

known), *furens*, *furensoides*, *haematopotus*, and *stellifer* (Jamnback, 1965). The opercular teeth are short, the *a.d.* setae are very unequal, the caudal segment has a patch of spines on its dorsum, and the respiratory horn is transversely convoluted, although weakly, near its midlength. Two characters of the respiratory horns are, however, notably divergent. There is but a single lateral spiracular opening and there is the absence of a pronounced dorsally directed protuberance for this opening. Undoubtedly, with the examination of additional pupal pelts, variation will be found in some of the characters described in this paper.

ACKNOWLEDGEMENTS

The author is appreciative of the assistance of Dr. W. W. Wirth, U. S. National Museum, who confirmed the identification of the adult specimen and of Dr. B. V. Travis, Department of Ento-

mology, Cornell University, who read the manuscript and helped in the interpretation of structures.

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EVALUATION OF AERIAL APPLICATIONS OF FLIT® MLO FOR THE CONTROL OF ORGANOPHOSPHORUS-RESISTANT *Aedes nigromaculis* AND *Culex tarsalis*

PATRICIA A. GILLIES,¹ GILBERT V. CHAMBERS² AND DON J. WOMELDORF¹

INTRODUCTION

Two major species of California mosquitoes have become resistant to all the available organophosphorus and organochlorine larvicides. The irrigated pasture mosquito, *Aedes nigromaculis* (Ludlow), is extremely resistant in two areas of the San Joaquin Valley and in one part of the Sacramento Valley, and is developing

resistance in the rest of the Central Valley, (Womeldorf *et al.*, 1971). *Culex tarsalis* Coquillett, the primary vector of St. Louis and Western equine encephalitis in California, has become highly resistant in the southern and central San Joaquin Valley and possibly in other locations in the State as well, (Georghiou *et al.*, 1969; Womeldorf *et al.*, 1971). This critical situation has renewed interest in the petroleum-derived control agents.

FLIT® MLO has been extensively studied in the laboratory against *C. pipiens quinquefasciatus* Say and *A. aegypti* (L.). The effects of the material, expressed as mortality, developmental retardation, and physiological manifestations, have been

¹ California Department of Public Health, Bureau of Vector Control and Solid Waste Management, 2151 Berkeley Way, Berkeley, California 94704.

² ESSO Research and Engineering Company, Baytown Petroleum Research Laboratory, Baytown, Texas 77520.

elucidated (Micks *et al.*, 1967, 1968, 1969; Micks, 1970).

Little information has yet been published relating to observations of the effect of FLIT MLO on field populations. Observations during operational and experimental applications against permanent-water mosquitoes have been reported by several workers in Southern California. McFarland (1968) reported that 5 to 6 gal/acre were required to kill larvae and pupae (species not given) and commented that wind conditions on open water may have been the cause of observed larval survival at rates of 1.5 and 2 gal/acre. Mulla *et al.* (1969) showed that FLIT MLO at 2 and 4 gal/acre gave complete control of larval *C. tarsalis* for 3 weeks but did not yield a high level of control at 1 gal/acre. Shanafelt (1969) treated cemetery urns producing *C. p. quinquefasciatus* and *Culiseta incidens* (Thomson) at the rate of 2.5 ml/urn and stated that 24-hour kills were complete and that first reinfestation was observed 36 days following treatment. Kimball and Perruzzi (1970), working with *C. p. quinquefasciatus*, *C. peus* Speiser, and *C. incidens* in urban sources, found that FLIT MLO killed both larvae and pupae in 24 hours at rates of 2 to 3 gal/acre and provided control for 14 days.

Three species of intermittent-water mosquitoes have received attention. Bearden and Steelman (1971), who applied FLIT MLO against first instar larvae of *Psorophora confinnis* (Lynch-Arribálzaga) in Louisiana rice fields using a back pack mist blower, saw mortalities of 94.5 percent and 100 percent at rates of 1 and 2 gal/acre, respectively. Armstrong (1969), having treated pools containing *A. taeniorhynchus* (Wiedemann) larvae with a Hudson sprayer, stated that 2 gal/acre produced an estimated 90 percent-95 percent kill, based on evaluations at 24 to 48 hours in water ranging from fresh to seawater. Murray (1968a, b) found that an application of 2 gal/acre was sufficient to kill organophosphorus-resistant larval *A. nigromaculis* in 24 hours but saw

emergence from some treated pupae and no observable effect upon treated adults. Gillies and Womeldorf (1968) noted that hand-can application of 2 gal/acre and aerial application of 3 gal/acre were the lowest rates that produced acceptable kills of organophosphorus-resistant *A. nigromaculis* larvae when the tests were evaluated at 24 hours. Chambers (1968) pointed out the importance of continuing field observations of larval mortality for at least 24 and up to 72 hours and suggested basing control evaluation on adult production.

