EFFECTS OF A NEW FLUORESCENT INSECT GROWTH REGULATOR ON THE LARVAL INSTARS OF *AEDES AEGYPTI*[1]

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ABSTRACT. A new fluorescent compound (5-[[[5 - (dimethylamino)-1-naphthalenyl]sulfonyl]-amino]-1,3-benzodioxole) tested against all instars of larvae of *Aedes aegypti* (L.) was found to produce morphogenetic effects similar to those produced by synthetic insect growth regulators. Larvae failed to molt completely to the next larval stage, larval-pupal intermediates were formed; or the surviving larvae required more time for pupation.

INTRODUCTION. A wide variety of synthetic insect growth regulators (IGRs) has been tested for control of mosquito species (Arias and Mulla 1975; Ittycheriah et al. 1974; Jakob 1972; Patterson 1974; Spielman and Skaff 1967; Spielman and St. Onge 1974; and Steelman and Schilling 1972). However, none yet tested is easily traced to sites of absorption, accumulation, and/or ingestion in mosquito larvae. Some of us (Mayer et al. 1976) conceived that incorporation of fluorescence into a compound with the characteristics of an IGR to produce a fluorescent insect growth regulator (FIGR) would make it possible to determine the mode(s) and/or site(s) of action of the compound. We therefore tested one, 5-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1,3-benzodioxole, on a variety of insects to ascertain its production of morphogenetic effects and the presence of fluorescent characteristics that might be useful. Our microspectrofluorometric observatins of mosquito larvae confirmed the presence of the FIGR in the pharyngeal, midgut, and anal papillae regions (Mayer and Cocke 1976). When subsequent preliminary screening experiments with *Aedes aegypti* (L.) demonstrated that all stages of development were affected, we made a more detailed study of the effect of the FIGR on the various developmental stages of *Ae. aegypti* larvae to determine its morphogenetic effects and lethality and compared its effect with those of a natural and a synthetic IGR.

MATERIALS AND METHODS. *Ae. aegypti* eggs obtained from our rearing colony were flooded with distilled water, and several drops of TetraMin®[4] fish food slurry were added to provide an adequate supply of larval food. Emerging larvae (100 of the same age and instar) were placed in porcelain pans (35 x 23 cm) containing 1 liter of distilled water, and 10 drops of TetraMin slurry. The FIGR was synthesized according to Mayer et al. (1976), dissolved in acetone (0.5 ml), and added to the culture pans to provide treatments of 0.1, 1.0, 2.5, 5.0, 7.5, 10.0 and 15.0 parts per million (ppm). Control pans were treated with 0.5 ml of acetone. Each pan was covered with Saran® wrap to reduce evaporation; small holes were made in the cover to assure an adequate air supply. The rearing room temperature was maintained at 26 ± 1°C, and the photoperiod was constant at 14 hr light and 10 hr dark throughout the test period. Each test was replicated 3 times. Also, the synthetic (cecropia) JH

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[4] This paper reports the results of research only. Mention of a pesticide or a commercial or proprietary product in this paper does not constitute a recommendation for use by the U. S. Department of Agriculture nor does it imply registration under FIFRA as amended.

(methyl 10,11 - epoxy - 7-ethyl-3, 11-dimethyl - 2, 6 - tridecadienoate, obtained from Hoffman-LaRoche, Inc. Nutley, N.J., 99% pure) was tested on 3rd instars at similar concentrations. Fourth instars were treated with methoprene, [isopropyl (*E,E*) - 11 - methoxy-3,7,11-trimethyl-2,4-dodecadienoate, obtained from the Zoecon Corp., Palo Alto, Calif. (95% pure)] at 0.01 ppm.

