ADDITIONAL STUDIES WITH JUVENILE HORMONE-TYPE COMPOUNDS AGAINST MOSQUITO LARVAE [1]

W. L. JAKOB

The potential of recently developed compounds with juvenile hormone (JH)-like activity as insect control agents has been reported (Sacher, 1971; Staal, 1971; Jakob and Schoof, 1971). These materials, with one exception, permit continued development of larvae to pupae and then inhibit or prevent emergence of adults. The exception (MON 585) [2] also permits continued larval development but results in the death of newly formed pupae. Thus, their potential use will require adjustments in evaluative procedures as compared to those usually employed with conventional mosquito larvicides.

Data obtained in laboratory studies against mosquito larvae with nine compounds having JH-like activity are presented.

MATERIALS AND METHODS. LC-95 determinations against 3rd instar *Culex pipiens quinquefasciatus* (DDT-dieldrin resistant), *Aedes aegypti* (DDT-resistant), *Anopheles albimanus* (dieldrin resistant), and *Anopheles stephensi* (susceptible) were conducted by the procedures previously described (Jakob and Schoof, 1971).

In certain tests 3rd instars were exposed in treated tap H_2O for specific periods of time, removed by straining onto nylon tulle, washed lightly and then placed in untreated tap H_2O to complete development to pupae. In such tests no food was provided specimens exposed for 6 hours or less until the larvae had been transferred to untreated H_2O whereafter food was provided daily until approximately half the specimens had pupated. The number of dead larvae and pupae removed daily was recorded. Living pupae were removed daily and placed in untreated H_2O in paper cups for observation of subsequent adult emergence (Jakob and Schoof, 1972). Results, as in LC-95 determinations, are expressed as percent mortality, based on the number of "normal" adults emerged in relation to the number of larvae tested.

In other studies graham flour of 53–125μ size particles (Wilton *et al.*, 1972) was treated with solutions of the compound and the solvent was removed under vacuum on a flash evaporator. Specific amounts were provided 25 larvae in glass beakers (surface area–50 sq cm) or 50 larvae in stainless steel pans (surface area –177 sq cm) as larval food once daily for 1 or 2 days with all subsequent daily feedings being made with untreated laboratory chow. Unconsumed treated food was not removed from the test containers. All other procedures were as described above. Controls were exposed to solvent only or provided untreated graham flour.

The compounds tested and their chemical compositions are listed (Table 1).

RESULTS AND DISCUSSION. The data (Table 2) indicate that all compounds, except ZR–0445 and ZR–0450, effected at least 95 percent reductions in adult emergence of one or more of the test species at a concentration of 0.1 ppm or less. R–20458, RO 20–3600 and ZR–0436 were notably more effective against *C. p. quinquefasciatus* than the other test species, whereas ZR–0515 was most active against *A. albimanus*. Data from the manufacturer have indicated that 4th instar *Ae. aegypti* are 100-fold more responsive to ZR–0515 than are 3rd instars.

All compounds inhibited adult emergence. No marked toxicity to larvae or

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

[2] 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.

TABLE 1.—Chemical structure of compounds tested.

Compound	Structure
MON 585	2,6-di-*t*-butyl-4-(α,α-dimethylbenzyl)phenol
R–20458	1-(4′-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene
RO 20–3600	6,7-Epoxy-3-methyl-7-ethyl-1-[3,4-(methylenedioxy)-phenoxy]-2-*cis/trans*-octene
ZR–0436	1-[6′,7′-epoxy-3′,7′-dimethyl oct-2′-enyl]-4-ethylphenyl ether
ZR–0442	1-[6′,7′-epoxy-3′,7′-dimethyloctanyl]-4-ethylphenyl ether
ZR–0445	1-[3′,7′-dimethyloctanyl]-4-ethylphenyl ether
ZR–0450	Ethyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate
ZR–0485	Isopropyl 3,7,11-trimethyldodeca-2,4-dienoate
ZR–0515	Isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate

TABLE 2.—Concentration (ppm) of JH-type compounds required for 95 percent mortality [1] of mosquito species exposed as 3rd instars.