Although the early work on irrigated pasture mosquitoes in California was inconclusive, the results were encouraging enough to warrant operationally-oriented applications under close observation. In August, 1970, the University of California, the Tulare Mosquito Abatement District, the California Department of Public Health, and ESSO Research representatives cooperated in a number of test applications in Tulare County. Observations relating to those applications have also been discussed by Schaefer and Ramke (1971). In September, 1970, the Turlock Mosquito Abatement District cooperated in a similar series of tests in Stanislaus County.

There were three principal test objectives: (1) to determine effects of treatment upon immature mosquitoes, (2) to clarify procedures for field evaluation of a treatment in which the desired results might not appear until after 24 hours, and (3) to allow the mosquito control workers to observe applications of the material and to determine whether it could fit into use patterns developed for conventional insecticides. This paper will, for the most part, confine itself to the first two objectives.

MATERIALS AND METHODS

The larvicides evaluated were FLIT MLO at Tulare and the experimental larvicide BPRL 5337-2 at Turlock. These petroleum-derived materials have similar

density, viscosity, surface tension and volatility. The experimental larvicide differs in larvicidal activity from the commercial FLIT MLO, being slightly more active against *C. p. quinquefasciatus* (Table 1).

TABLE 1.—Percent mortality of laboratory fourth instar *C. p. quinquefasciatus* larvae to samples of materials used at Tulare and Turlock, by equivalent dose and time after treatment. (WHO-M-18 procedure, 4 replicates of 25 larvae)

| Material | Equivalent dose, g/a | Hours after treatment | |
|--------------------------|----------------------|-----------------------|----|
| | | 24 | 48 |
| FLIT MLO (Tulare) | 0.5 | 58 | 70 |
| | 1.0 | 79 | 83 |
| BPRL 5337-2 (Turlock) | 0.5 | 58 | 71 |
| | 1.0 | 84 | 92 |

AERIAL APPLICATION. Treatments were applied with district-owned PA-25-235 aircraft equipped with a standard trailing wing boom and air-driven pump system. Nozzles were positioned at a 45° angle aft to the thrust of the aircraft.

At Tulare, the desired application rate was obtained by varying line pressure and by the addition of Tee-Jet D8-45 nozzles to the fourteen D6-45 nozzles already being employed in the daily operational application of other mosquito control formulations. The nozzles were not evenly distributed on the boom. Swath interval was 33 feet, with the exception of one application at a 40-foot interval. Tests were flown at an altitude of 15-25 feet.

In Turlock, twelve D8-45 nozzles were evenly distributed on the boom. A working pressure of 23 psi produced a discharge rate of 8 gal/min. Swath intervals of 20 feet and 40 feet provided calculated application rates of 2 and 1 gal/acre respectively, when flown at an altitude of 25-35 feet. The aircraft in both test series were flown at 100 mph.

Swath intervals were premeasured, marked and flagged. Applications were made between 0600 and 0930 hours during favorable wind conditions. Wind velocity,

measured with a Dwyer anemometer, ranged from less than 1 mph to 6 mph during the tests. Spraying began at the downwind side of the field and continued upwind. At the time of application, the air temperature range at Tulare was 66° to 73° F and at Turlock the range was 52° to 72° F. Water temperatures, measured near the surface and in the open, ranged from 68° to 74° F at Tulare and 50° to 65° F at Turlock. Details of application conditions on selected fields are summarized in Table 2.

FIELD OBSERVATIONS. Test fields ranged from 15 to 80 acres and were flood-irrigated pastures typical of the San Joaquin Valley. The two general types encountered were graded improved pastures and unimproved natural-terrain fields. Basic criteria for choosing test fields were that larval or pupal numbers were adequate to assess treatment results and that the amount of irrigation water appeared sufficient to remain for several days following treatment. An additional criterion applied at Turlock was that only fields with late instar larvae or pupae were selected for the treatment. Most fields contained only *A. nigromaculis*, a few had mixtures of *A. nigromaculis* and *C. tarsalis*, and two had only *C. tarsalis*.

Immature mosquitoes were sampled by dipping, and counts were made by instar. Depending on field size, 5 to 20 stations were sampled. At least 10 dips were taken per station unless precluded by water scarcity. Station orientation across a field was arranged to intercept several swaths. No attempt was made to observe regular spacing between stations since the water usually was distributed unevenly across the field.

Initially, at Tulare, the counts recorded were: pretreatment (the afternoon before treatment) and about 8, 24, 48, and 72 hours posttreatment. As the Tulare study progressed, the time of the pretreatment count was changed to the morning of treatment. At the conclusion of the Tulare work it was learned that it was not realistic to terminate observations at the end of 3 days, so at Turlock, the treated

TABLE 2.—Application conditions of aerial sprays of FLIT MLO at Tulare (1-7) and of the experimental larvicide BPRL 5337-2 at Turlock (8-12), 1970.

