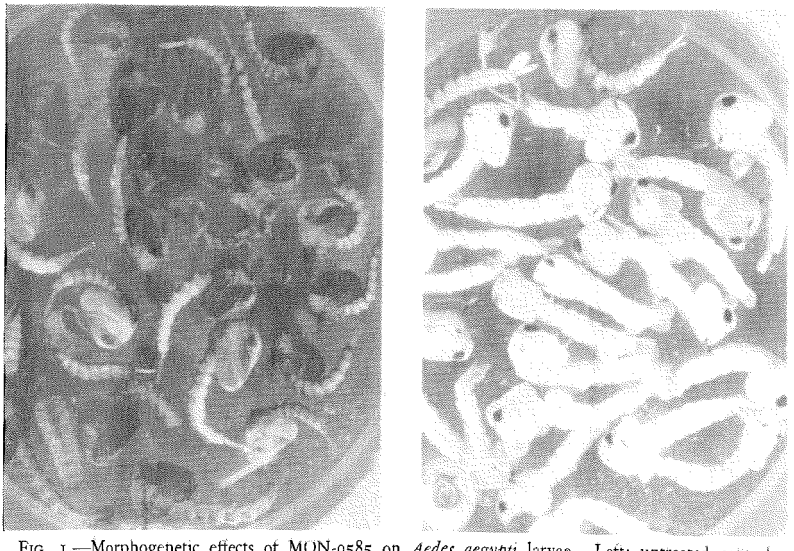

FIG. 1.—Morphogenetic effects of MON-0585 on *Aedes aegypti* larvae. Left: untreated control pupae; right: affected prepupae (treated at 2nd instar with 0.2 ppm MON-0585).

TABLE 2.—Effect of varying the exposure time of mosquito larvae to MON-0585 on adult emergence.

Length of Exposure (Hours) *	Dose (ppm)	Emerging Adults (%)		
		Larval Instar Treated		
		2nd	3rd	4th (Early)
1	0.1	100	98	98
	1.0	100	90	8
	2.0	100	48	0
3	0.1	98	88	28
	1.0	100	13	0
	2.0	80	10	0
6	0.1	100	35	10
	1.0	90	0	0
	2.0	70	10	0
8	0.1	98	38	13
	1.0	95	3	0
	2.0	70	0	0
14	0.1	92	30	0
	1.0	25	0	0
	2.0	30	0	0
24	0.1	80	30	5
	1.0	10	0	0
	2.0	10	0	0
Continuous	0.1	40	20	0
	1.0	0	0	0
	2.0	0	0	0

* Larvae transferred to distilled water after the indicated exposure periods.

gardless of the stage in which the mosquito larvae were treated, identical response was obtained in all cases, complete larval development followed by prepupal death. Furthermore, in contrast to conventional insecticides, late instars were as susceptible to the chemical as the early instars.

The results of variation in the exposure time of mosquito larvae to MON-0585 are shown in Table 2. In this test larvae were exposed to the chemical for a specific length of time and were subsequently transferred to distilled water for completion of their life cycle. The results show that the fourth instar larvae are most sensitive to the chemical and as little as one hour exposure at 1.0 ppm was sufficient to elicit 92 percent response.

TABLE 3.—Effect of MON-0585 on 4th instar larvae of *Aedes aegypti.*

Hours Prior to First Pupation	Stage	% Control 0.1 ppm	% Control 1.0 ppm
18	Early 4th	95	100
15	Early 4th	83	97
12	Middle 4th	75	90
7	Mature 4th	60	77
+3	5% pupae	50	70

Early instar larvae show a gradation of response, thus as much as 24 hours exposure were required to show the above effect in the second instar.

The results in Table 3 indicate that MON-0585 might specifically interfere with a differentiation process. There is a typical "deadline" during the fourth instar, after which the larvae won't be affected by the chemical and would proceed in their normal development.