Compound	*Ae. aegypti*	*C. p. quinq.*	*A. albimanus*	*A. stephensi*
R–20458	0.1	0.0025	0.1	0.1
RO 20–3600	0.05	0.005	0.05	0.05
ZR–0436	0.25	0.025	0.25	0.1
ZR–0442	0.1	0.1	0.5	0.25
ZR–0445	>2.5	2.5	>2.5	2.5
ZR–0450	>0.25	>0.25	>0.25	>0.25
ZR–0485	0.1	0.025	0.025	0.1
ZR–0515	0.25	0.025	0.0025	0.05

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

prolongation of the developmental cycle was attributable to any concentration of the test materials. Development of treated larvae proceeded normally (comparable to controls) to pupation but emergence of imagoes was adversely affected.

TABLE 3.—Mean percent mortality of *C. p. quinquefasciatus* and *Ae. aegypti* following exposure of 3rd instars to ZR–0515 treatments for specified periods of time.

Conc.	Exposure	*C. p. quinq.*	*Ae. aegypti*
0.1 ppm.	24 hr.	94	..
0.25 ppm	4 hr.	39	..
	6 hr.	38	..
	24 hr.	>99	..
0.5 ppm	2 hr.	50	..
	4 hr.	64	..
	6 hr.	81	..
1.0 ppm	1 hr.	47	..
	2 hr.	77	28
	4 hr.	99	73
	6 hr.	98	72
2.5 ppm	2 hr.	..	60
	4 hr.	..	74
	6 hr.	..	95

Results from exposure of larvae to ZR–0515 for specific periods of time are given (Table 3). Exposure of *C. p. quinquefasciatus* 3rd instars for 24 hours to concentrations of 0.1 or 0.25 ppm resulted in 90 percent or higher mortality and exposures of 4 or 6 hours to 1 ppm were completely effective. With *Ae. aegypti* exposure for 6 hours to 2.5 ppm effected 95 percent mortality.

Studies involving feeding of graham flour treated (w/w) with MON 585 or ZR–0515 are summarized (Table 4). The feeding of 50 mg of 0.05 percent MON 585 flour, on the initial test day only, resulted in 90 percent or higher mortality of *C. p. quinquefasciatus* and *A. albimanus.* Generally, similar results were obtained when 25 mg of food treated at a given rate were provided for 2 consecutive days, and when 50 mg were provided on the initial test day only. Results with *C. p. quinquefasciatus* indicate that 25 or 50 mg of 0.1 percent ZR–0515 flour effected at least 93 percent mortality. The

TABLE 4.—Percent mortalities of mosquito species following ingestion by larvae of graham flour treated with JH-type compounds.

Conc. on flour (w/w)	Amount (mg)*	No. days	MON 585		ZR-0515	
			C. p. quinq.	*A. alb.*	*C. p quinq.*	*Ae. aeg.*
0.025%	50	1	38, 63	54	......	....
		2	92, 71	94	......	....
0.05%	25	1	59, 60	...	67	....
		2	92, 93	...	48	43
	50	1	92, 100	90	39, 94	71, 99
		2	99, 98	100	......	....
0.1%	25	1	......	90	93, 100	....
		2	......	98	100	....
	50	1	......	98	95, 100	100, 100
		2	......	100	......	....
0.5%	50	1	......	...	97	100
1.0%	50	1	......	...	100	100

* Mg of food provided per beaker (surface area—approximately 50 sq cm).

response of *Ae. aegypti* to ZR-0515-treated flour was somewhat greater than anticipated in view of the previously noted refractory nature of 3rd instars of this species to the compound.