| Experiment number | Nozzles number and size | Line pressure psi | Wind mph | Ambient temp. ° F | Water temp. ° F | Estimated altitude ft. |
|-------------------|-------------------------|-------------------|----------|-------------------|-----------------|------------------------|
| 1 | 14-D6,13-D8 | 22 | <2 | 66 | 68 | 15 |
| 2 | 14-D6,13-D8 | 22 | <2 | 66 | 68 | 25 |
| 3 ^a | 14-D6,13-D8 | 22 | 4-6 | 70 | 73 | 25+ |
| 4 | 14-D6,13-D8 | 22 | 4-6 | 70 | 72 | 15 |
| 5 | 14-D6,13-D8 | 22 | <2 | 77 | 72 | 15 |
| 6 | 14-D6,6-D8 | 22 | <2 | 73 | 74 | .. |
| 7 ^b | 14-D6,6-D8 | 22 | <2 | .. | .. | 25 |
| 8 | 12-D8 | 25 | <2-3 | 52 | 50 | 30 |
| 9 | 12-D8 | 25 | <2-3 | 54 | 52 | 30 |
| 10 | 12-D8 | 23 | <2 | 62 | 58 | 30 |
| 11 | 12-D8 | 23 | <2 | 71 | 62 | 30 |
| 12 | 12-D8 | 23 | <2 | 72 | 65 | 30 |

^a Examination of nozzles revealed one nozzle plugged and majority of others partially blocked.

^b Line pressure and/or number of D-8 nozzles are probably in error.

area was observed until complete larval mortality had occurred or the field became completely dry. Adult emergence, if any, was noted.

RECOVERY MEASUREMENTS. Although 1 to 4 test applications were made on most days over the 3 weeks of observations, it was possible to perform recovery measurements on only 1 or 2 test applications on any given day. For percent recovery tests and distribution pattern tests, a sampling line 120 feet long was established at right angles with the line of flight. Stations at 10-foot intervals were employed when determining percent recovery alone. Additional stations were added at 5-foot intervals to obtain distribution patterns. Hydrocarbon sensitive dye cards (oil sensitive cards for airplane spray, Home and Farm Chemical Co., P.O. Box 6055, Charlotte, N.C.) were used at the 10-foot stations for visual comparison.

To recover applied materials, a sheet of an analytical grade of aluminum foil was attached to a thin aluminum platform (5" x 6") and placed horizontally above the vegetation. The foil was removed from the field within 15 minutes after the application, folded inward, and placed in a wide-mouthed bottle which was capped to minimize evaporation. In the laboratory the droplets were washed from the foil using 100-150 ml of pure grade iso-

octane (2,2,4-trimethyl pentane). The amount of recovered material was determined by gravimetric techniques after evaporation of the iso-octane. The iso-octane washing was funneled through glass wool, to catch foreign matter, into a 200 ml anti-creep beaker (United States Testing Co., 1415 Park Avenue, Hoboken, N.J.). The iso-octane was slowly evaporated from beakers with the aid of dry compressed air or nitrogen. A calcium sulfate drier in the compressed air or nitrogen line was usually needed; but with the extremely low humidity in California's Central Valley, moving air over the beakers with a fan was adequate. Quantitative determinations were made by correcting residual weights by the residual weight of 125 ml of iso-octane and the examination of two reference standards evaporated at the same time. The reference standards are one drop and two drops of FLIT MLO weighed to the nearest 0.1 milligram with 125 ml of iso-octane being added. Gallons per acre were calculated from corrected recovered weights, size of target and density of material.

The distillation characteristics of recovered material were compared to the applied materials with gas chromatographic techniques in order to evaluate evaporation losses of the applied materials. The

gas chromatographic technique employed a programmed temperature gas chromatograph with thermal conductivity detector (RHEN-TUNG matched filaments code WX mount 9225), using a 182 cm column of .63 cm stainless steel packed with 2 wt. % SE₃₀ on regular grade Chromasorb. The operating conditions were: injection port temperature, 375° C; detector temperature, 325° C; filament current, 150 ma; helium carrier flow, 50 ml per minute minimum; and temperature programmed for 30° C increase per minute.

BIOASSAYS. Larvae and water for bioassay were collected from the field immediately prior to treatment. A series of aluminum pans (9" x 9" x 1.75") containing approximately 50 larvae each was placed on the ground at 5 of the 10-foot interval stations and exposed to the spray. After treatment, each pan was covered with a similar pan, fastened with a clip. A second series, used for untreated controls, was subjected to the same conditions as the aerially treated larvae, but protected from the spray. A third series was used to provide treated controls by applying known amounts of the larvicide with a micro-syringe to obtain various equivalent dosage levels. Additionally, some larvae

were sorted by instar and treated with measured amounts of material to gain information on instar susceptibility differences. All pans were left in the field, and temperature and mortality were recorded at intervals. Test larvae were fed a high protein pelletized livestock food. Untreated field water was added to the pans to replenish losses from evaporation.

RESULTS AND DISCUSSION

APPLICATION MEASUREMENT. In ten experiments the average recovery per experiment of the aerially applied material varied from 59 percent to 95 percent of the calculated rate of application (Table 3). Percent recovery was not computed for Experiments 3 and 7 because the calculated rate of application was questionable due to operational problems. The average of the Tulare experiments was slightly higher than that of Turlock experiments (73 percent vs 68 percent). However, this difference is not statistically significant by the Mann-Whitney test (Siegel 1956).

