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W. L. JAKOB AND H. F. SCHOOF

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9. ABSTRACT

Results obtained at the Technical Development Laboratories with MON 585, a synthetic hormonomimetic compound with unique activity for larve of six mosquito species are presented. Technical MON 585 contains only carbon, hydrogen and oxygen, and has the chemical structure of 2,6-di-t-butyl-4-(a, a-dimtthylbenzyl) phenol. Data from the manufacturer show that the compound has an acute oral LD-50 of 1,890 mg./kg. to female rates. Technical MON 585 was dissolved in 95 percent ethanol and serially diluted so that the addition of I ml. of solution to 250 ml. H20 effected the desired test concentration. A 3 lb. MON 585/gal. emulifiable concentrate (e.c.), used in certain tests, was diluted with H20 and pipetted into the medium.

Laboratory and simulated filed studies show that Mon 585, a synthetic juvenile hormone mimic, was effective against larvae of Ae, aegypti, C. p. quinquefasciatus, C. tarsalis, Ae. taenirohynchus, A. albimanus and A. stephensi. Activity of the compound is expressed in death of newly formed pupae. Exposure of third instars to relatively high concentrations for intervals of 6 hours or less similarly resulted in mortality at the larval-pupal moult. The compound did not cause any larval mortality.

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MOSQUITO LARVICIDE STUDIES WITH MON 585, A JUVENILE HORMONE MIMIC[1]

W. L. JAKOB AND H. F. SCHOOF

The potential of synthetic compounds with juvenile hormone (JH)-like activity as "third generation pesticides" (Williams, 1967) has been investigated in laboratory studies with mosquito larvae (Jakob and Schoof, 1971) in which several synthetics were shown to be active against one or more of four test species at 0.1 ppm or less. The compounds were characterized by their inhibition or prevention of emergence of adults, such as occurred in earlier studies with farnesol analogs and crude insect extracts against mosquito larvae (Lewallen, 1964; Spielman and Williams, 1966).

Results obtained at the Technical Development Laboratories with MON 585,[2, 3] a synthetic hormonomimetic compound with unique activity (Sacher, 1971), for larvae of six mosquito species are presented.

Materials and Methods. Technical MON 585 contains only carbon, hydrogen and oxygen, and has the chemical structure of 2,6-di-*t*-butyl-4-(*a*,*a*-dimethylbenzyl)phenol. Data from the manufacturer show that the compound has an acute oral LD-50 of 1,890 mg. kg. to female rats. Technical MON 585 was dissolved in 95 percent ethanol and serially diluted so that the addition of 1 ml. of solution to 250 ml. H_2O effected the desired test concentration. A 3 lb. MON 585/gal. emulsifiable concentrate (e.c.), used in certain tests, was diluted with H_2O and pipetted into the medium.

The procedures and criteria for LC-95 determinations have been outlined (Jakob and Schoof, 1971). All tests were begun with third instars, and the effectiveness of the compound was based on the emergence of apparently "normal" adults in relation to the number of larvae exposed. In specified instances the tap (well) H_2O available at the facility was replaced as the larval medium by 1 percent salt H_2O or sewage treatment plant effluent. The species tested were: *Aedes aegypti* (DDT resistant), *Culex pipiens quinquefasciatus* (DDT-dieldrin resistant), *Culex tarsalis* (susceptible), *Aedes taeniorhynchus* (susceptible), *Anopheles albimanus* (dieldrin resistant) and *Anopheles stephensi* (susceptible). All laboratory tests were conducted at 76–80° F, 40–60 percent R.H. and a 12:12-hour photoperiod.

In special tests third and fourth instars were exposed in treated tap H_2O for specific intervals, removed by straining onto nylon tulle' washed lightly with clean tap H_2O and placed in untreated H_2O for the remainder of their development cycle. In such tests no food was provided specimens exposed for 12 hours or less until the larvae had been transferred to the untreated H_2O. Activity or effectiveness, as in LC-95 determinations, was based on the percent emergence of "normal" adults.

A simulated field test was conducted out of-doors in 55-gallon drums (containing 50 gal. tap H_2O). After conditioning for 3 days the drums were treated with MON 585 e.c., diluted so that the addition of 15 ml. of emulsion gave the desired concentration. The treatment was thoroughly distributed by stirring the medium with a perforated paddle. Fifty third-instar *C. p. quinquefasciatus* were confined in a cage (approximately 5 inches square x 14 inches deep) formed by stretching nylon hose over a stainless steel framework. Daily, a small amount

[1] From the Biology Section, Technical Development Laboratories, Laboratory Division, Center for Disease Control, Health Services and Mental Health Administration, Public Health Service, U.S. Department of Health, Education, and Welfare, Savannah, Georgia 31402.