Both compounds thus have been shown to be effective by ingestion as well as by exposure of larvae in water treated with solutions of the compound. However, in similar studies with larvae in stainless steel pans, the amount of treated food required to effect 90 percent or higher mortality of *C. p. quinquefasciatus* as an initial feeding only was 15 or 10 mg of 0.1 percent or 0.2 percent MON 585, respectively. With ZR-0515 <70 percent mortality of *C. p. quinquefasciatus* was obtained when 25 mg of 0.5 percent treated flour was similarly provided. Thus ZR-0515 was less effective than MON 585 when presented to *C. p. quinquefasciatus* in bait form despite the fact that in LC-95 determinations ZR-0515 was markedly more active than MON 585 (Jakob and Schoof, 1972). Differences in activity when the compounds are presented on food may relate to differences in water solubility or the physical state of the technical material (MON 585 is a solid, ZR-0515 a liquid).

CONCLUSIONS. Several additional compounds which inhibit development of mosquitoes were found to be effective against one or more test species at concentrations of 0.1 ppm or less. Differences in response among species occurred, as has been the experience with conventional toxicants.

Third instars exposed to high concentrations of ZR-0515 for limited periods responded similarly to those exposed continuously to much lower concentrations. Late 4th instars were found the most responsive stage with ZR-0515 (Schaefer and Wilder, 1972) whereas early 4th instars were most readily affected by MON 585 (Sacher, 1971; Schaefer and Wilder, 1972). However, timing of field applications to coincide with the most sensitive stages is often not practical. The effect obtained with limited exposures of 3rd instars to ZR-0515 and MON 585 (Jakob and Schoof, 1972) suggest that high dosages of JH mimics may be effective against younger larvae and in situations subject to dilution. Such higher dosages may indeed be necessary to satisfactorily control mosquitoes with asynchronous broods since the earlier instars are known to be generally less responsive than more mature larvae.

Activity of ZR-0515 and MON 585 through ingestion of treated food particles, of the size preferred by 3rd instar *A. albimanus* (Wilton *et al.*, 1972), was demonstrated and thus provides another potential means of control. Field trials of such applications are necessary to determine the acceptance of JH baits presented in competition with the food normally available.

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SOME INFLUENCES OF AQUATIC VEGETATION ON THE SPECIES AND NUMBER OF CULICIDAE (DIPTERA) IN SMALL POOLS OF WATER

BRUCE M. FURLOW AND KIRBY L. HAYS

Department of Zoology-Entomology, Agricultural Experiment Station, Auburn University, Auburn, Alabama 36830

ABSTRACT. Physical configuration of the aquatic vegetation in 10 ft. diameter plastic pools in the southeastern United States appeared to influence qualitative parameters of mosquito populations. There were definite trends reflecting the association of particular species of Culicidae with a particular type of aquatic plant: submerged, floating, emergent or none. Even though apparent quantitative differences were observed in culicid populations among the various types of vegetation, vegetation had no significant effect on population sizes. The floating plant, *Spirodela oligorrhiza* (duck weed), had a detrimental effect on mosquito survival when it completely covered the surface of the water.

INTRODUCTION. Most mosquito breeding is associated with aquatic vegetation (Bates, 1949). Thus, understanding the mosquito-aquatic plant relationship is important. Plants may influence mosquitoes in many ways. They may physically restrict oviposition either by disrupting the behavior of the female or by rendering the surface of the water inaccessible (Macan and Worthington, 1951). Conversely, metabolites or biochemical processes of plants may act as attractants to the female, leading her to favorable oviposition habitats (Rapp and Emil, 1965). Happold (1965) observed that the distribution of mosquito larvae in nature was influenced by the female's behavioral reaction to vegetation, amount of shade, and habitat location.

The influence of plants is important to both adult and immature mosquitoes. Aquatic vegetation influences many of the physiochemical and ecological relationships that contribute to the success of immature forms. Plants reduce wave action of the water and thus provide a more