Selected distribution patterns of recovery measurements are shown in Figures 1A and 1B and measures of variability for the experiments are given in Table 3.

TABLE 3.—Recovery of material on fallout plates with aerial sprays of FLIT MLO at Tulare (1-7) and of experimental larvicide BPRL 5337-2 at Turlock (8-12), 1970

| Experiment number | Swath width ft. | Calc. rate applied g/a | No. of stations | Mean rate recovered g/a | Coefficient of variation % | Percent recovery |
|-------------------|-----------------|------------------------|-----------------|-------------------------|----------------------------|------------------|
| 1 | 33 | 2.1 | 13 | 1.73 | | 82 |
| 2 | 33 | 2.1 | 13 | 1.54 | | 73 ^a |
| 3 | 33 | 2.1 | 13 | 0.94 | | |
| 4 | 40 | 1.7 | 13 | 1.21 | | 71 |
| 5 | 33 | 2.1 | 18 | 1.24 | 32 | 59 |
| 6 | 33 | 1.5 | 19 | 1.23 | 27 | 82 |
| 7 | 33 | 1.5 | 18 | 2.15 | 14 | 68 ^b |
| 8 | 20 | 2.4 | 19 | 1.42 | 20 | 59 |
| 9 | 40 | 1.2 | 19 | 0.72 | 53 | 60 |
| 10 | 20 | 2.0 | 12 | 1.90 | | 95 |
| 11 | 40 | 1.0 | 19 | 0.69 | 26 | 69 |
| 12 | 40 | 1.0 | 19 | 0.61 | 34 | 61 |

^a Examination of nozzles revealed one nozzle plugged and majority of others partially blocked.

^b Line pressure and/or number of D-8 nozzles are probably in error.

There is some indication of a trough or peak in certain patterns (Figure 1A, Experiments 5 and 6). The patterns of deviations above and below the average for the experiment were analyzed using the total number of test runs (Bradley 1968). The only significantly non-random pattern was that of Experiment 9 in which a plateau of low values occurred from Stations 4 through 8.

Experiment 9 also showed greater relative variability than the other tests (coefficient of variation, Table 3). With the exception of Experiment 9, the degree of variability was not markedly different for tests at the three swath widths. An attempt to "fill" the distribution trough by rearranging nozzles in Experiment 7 at Tulare resulted in lower relative variability.

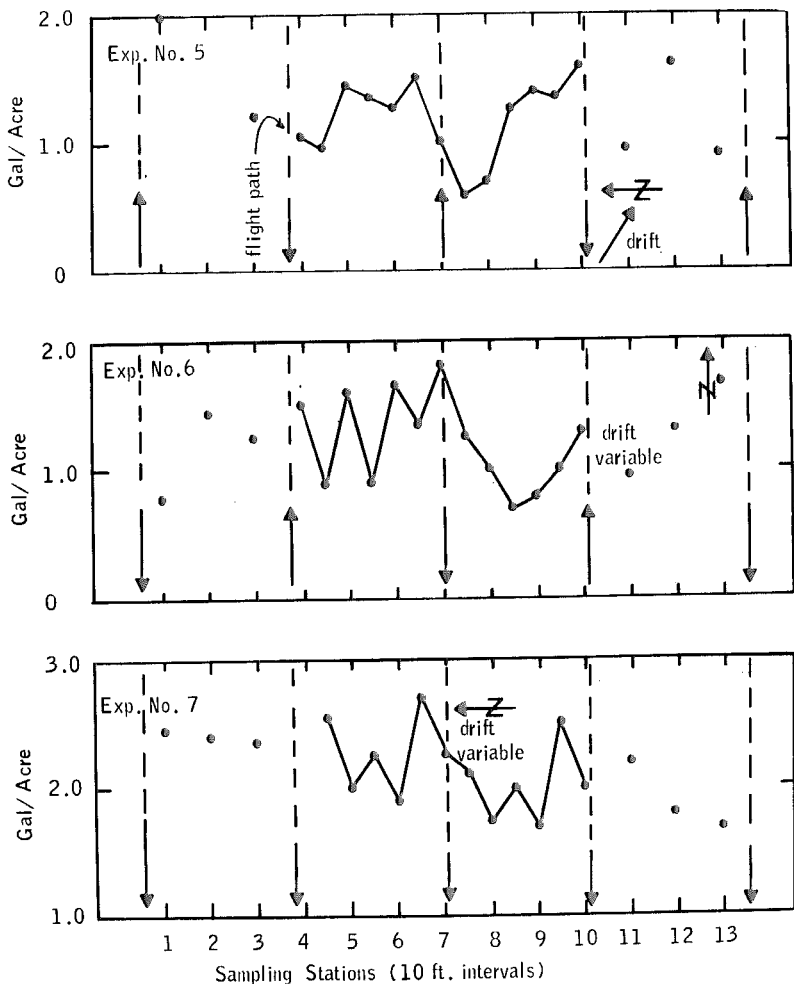


FIG. 1A.—Distribution patterns of recovery measurements of FLIT MLO in the Tulare Mosquito Abatement District.