SELECTIVITY OF MON-0585. MON-0585 is a highly selective mosquito larvicide. The chemical is equally effective against susceptible, organochlorine resistant and organophosphate resistant mosquitoes. A total of 8 susceptible and 6 resistant mosquito species have been tested to date, as listed in Table 4. However, MON-0585 is practically inactive against other organisms including several non-target aquatic and terrestrial species. Insects tested include *Aedes aegypti,* adults inactive at 500 mg/ft.[2]; *Acryrthosiphos pisum,* adults inactive at 0.01 percent; *Diabrotica virgifera,* larvae inactive at 20 ppm; *Epilachna varivestis,* larvae inactive at 0.01 percent; *Musca domestica,* larvae inactive at 20 ppm; *Musca domestica,* adults inactive at 0.01 percent; *Prodenia eridania,* larvae inactive at 0.01 percent and *Tetranychus atlanticus,* adults inactive at 0.01 percent. Furthermore, it has favorable fish toxicity and is extremely safe to mammals (Table 5).

TABLE 4.—Mosquito species susceptible to MON-0585

Culex pipiens quinquefasciatus[1]
C.p. quinquefasciatus[2]
Culex restuans
Culex tarsalis[2]
Aedes aegypti
Ae. aegypti[1]
Ae. nigromaculus[2]
Aedes sollicitans
Ae. taeniorhynchus
Anopheles albimanus
An. albimanus[1]
An. stephensi
An. quadrimaculatus
P. Psorophora sorophora confinnis

[1] Chloronated hydrocarbon resistant strain.
[2] Organophosphate resistant strain.

FAVORABLE ENVIRONMENTAL PROPERTIES. Since MON-0585 contains only carbon, hydrogen and oxygen, the chances of

TABLE 5.—Toxicology of MON-0585.

Fish	LD_{50}	MLD[a]
Rainbow trout		>10 ppm[b]
Black bullhead		>10 ppm
Guppy		>10 ppm
Carp		>10 ppm
Green sunfish		>50 ppm
Goldfish		>50 ppm
Bluegill		>50 ppm
Gambusia	*ca.* 15 ppm	
Mammals		
Rat acute oral	1890 mg/kg	
Rabbit acute dermal		>3160 mg/kg

[a] Minimum lethal dose.
[b] Twenty-four hours reading for trout, 96 hours for other fish.

environmental persistence and contamination are very small. Indeed, both bioassay and gas chromatography have shown that the half-life of 10 ppm MON-0585 in soil was approximately two days. The chemical has, however, sufficient hydrolytic and photostability to control mosquito larvae in the field. Preliminary field tests indicate that MON-0585 gives complete control of mosquito larvae at approximately 1.0 lb./A.

SUMMARY. MON-0585 is a specific, low toxicity material for control of mosquito larvae at their breeding sources. It is a new type of material which exhibits a different mode of action against immature mosquitoes. The compound appears to have a relatively low order of environmental persistence and a potential for minimal environmental contamination.

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AUTOMATIC DEVICES FOR RECORDING OVIPOSITION PERIODICITY OF MOSQUITOES IN THE LABORATORY

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INTRODUCTION. It is often necessary to know the temporal pattern of oviposition before other aspects of this activity can be studied critically. We describe here two devices that can be used to obtain such information, relatively simply, for most species of mosquito that will oviposit in the laboratory.

In devising a method of recording the temporal pattern of oviposition, we proceeded differently for (1) species that normally lay on a moist substrate close to water (e.g., *Aedes aegypti* (L.)), and for (2) those that normally lay on the surface of free water (e.g., *Culex pipiens* L.). In each case our device periodically presents, and withdraws, an oviposition surface; but in one design (for *A. aegypti*) the substrate is withdrawn without the mosquitoes remaining on it, and in the other design it is withdrawn with the mosquitoes still on it and then immediately rendered less suitable for oviposition. These two devices will be described separately. Both are electrically driven and regulated by an electric clock [2] set to provide impulses hourly on a 24-hour cycle.

THE SUNSET STRIP (*Aedes aegypti*) (Figures 1–3). This apparatus is fitted to the outside of a modified cage and is exposed by opening a trapdoor in the cage wall; it can therefore be installed, operated, and removed with minimal disturbance to mosquitoes in the cage.

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[2] 24-hr. dial Model 8682 Remind-O-Timer.