[2] Furnished through the courtesy of Monsanto Company, St. Louis, Mo.

[3] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

of larval food was provided, and living pupae were removed to paper cups for observation of subsequent emergence. Effectiveness of treatments was again based on the number of "normal" adults emerged in relation to the number of larvae exposed. Water temperature during the test period ranged from 72 to 81° F. At specified intervals thereafter aliquots (1,000 ml. each) were removed from the drums, pooled according to treatment level and used as media in laboratory tests with third instar *C. p. quinquefasciatus*. The larvae thus exposed were then processed as outlined for LC–95 determinations. Activity of the treatments is expressed in terms of percent mortality based on emergence of "normal" adults.

In tests with *Ae. taeniorhynchus*, samples of *Distichlis* spp. sod (6 inches diameter) were obtained from the field, placed in enamel pans, and flooded with 1,500 ml. 1 percent salt H_2O. MON 585 emulsion was pipetted into the medium to give the desired concentration. Fifty or 75 third instar *Ae. taeniorhynchus* were introduced into each pan, fed daily, and allowed to complete development to adults. A nylon tulle covering over the pans prevented escape of the adults. Effectiveness of treatments was based on emergence of "normal" adults in relation to number of larvae exposed.

RESULTS. The concentration of MON 585 required to produce at least 95 percent mortality of mosquitoes exposed as third instars was:

0.25 ppm—*Ae. aegypti, A. stephensi*
0.1 ppm—*C. p. quinquefasciatus, C. tarsalis, A. albimanus, Ae. taeniorhynchus*

C. p. quinquefasciatus was equally sensitive whether the medium was tap H_2O or sewage effluent. Determinations with *Ae. taeniorhynchus* were conducted with 1 percent salt H_2O as the larval medium.

Activity of MON 585 against all species was characterized by death of specimens shortly after the change from larva to pupa. The unmelanized pupa characteristically found, and the anomalous pupa occasionally seen, are shown in Figure 1. Thus, MON 585 is unique among hormonomimetics in that development is interrupted at the fourth instar larva-pupa moult, whereas all other compounds tested to date (Jakob and Schoof, 1971) usually permitted apparently normal development to the pupal stage but inhibited adult emergence.

Exposure of third instar larvae for specified intervals effected marked mortality in the two species tested (Table 1). The number of adults was reduced 100 and 99 percent with exposure of *C. p. quinquefasciatus* to 0.25 ppm MON 585 for 6 hours or to 0.5 ppm for 4 hours, respectively. Exposures to 1.0 ppm for 2 hours gave comparable results whereas exposures of only 1 hour to this concentration decreased the mortality to 84 percent. Similar exposures with *A. albimanus* also produced marked mortality, but longer exposures and/or higher concentrations were required with this species (*e.g.*, 0.25 ppm for 24 hours or 0.5 ppm for 6 hours to cause greater than 90 percent mortality). Mortality from such exposures also was characterized by death of pupae shortly after casting of the last larval skin. The data suggest that the treatments had an irreversible, delayed lethal effect on the development of the immature.

Studies in drums seeded with third instar *C. p. quinquefasciatus* gave 100 percent mortalities at concentrations ranging from 0.25 to 2.5 ppm in turbid H_2O with rust on the sides of the drums. Mortality in untreated drums (three replicates) averaged 11 percent. Water samples taken from the drums 10 days after treatment with 0.25 ppm MON 585 produced less than 70 percent mortalities of *C. p. quinquefasciatus* and *A. albimanus*. Mortality of these species in treatments (10 days old) of 0.5 and 1.0 ppm ranged from 89–98 percent. Water samples taken 38 days after treatment with 1.0 and 2.5 ppm produced less than 30 and 50 percent mortalities, respectively, of both species. How-

FIG. 1.—Anomalous pupa (above) and typical unmelanized newly formed pupa (lower) of *A. albimanus* obtained from exposure of third instars to 0.5 ppm MON 585.

ever, these tests were conducted in the fall of the year; more rapid breakdown of MON 585 treatments may occur with higher temperatures and longer periods of daylight.

Effectiveness of MON 585 against *Ae. taeniorhynchus* in saline water over sod samples was obtained with concentrations of 0.25 ppm. In two tests this dosage, from an e.c. formulation or as technical grade in ETOH solution, produced 99 percent mortality. A dosage of 0.1 ppm effected a mean of 64 percent mortality. Control mortality averaged <5 percent. Under these test conditions removal of dead and living pupae was not feasible but the treatment was observed to cause death of newly emerged pupal forms, activity typically seen and recorded in LC-95 determinations with this material.