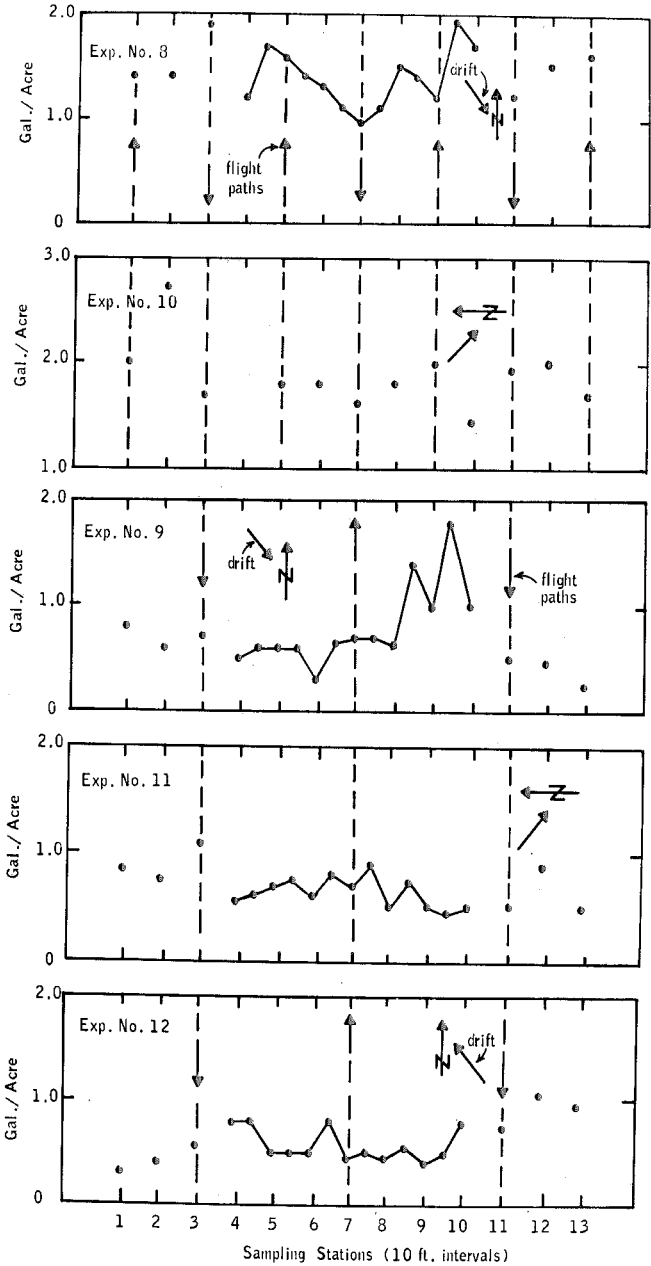


FIG. 1B.—Distribution patterns of recovery measurements of BPRL 5337-2 in the Turlock Mosquito Abatement District, 1970.

Comparison of the distillation characteristics of the applied material to that of the recovered material indicates an average 10 volume % change at Tulare and 5 volume % at Turlock. These changes represent the cumulative loss resulting from evaporation during the fall of the droplets, further evaporation in the field prior to removal of the foil from the fallout plate, and the loss of the more volatile material during the recovery procedure. A loss of 7 volume % and 5 volume % for Tulare and Turlock, respectively, occurred during the laboratory recovery procedure as reflected by the change in weight during the recovery procedure for the 1 drop standards. Furthermore, the weight loss for 1 gal/acre on foil was found to be 3 percent when exposed for 15 minutes under laboratory conditions of little wind movement and 74° F. In view of the possible weight losses during the recovery procedure, both in the field and in the laboratory, and since the more volatile components should be lost first, the noted small changes in volatility characteristics are considered to result mainly from evaporation losses during recovery. Therefore, airborne evaporation losses are considered insignificant.

The applied material is subject to a greater evaporation loss as a film on the water surface than as droplets, since the surface area of the film is greater than the surface area of the falling droplets. For example, 1 gallon divided into 100 micron droplets has a surface area of 0.06 acre, but the resultant unbroken surface film can cover 1 acre. If the droplet size is greater than 300 microns (a surface area of less than 0.02 acre per gallon), it could be assumed that the evaporation of the falling droplets would be even further reduced.

Owing to the relatively low volatility of FLIT MLO, small airborne particles from one pass may impinge on the aircraft windshield on succeeding passes. This problem was also noted by Murray (1968a, b) and King (1971). Dust adhering to the resultant film reduced visibility from

the cockpit, particularly when flying into the sun. This problem was partially alleviated by flying swaths crosswind and beginning the swaths so as to have succeeding passes upwind. A windshield washer (Ag Aircraft Safety Devices Co., P.O. Box 137, Cotton Center, Texas 79021) was installed on the Turlock plane, following the test series, and has apparently provided a solution since water readily removes the hydrocarbon film.