Culture pans were examined daily for larval deaths, abnormal ecdyses, and pupal deaths. Live pupae were removed daily and placed in jars containing approximately 50 ml of the original culture medium. These jars were then placed in constant-humidity lamp chimneys where pupal death, incomplete emergence (adults only partially removed from pupal exuvia), and complete emergence were monitored. The resulting data were pooled with data obtained from the treatment pans. Mortality was corrected by using Abbott's formula, and LC_{50} values were determined by a probit analysis program on a programmable calculator. A

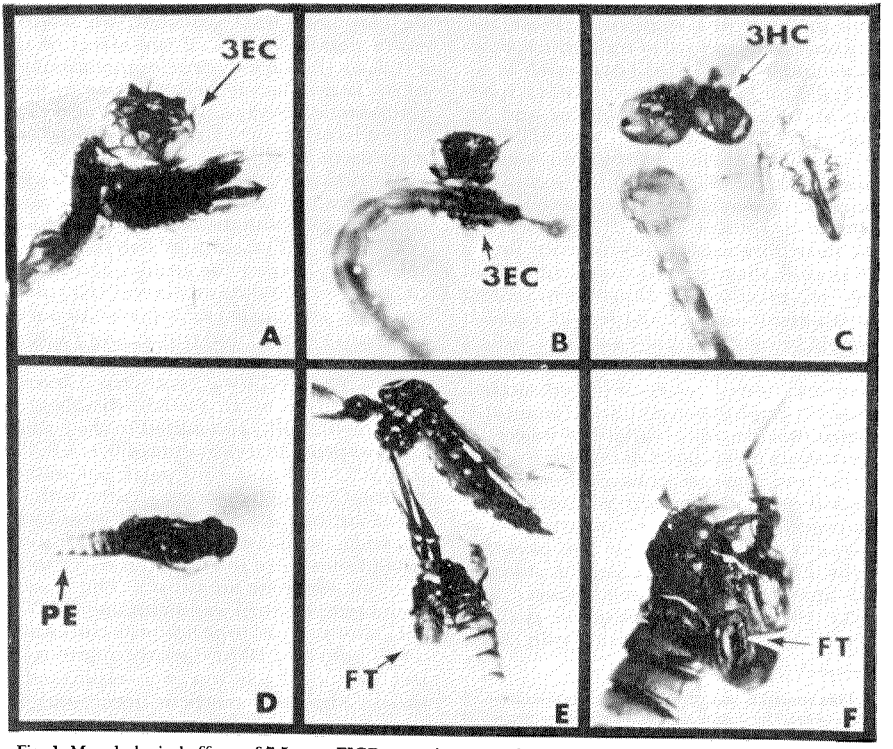

Fig. 1. Morphological effects of 7.5 ppm FIGR on various growth stages of *A. aegypti*. A. fourth-instar larva with third-instar exocuticle (3EC) attached to siphon and anal papillae region, × 35; B. fourth-instar larva showing wrinkled third-instar exocuticle (3EC) attached at the seventh abdominal segment, × 25; C. fourth-instar larva showing third-instar head capsule (3HC) attached to new fourth-instar head capsule, × 25; D. adult mosquito head, thorax, and abdomen extending from pupal exuvia (PE), × 15; E. adult male mosquito incompletely emerged with folded tarsi (FT), × 25; F. pupal exuvia showing folded tarsus (FT) remaining after adult emergence, × 40.

one-way analysis of variance program was used to determine significance variation in mean developmental times.

RESULTS AND DISCUSSION. Treatment of *Ae. aegypti* larvae with various concentrations of the FIGR produced morphogenic effects that were similar to those produced by methoprene. Thus some larvae (Fig. 1A–1C) were unable to shed the old cuticle from the abdominal and head regions. The partially shed exuviae remained compacted in many larvae and attached in the siphon and anal papillae areas. Other larvae retained the larval head capsule attached at some point on the new head capsule. Some extruded the hindguts, which became entangled with old cuticle in the anal papillae region. However, there seemed to be no difficulty in larvae removing the thoracic and upper abdominal regions from the old skin. In the pupal stage, the effects of the FIGR were often manifested by the formation of a type of larval-pupal intermediate: the pupa retained the larval head capsule and portions of larval cuticle in the lower abdominal regions. The thorax of these specimens was free from 4th-instar cuticle. Wing pads and trumpets were extended, and there were pupal palmate hairs on the upper abdominal regions. Specimens that formed apparently normal pupae often died before the adult emerged.