Differences in response to MON 585 between instars of *C. p. quinquefasciatus*, *Ae. aegypti* and *Ae. taeniorhynchus* were shown (Table 2) in studies with exposures of specified intervals. The data indicate that fourth instars were generally more readily affected than were younger larvae. The specific reason for these differences was not investigated but may help to explain variations in results among researchers using larvae in different stages of development. The results from exposure of *C. p. quinquefasciatus* to 0.25 ppm for 2, 4 and 6 hours in this study were lower than the mean values obtained (Table 1) in other tests involving similar exposures. Such data reflect the normal range of sensitivity of larvicide tests, particularly those which, of necessity, are of prolonged duration.

DISCUSSION. The data reported indicate that MON 585, a synthetic hormonomimetic compound, is highly effective against larvae of a number of mosquito species. The expression of its effect—interruption of metamorphosis at the larva-pupa moult—is unique among compounds tested to date. This type of activity was obtained in laboratory tests with sewage treatment effluent, with 1 percent salt

TABLE 1.—Mean percent mortality of mosquitoes from exposure of third instars to MON 585-treated water for specified periods of time.[1]

Concentration (ppm)	Hours Exposed					
	1	2	4	6	12	24
			C. p. quinquefasciatus			
0.1	..	..	..	73	92(2)	95(2)
0.25	..	60(2)	87(3)	100(3)	99(3)	99(3)
0.5	44(3)	81(3)	99(3)	100(2)	..	100
1.0	84(3)	96(3)	100(2)	100	..	100
ETOH control	..	9	..	5(5)	..	..
			A. albimanus			
0.1	..	..	..	43	67	64
0.25	..	40	37	77	50	100(2)
0.5	17	58(2)	87(2)	92(2)	..	..
1.0	78(2)	85(2)	88(2)	100	..	..
ETOH control	..	12	..	11(3)	..	..

[1] Mortality based on number of "normal" adults in relation to the number of larvae exposed. Where more than one test, the number is indicated in parentheses.

H_2O, and with turbid H_2O in rusty 55-gallon drums. The compound, at LC–95 concentration, was nontoxic to the larval stages tested, but studies to define such limits were not conducted.

Although most studies involved continuous exposure of larvae in treated water, MON 585 was also effective when larvae were exposed to relatively high concentrations for limited periods. For example, exposure for 2 hours to 1 ppm resulted in 96 percent and 85 percent mortality of *C. p. quinquefasciatus* and *A. albimanus*, respectively. Similarly, exposure of third instars of these species for 4 or 6 hours to 0.5 ppm resulted in more than 90 percent mortality. Although the LC–95 of MON 585 against both species was 0.1 ppm, *C. p. quinquefasciatus* was slightly more responsive to such exposures than was *A. albimanus*. Studies comparing exposure of third and fourth instars of two species for varying intervals showed that mortality was consistently

TABLE 2.—Percent mortality[1] of *C. p. quinquefasciatus*, *Ae. aegypti* and *Ae. taeniorhynchus* following exposure of indicated instars to MON 585 treatments for specified periods of time.

Conc.—Exposure	*C. p. quinq.*		*Ae. aegypti*	
	Third	Fourth	Third	Fourth
0.25 ppm — 2 hr.	27	48	4	3
— 4 hr.	58	78	4	11
— 6 hr.	80	90	3	26
— 12 hr.	97	100	1	65
— continuous	100	100	90	100
ETOH control — 6 hr.	4	11	9	3

	Ae. taeniorhynchus	
	Second	Fourth
0.5 ppm — 2 hr.	71	83
— 4 hr.	87	92
— 6 hr.	99	97
1.0 ppm — 2 hr.	64	87
— 4 hr.	88	100
— 6 hr.	100	100
ETOH control — 6 hr.	23	13

[1] Mortality based on number of "normal" adults in relation to the number of larvae exposed.

higher with the more mature larvae. Differences in mortality between instars were less marked with *C. p. quinquefasciatus* than with *Ae. aegypti*. Mortality of fourth instar *Ae. taeniorhynchus* was neither consistently nor markedly higher than that of second instars. The effect of such limited exposures was expressed by the death of newly emerged pupae, the response also typically obtained in continuous exposure tests. Thus, MON 585 appears to effect an irreversible, lethal interference or disruption of a process (or processes) controlling development of larvae to pupae.

SUMMARY. Laboratory and simulated field studies show that MON 585, a synthetic juvenile hormone mimic, was effective against larvae of *Ae. aegypti*, *C. p. quinquefasciatus*, *C. tarsalis*, *Ae. taeniorhynchus*, *A. albimanus* and *A. stephensi*. Activity of the compound is expressed in death of newly formed pupae. Exposure of third instars to relatively high concentrations for intervals of 6 hours or less similarly resulted in mortality at the larval-pupal moult. The compound did not cause any larval mortality.

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