OBSERVATIONS ON *Aedes nigromaculis*. Table 4 presents examples of fields treated at Turlock, listing pretreatment and the several post-treatment observations. Although the test fields generally had large areas of water at pretreatment inspection, these dried to potholes or hoof prints as post-treatment observations continued, concentrating survivors into the remaining water. By the second or third day following treatment, this had totally obscured mortality assessment on a per dip basis.

Even the definition of actual mortality at any particular interval following treatment was difficult because of the delayed effect of the material. Affected larvae were sometimes observed at the first post-treatment inspection, as early as 4-6 hours after the application. Within 24 hours, a mixture of dead, moribund, and apparently normal larvae or pupae could frequently be dipped from the same source. Larvae and pupae continued to die through the third day following field treatment. Bioassay results confirmed that for third instar larvae no appreciable mortality occurs within 24 hours and that mortality continues through 96 hours. Figure 2 illustrates this for treatment with the equivalent of 1.2 gal/acre of BPRL 5337-2.

Although delayed mortality complicated field assessment of treatment results, still it was evident that the percent mortality at a given point in time varied with the age of the immatures, as shown by Micks *et al.* (1969) and Micks (1970). This was substantiated by the bioassays of field samples treated with known concentrations. All fourth instar larvae and pupae were killed at 48 hours even with the lowest

TABLE 4.—Field observations on selected applications of FLIT MLO vs. *A. nigromaculis* in the Turlock MAD.

| Experi- ment number | Acres | Rate g/a | hrs | No. stations | | No. dips | Stage of survivors ^a | | | | Remarks | |
|---------------------------|-------|-------------|------|--------------|-------------|-------------|---------------------------------|-------------|-----|----|---------|---|
| | | | | W/ water | W/ mosq. | | 1 | 2 | 3 | 4 | | P |
| 10 | 45 | 2.0 | —2 | 15 | 15 | 150 | 353 | 850 | | | | Water ponded; to 6" deep. Unimproved flooded pasture. |
| | | | 27 | 15 | 14 | 150 | | —no counts— | | | | Live, dead, and affected larvae. |
| | | | 51 | 15 | 13 | 150 | | 186 | | 1 | | Dead and affected larvae, water receding. |
| | | | 75 | 13 | 12 | 130 | 2 | 52 | 49 | | | Newly dead larvae. |
| | | | 105 | 10 | 10 | 95 | | 34 | 98 | | | |
| | | | 129 | 10 | 7 | 76 | | 14 | 55 | | X | New adults at 2 stations. |
| | | | 155 | 3 | 3 | 30 | | | | | X | A few new adults at 4 stations. |
| | | | 179 | 0 | 0 | 0 | | | 5 | | X | A few adults. |
| 11 ^b | 67 | 1.0 | —2.5 | 5 | 5 | 50 | 100 | 210 | | | | Water ponded; to 6" deep. Unimproved flooded pasture. |
| | | | 26 | 5 | 5 | 50 | | —no counts— | | | | 4th appear unaffected; not as numerous. |
| | | | 50 | 5 | 5 | 50 | 1 | 97 | | | | Also dead and moribund larvae. |
| | | | 74 | 4 | 3 | 40 | | 9 | 7 | | | |
| | | | 92 | 3 | 3 | 30 | | 1 | 25 | | | |
| | | | 116 | 2 | 0 | 20 | | | | | | |
| 11 ^b | 67 | 1.0 | —2.5 | 5 | 5 | 50 | 11 | 48 | 246 | | | Water ponded; to 6" deep. Unimproved flooded pasture. |
| | | | 26 | 5 | 3 | 50 | | 1 | 2 | | | Many dead pupae. |
| | | | 50 | 4 | 0 | 40 | | | | | | |
| Check | | | 0 | 5 | | 50 | 10 | | | | | |
| | | | 22 | 5 | | 50 | | | | | | |
| | | | 46 | 5 | | 50 | 36 | | | | | |
| | | | 70 | 5 | | 50 | 9 | | | 13 | | |
| | | | 94 | 5 | | 50 | | | | 42 | | |

^a Moribund or obviously affected individuals not counted.^b Separate sub areas within same field.

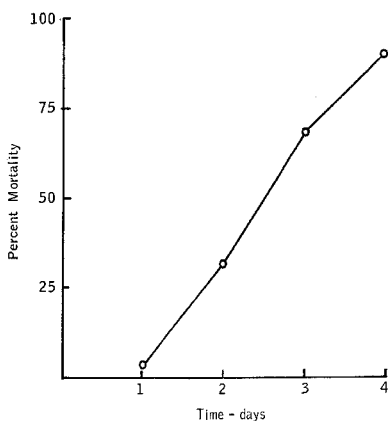


FIG. 2.—Mortality of third instar *A. nigromaculis* to BPRL 5337-2, by time after treatment. (dose equivalent to 1.2 gal/acre)

rate of FLIT MLO tested, 0.3 gal/acre. No small larvae were tested at that dosage; but at 0.6 and 1.2 gal/acre, the difference in mortality was obviously related to larval size (Figure 3).