The most obvious effect of the FIGR was noted at the time when adults attempted to extricate themselves from the

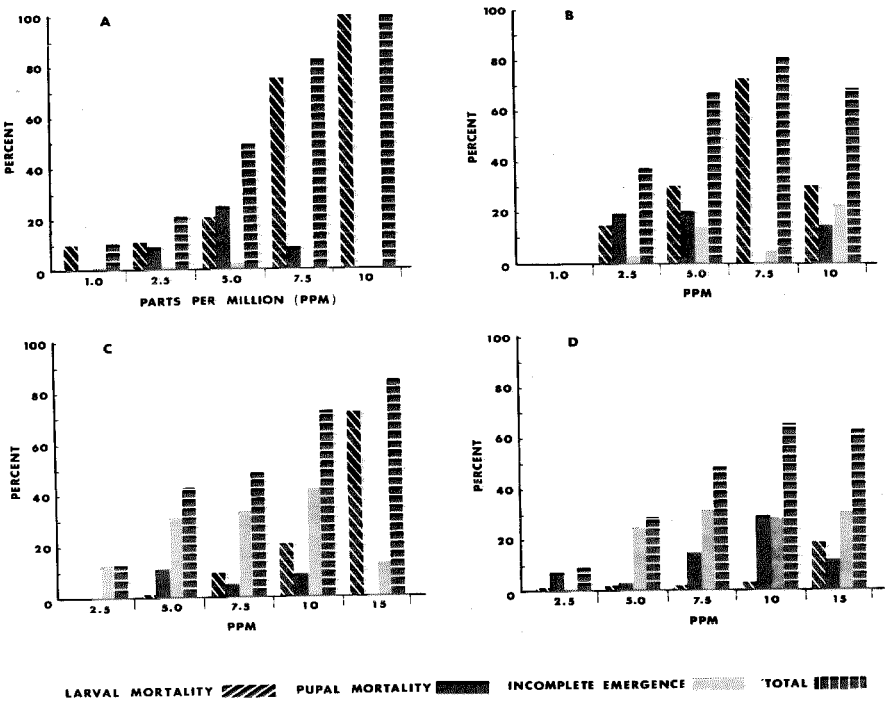

Fig. 2. Corrected mortality of *A. aegypti* larvae exposed to the FIGR at various concentrations. A. First instar; B. second instar; C. third instar; D. fourth instar.

pupal skin (Fig. 1D,E). Many adults extruded only the head and thorax; others were able to remove their whole body but remained bound by their legs and tarsi to the pupal skin (Fig. 1F). However, any adult male mosquitoes that emerged normally did complete genitalia rotation, a process that is sometimes inhibited by IGR treatments (Spielman and Skaff 1967).

Mortality was higher when 1st and 2nd instars were exposed to a given concentration of the FIGR (Fig. 2A,B) than when 3rd and 4th instars were exposed to similar concentrations (Fig. 2C,D). For example, 74 and 73% of the young larvae were killed by exposure to 7.5 ppm FIGR, and treatment with 10 ppm produced 100% kill of 1st instars and 73% kill of 2nd instars. In contrast, only 30% or less of 3rd and 4th instars were killed by treatment with 15.0 ppm.

No trends were apparent in the deaths occurring at the pupal stage due to exposure of larvae to the FIGR. Deaths varied inconsistently with the duration of treatment, instar treated, and the concentration of FIGR. The numbers of adults that emerged incompletely from the pupal skins did increase with increasing dose. Larvae treated with 5–10 ppm FIGR as 3rd and 4th instars produced a higher percentage of partially emerged adults than younger larvae treated with the same concentrations. Third instars treated with 5.0, 7.5 and 10 ppm FIGR produced 32, 34, and 43% partially emerged adults, respectively. The LC_{50} values based on total mortality of the FIGR for the various larval instars are given in Table 1.