Mortality was a major cause of control, but retardation of larval development resulting from the treatment provided further indirect control by allowing the fields to dry before the survivors could complete their life cycle. Field data from the Tulare test series, where all stages were treated, afforded an opportunity to compare rates of development of various instars. Observations from stations at which only one stage was present in pretreatment samples were used to estimate the elapsed time from treatment to fourth instar (Table 5). These estimates were compared with development for larvae from an untreated field. Since it became necessary to spray the check field, comparisons beyond fourth instar were impossible. Comparing the larval development rate in the treated fields with that of the untreated check in Turlock (Table 5) provides additional evidence of retardation.

The bioassay yielded further confirmation. Although only 9 of the treated third instar larvae survived at 96 hours,

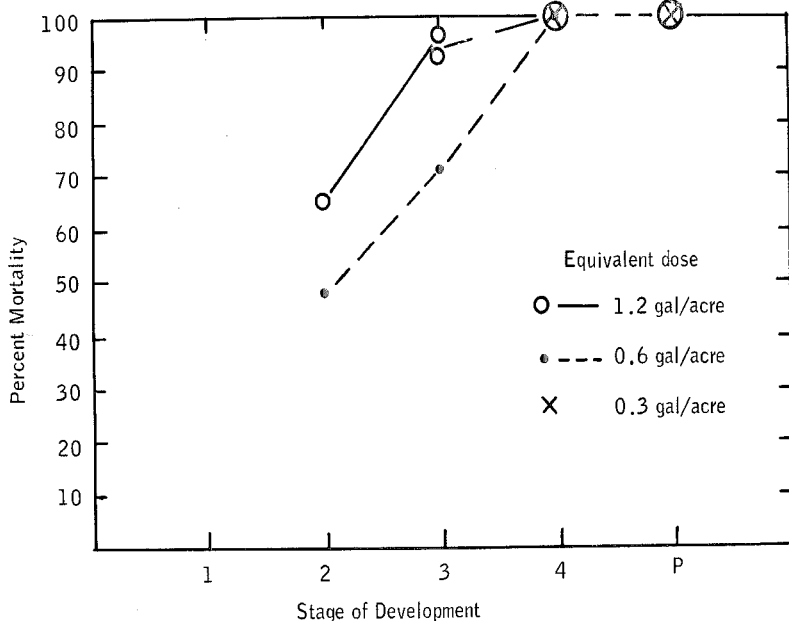


FIG. 3.—Mortality at 48 hours of field collected immature stages of *A. nigromaculis* to known doses of FLIT MLO.

TABLE 5.—Estimated retardation of larval development of *A. nigromaculis* in the field after treatment with 2.1 gal/acre FLIT MLO compared to development of larvae from an untreated field.

| Instar treated | Hours to fourth instar from treatment time | |
|----------------|--|-------------------|
| | Treated larvae | Untreated control |
| First | 78 | 40 |
| Second | 66 | 26 |
| Third | 34 | 13 |

the observation of 8 third and 1 fourth instars and no pupae contrasted sharply with the 89 untreated survivors which had all reached fourth instar with 2 in the pupal stage.

Pelletized livestock food, continually available to the larvae in the pans, appeared undisturbed throughout the 96-hour count. This apparent lack of active food intake was also observed at the lowest rate evaluated, 0.6 gal/acre. These observations are compatible with those of Micks (1970) on the absence of visible food in the gut of treated *C. p. quinquefasciatus* and *A. aegypti*.

The selection of *A. nigromaculis* test fields on the basis of sufficient water for an extended post-treatment evaluation biased test results by affording greater opportunity for adult emergence. Nevertheless, the numbers of adults that were observed to emerge from the treated fields were very low compared with the potential, as evidenced by the high numbers of larvae and pupae at the pretreatment counts. Although control was not total, it must be pointed out that the areas of treatment were characterized by populations of mosquitoes exhibiting extremely high levels of resistance to the organophosphorus compounds. Conventional applications of these compounds had, in the recent history of these fields, resulted in an unacceptable degree of control.

The reasons for incomplete control using FLIT MLO are not known; however, differential exposure to the larvicide at the water surface may offer at least a partial explanation for larval survival in the field.

Incomplete coverage may result from treatment of areas with exceedingly dense vegetation. It is possible that larvae which remain in these areas during the surface life of the film are protected from contact with the material. In this event, higher application rates would probably not appreciably increase mortality.

No appreciable difference in control effectiveness was noted between the 1 and 2 gal/acre rates applied in the Turlock tests. Lower application rates should be tested to evaluate their effectiveness. However, lower application rates of FLIT MLO are accompanied by a lower dosage rate, since this larvicide is not applied with a diluent. Application factors such as swath placement and distribution pattern thus become extremely critical with lower rates. This is unlike application procedures using organophosphorus materials. The dosage rate applied with these materials is many times that necessary to produce mortality and the application rate can be varied from ULV to many gallons per acre by dilution, with the dosage level remaining constant.

Treatment of larvae at third instar or later could have the benefit of decreasing the total acreage treated where the size of the flooded areas decreases rapidly following each irrigation. However, uneven irrigation patterns could complicate selective application, with various instars present at the same time.

OBSERVATIONS ON *Culex tarsalis*. Immature *C. tarsalis* were occasionally present with *A. nigromaculis* at the pretreatment inspection and were observed following treatment. In other tests, no *C. tarsalis* larvae were noted at the pretreatment inspection but were found in varying numbers at subsequent times. In several instances *C. tarsalis* larvae were seen even after all *A. nigromaculis* immatures had been killed. Although the data from these field observations were inadequate for analysis, it appeared that the mortality rates of larval *C. tarsalis* were lower than those of the *A. nigromaculis* with which they were associated. The susceptibility of *A. nigromaculis* relative to *C. tarsalis*

TABLE 6.—Effects of FLIT MLO upon immature *C. tarsalis*.

| Experiment number | g/a | Hours | No. dips | 1 | 2 | 3 | 4 | P | Total |
|-------------------|-----|--------|-----------------|----|-----|----|-----|----|-------|
| 13 ^a | 2.1 | -0.5 | 50 | 12 | 21 | 37 | 111 | 44 | 225 |
| | | +6.5 | 50 | 0 | 2 | 1 | 29 | 11 | 43 |
| | | +29.0 | 50 | 0 | 1 | 2 | 19 | 33 | 55 |
| | | +54.0 | 30 ^b | 0 | 14 | 3 | 12 | 11 | 40 |
| | | +71.0 | 30 | 1 | 5 | 8 | 9 | 7 | 30 |
| Egg rafts | | | | | | | | | |
| 10 | 2.0 | -0.5 | 25 | 6 | 117 | 87 | 98 | 2 | 310 |
| | | +28.0 | 25 | 0 | 7 | 20 | 31 | 0 | 58 |
| | | +50.0 | 25 | 0 | 3 | 12 | 20 | 12 | 47 |
| | | +74.0 | 25 | 0 | 3 | 6 | 11 | 7 | 27 |
| | | +102.0 | 25 | 0 | 2 | 5 | 19 | 3 | 29 |
| +120.0 | 25 | 0 | 0 | 3 | 5 | 2 | 10 | | |

^a Same application conditions as Experiment 5 (Table 2).

^b Two of five stations dried.

is in contrast to the susceptibility of *A. aegypti* relative to *C. p. quinquefasciatus* as reported by Micks *et al.* (1968).

Two treatments were made of sources containing *C. tarsalis* alone. The results are summarized in Table 6. A decline in numbers was obvious. No measurement was attempted of any adult emergence, and the lack of an untreated check field made it impossible to evaluate retardation of development.

ACKNOWLEDGMENTS

Special thanks are due Messrs. Dennis J. Ramke and Leonard K. McKean of the Tulare Mosquito Abatement District and Messrs. Stephen M. Silveira, Grover W. Force, and Floyd A. King of the Turlock Mosquito Abatement District who made this study possible. Operators of the two districts contributed by providing the essential inspections to aid in selecting experimental fields. Personnel of the Bureau of Vector Control and Solid Waste Management assisted in field evaluations, and Mr. C. W. Gordon, ESSO Research and Engineering Company, provided recovery data. Dr. C. H. Schaefer, Director, University of California Mosquito Control Research Laboratory at Fresno, coordinated and assisted in the Tulare study. Humble Oil and Refining Company provided material for the tests.

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STUDIES WITH JUVENILE HORMONE-TYPE COMPOUNDS AGAINST MOSQUITO LARVAE¹

W. L. JAKOB AND H. F. SCHOOF

Among alternate methods being investigated as substitutes for the conventional chemical control methods against arthropod vectors, the use of insect hormones or chemically-related substances is an exciting possibility. Recent reviews of the history, structure and action of insect hormones have been published (Roller and Dahm, 1968; Berkoff, 1969). The activity of early synthetic juvenile hormone (JH) mimics against mosquito larvae was in-

vestigated (Lewallen, 1964; Nair, 1967; Spielman and Williams, 1966). As part of its continuing search for new, safe methods to control insects of public health importance, this laboratory has evaluated synthetic compounds having hormone-like activity against mosquito larvae. The results obtained in laboratory tests with 12 compounds are presented.

MATERIALS AND METHODS. All tests were conducted in 600-ml. glass beakers containing 250 ml. of well water. Twenty-five third instars were introduced not less than ½ hour after treatment with the compound. Food was initially provided within 2 hours thereafter, with additional amounts provided daily until approximately half the specimens had pupated. The number of dead larvae and pupae re-

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. Presented at the annual meeting, AMCA, Denver, Colorado, March 21-24, 1971.