Table 1. LC_{50} values of the FIGR for each instar of *A. aegypti larvae.*

Instar tested	Slope	LC_{50} (ppm)
First	0.6585	2.188
Second	2.0548	3.938
Third	1.0982	7.256
Fourth	2.6666	7.914

Treatment with the FIGR significantly increased the mean duration of the 1st, 3rd, and 4th larval stages (from the time of treatment to the time of pupation) for those larvae that pupated (Table 2).

The effects of the methoprene on 4th instars were similar in many ways to the effects of the FIGR on similar larvae. They became soft and distended, and the larval cuticle appeared thin and transparent. Many pupae retained the larval skin on a portion of the abdomen. Others retained the 4th instar head capsule attached over the anterior portion of the cephalothorax of the newly formed pupa. Also, adults emerged incompletely or left the legs and tarsi attached in the pupal exuvia. This occurrence of morphogenetic forms in *Ae. aegypti* treated with methoprene compares well with the results obtained by Arias and Mulla (1975) using *Culex tarsalis* (Coquillett).

Likewise, 3rd instars treated with synthetic JH had morphogenetic abnormalities quite similar to those of larvae treated with the FIGR or methoprene.

Table 2. Developmental time to pupation when instars of *A. aegypti* larvae were exposed continuously to concentrations of the FIGR (surviving larvae only).

Larval instar at beginning of treatment	Mean time (hr) to pupation when larvae were exposed to indicated concentration (ppm) of FIGR						
	0.0	1.0	2.5	5.0	7.5	10	15
First	190	195	159	183	244[a]	NP[b]	—[c]
Second	166	197	213	295[a]	305[a]	322[a]	—[c]
Third	91	100	105	118[a]	145[a]	172[a]	167[a]
Fourth	50	60	53	61	69	74	100[a]

[a] Difference from no treatment (0.0), significant=0.05.
[b] NP=No pupation.
[c] Dose not tested.

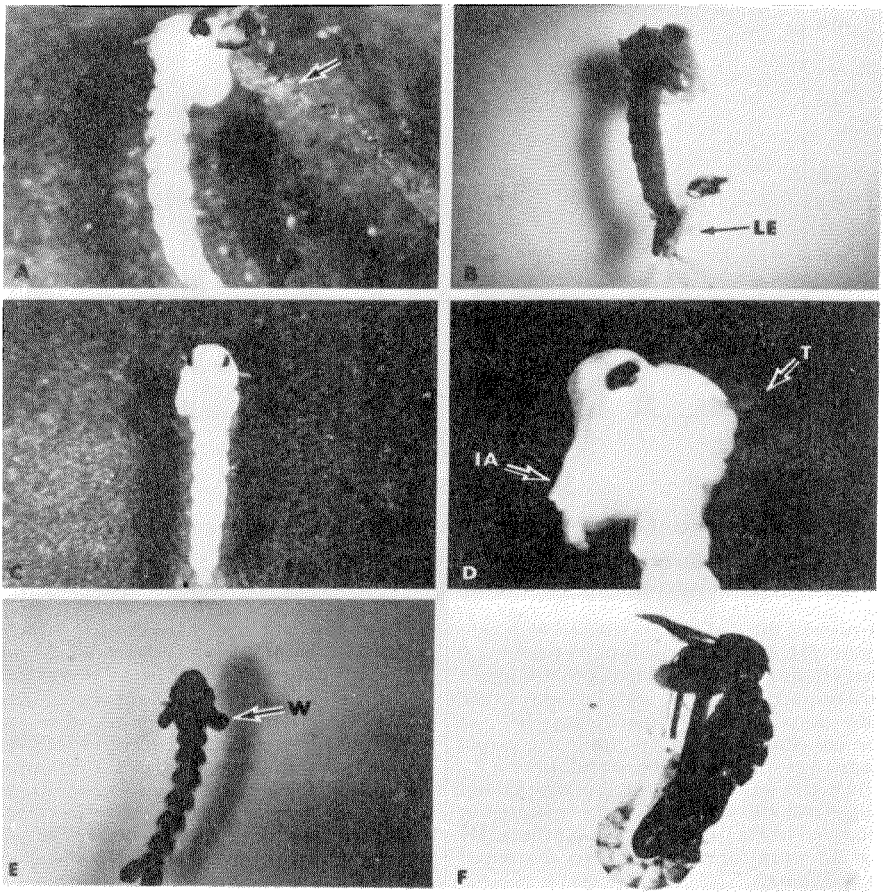

Fig. 3. Gross external morphogenetic effects of synthetic JH on *A. aegypti* larvae. A. Larval-pupal intermediate showing fourth-instar larval exuvia (LE) attached in the head region, × 20; B. larval-pupal intermediate showing fourth-instar larval exuvia (LE) compressed and attached to siphon area, × 13; C. unmelaninized pupa showing trumpets (T) and extended imaginal appendages (IA), × 30; E. melanized pupa showing wing pads (W) extended in exarate fashion, × 13; F. incompletely emerged adult trapped in pupal exuvia, × 18.

However, like methoprene, only the pupal stage was affected. Most pupae retained the 4th-instar cuticle (Fig. 3A,B), but those that pupated successfully often died as "albino" pupae (Fig. 3C); that is, they lacked the hardening and darkening of the cuticle and remained distended, exarate, with wing pads and legs free from the cephalothoracic region (Fig. 3D,E). Some pupae survived treatment with 10 ppm but produced adults that were incapable of freeing themselves from the pupal exuviae (Fig. 3F): their tarsi remained folded entrapping the newly emerging adult. Also, a few adults emerged legless and small compared with the controls.

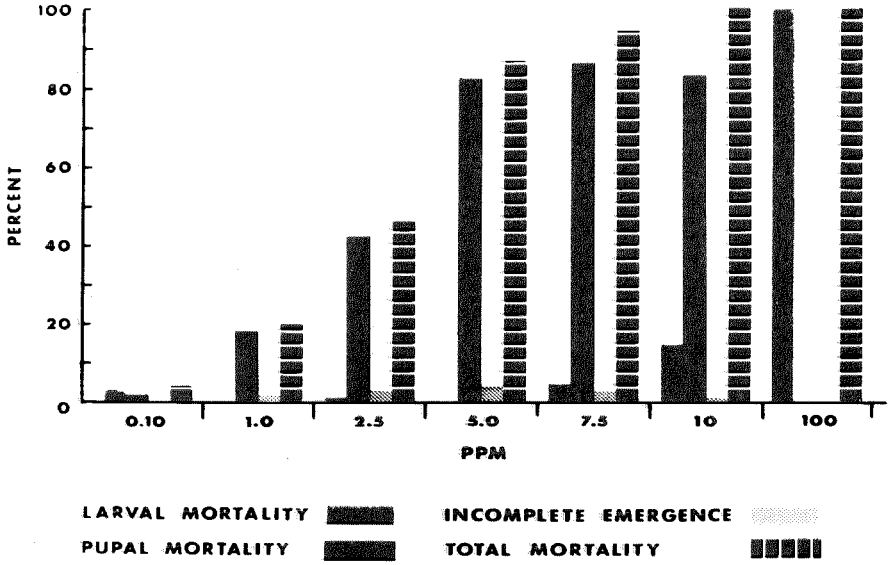

Fig. 4. Corrected mortality of third-instar *A. aegypti* larvae exposed to various concentrations of synthetic JH.

However, the 3rd instars treated with synthetic JH at concentrations between 1.0 and 10 ppm molted normally to 4th instar and pupated normally with no delay in the time to pupation. Indeed, regardless of the concentration to 10 ppm, the pupal stage was the point in development at which the lethal effects were manifested (Fig. 4); at 100 ppm, all deaths occurred in the third instar. The LC_{50} for synthetic JH was 3.5 ppm for *Ae. aegypti* larvae treated as 3rd instar.

CONCLUSIONS. *Ae. aegypti* larvae exposed to FIGR beginning in each of the 4 instars showed significant developmental responses, but 1st and 2nd instars appeared to be more susceptible to kill, possibly due to longer exposure. The FIGR produced larval-pupal intermediates closely resembling those produced by treatments with methoprene. Such forms cannot be interpreted as supernumerary intermediate stages (Ittycheriah et al. 1974) nor as "non-viable 5th-instar larvae" (Spielman and Skaff 1967) since pupalcephalothoracic cuticle with fully developed trumpets and abdominal palmate hairs were observed. Nevertheless, the morphogenetic effects and the increased developmental times resulting from treatment with the FIGR do seem to warrant classifying this compound as a growth regulator for this insect. This classification is also supported by the physiological data presented (Mayer et al. 1976), which showed the FIGR induced ovarian development in allatectomized *Ae. aegypti*.

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ORGANOPHOSPHORUS TOLERANCE IN *CULEX QUINQUEFASCIATUS* IN TEXAS

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ABSTRACT. Twelve collections of *Culex quinquefasciatus* from separate areas of Galveston County, Texas and 8 from various parts of Brazoria County were established as temporary strains in the laboratory for 2 or 3 generations to provide sufficient material for insecticide susceptibility tests. As compared with a standard susceptible strain (UTMB), the LC_{50}'s for malathion of 9 strains of larvae from Galveston County ranged from 0.0058 to 0.280 ppm or a maximum of 7X less susceptible. The LD_{50}'s of the adults ranged from 0.9% to > 5.0% malathion. Five strains of larvae from Brazoria County were 2X to 9X more tolerant to malathion than the standard, whereas the LD_{50}'s of the 8 strains of adults ranged from 1.3% to > 5.0%. Larvae from both counties exhibited tolerance to chlorpyrifos, ranging from 2X to 13X as compared with a susceptible strain. The larvae of 2 strains tested against a battery of other organophosphorus insecticides not used in mosquito control, exhibited decreased susceptibility which in the case of fenitrothion and parathion reached 16X and 20X, respectively, less susceptible than the UTMB strain.

Despite the rather intensive use of organophosphorus (OP) insecticides for the control of various mosquito species by mosquito control districts along the Texas Gulf Coast, and the presence of organophosphate resistance in *Culex pipiens quinquefasciatus* in California (Georghiou et al. 1975) and tolerance in Louisiana (Steelman and Devitt 1976), there have been no reports of resistance in Texas since the finding 15 years ago that this and several other species were resistant to the BHC-dieldrin group (Micks et al 1961). Shortly thereafter the use of malathion as an adulticide was initiated, sometimes supplemented by certain other OP insecticides. In addition, chlorpyrifos has been used as a larvicide for approximately the past 7 years for the control of *Cx. quinquefasciatus*.

Because of the fact that *Aedes sollicitans* and *Ae. taeniorhynchus* have been the principal target species of control programs, and that malathion-resistance was reported in *Ae. taeniorhynchus* in Florida after approximately 10 years of use (Gahan et al 1966), much of the insecticide susceptibility monitoring was concentrated on these *Aedes* species. However, the occurrence of outbreaks of St. Louis encephalitis in Harris County in 1975 and 1976 stimulated us to make a thorough investigation of the insecticide susceptibility status of *Cx. quinquefasciatus* in 2 adjacent coastal counties, i.e., Brazoria and Galveston, during 1976.

MATERIALS AND METHODS. During the last half of 1976, a total of 20 batches of larvae were collected from various areas of Brazoria and Galveston Counties. Each was given a specific strain designation and