

SUSCEPTIBILITY OF SELECTED MOSQUITO SPECIES TO FIVE CHEMICALS WHICH INHIBIT INSECT DEVELOPMENT

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ABSTRACT. The toxicological response of 12 species of mosquitoes was determined using the inhibitors of insect development Thompson-Hayward-6040 (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea) Monsanto-0585 (2,6-di-*t*,butyl-4-(*a,a*-dimethyl benzyl) phenol), Altosid® (isopropyl 11-methoxy-3,7,11-trimethyl-dodeca-2,4-dienoate), Stauffer-R-20458 (1-(4-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene) and Hercules-24108 (3-Butyn-2-yl N-(*p*-chlorophenyl) carbamate).

Dosage-mortality data revealed that the susceptibility of each mosquito species varied rela-

tive to the type of inhibitor of development used. The susceptibility of each species within a genus, relative to the type of compound, varied to such a degree that no comparative generalizations of genera susceptibility could be made. Some species differences in susceptibility varied as much as 10,000 times between compounds. *Aedes sollicitans* (Walker) was the most susceptible species to all 5 of the compounds tested. All 12 species of mosquitoes studied were more susceptible to Thompson-Hayward-6040 than to the other 4 chemicals tested.

The potential control value of recently developed compounds which inhibit mosquito development has been reported (Sacher, 1971; Schaefer and Wilder, 1972; Steelman and Schilling, 1972; Jakob, 1972; Randall and Strong, 1973; and Schaefer and Wilder, 1973). Data are lacking on the toxicological response of various species of mosquitoes to these chemicals. Hence, this study was initiated to determine the comparable susceptibility of 12 selected species of mosquitoes to 5 compounds which inhibit mosquito development.

MATERIALS AND METHODS. The following mosquito species were utilized in this study: *Aedes aegypti* (Linnaeus), *Aedes sollicitans* (Walker), *Aedes taeniorhynchus* (Wiedemann), *Aedes triseriatus* (Say), *Culiseta inornata* (Williston), *Culex pipiens quinquefasciatus* Say, *Culex salinarius* Coquillett, *Culex tarsalis* Coquillett, *Anopheles quadrimaculatus* Say, *Psorophora confinnis* (Lynch-Arribálzaga), *Psorophora ferox* (Humboldt) and *Psorophora varipes* (Coquillett). All mosquito species were obtained from the U.S.D.A. Gulf Coast Research Laboratory, Lake Charles, Louisiana, except *C. p. quinquefasciatus* which was reared in the labora-

tory at Louisiana State University in Baton Rouge and *A. sollicitans* which was field-collected as adults, blood-fed in the laboratory and held for egg production at the Lake Charles Laboratory. The mosquito species obtained from the U.S.D.A. Mosquito Research Laboratory were colonized from naturally occurring populations in Louisiana with the exception of *C. tarsalis* which was obtained from California.

The eggs of 11 species of mosquitoes were transported to the laboratory in Baton Rouge via surface transportation. *Aedes* and *Psorophora* eggs were stimulated to hatch in a hatching solution prepared by mixing ca. 0.1 g of nutrient broth with 2.0 l of water which was added to 40 x 20 x 4.5 cm enamel pans containing the eggs. *Anopheles*, *Culex* and *Culiseta* eggs were placed on the water surface of 40 x 20 x 4.5 cm enamel pans which contained ca. 2.0 l of water for hatching. Twenty-four hours after hatching the 1st instar larvae were transferred to 28 x 17 x 4.5 cm enamel pans containing 1.0 l of water. Each pan of larvae was aerated continuously. Approximately 300 larvae were placed in each pan for rearing.

All larvae were reared according to the procedures described above except for *An. quadrimaculatus*. These larvae were not aerated and were fed 0.1 g of finely ground liver meal. Culicine larvae were fed ground rabbit pellets. Larvae were reared in pans

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until early 3rd instar, whereupon they were randomly removed for dosage-mortality studies.

The five chemical compounds which inhibit mosquito development were: Altosid® (isopropyl 11-methoxy-3,7,11-trimethyldeca-2,4-dienoate) 4 lb AI/gal EC, Hercules-24108 (3-Butyn-2-yl N-(p-chlorophenyl) carbamate) technical, Monsanto-0585 (2,6-di-t-butyl-4-(d,d-dimethylbenzyl) phenol) 3 lb AI/gal EC, Stauffer-R-20458 (1-(4-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene) 4 lb AI/gal EC, and Thompson-Hayward-6040 (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea) 5% EC. The four emulsifiable concentrate compounds were serially diluted with distilled water and each serial dilution was pipetted into 1.0 l of water per pan to give the desired concentrations. Hercules-24108 was serially diluted with acetone, after which each serial dilution was pipetted into 1.0 l of water, per pan.

A minimum of 4 replications, each consisting of the 5 concentrations plus the untreated checks were used for testing each compound. Ten 3rd instar larvae were placed in each treatment-concentration and concurrently a similar number was placed in each untreated check.

The larvae, pupae or adults killed by various concentrations were removed at 24-hr intervals after treatment. All live pupae removed from the test pans were held in small plastic soufflé cups that were covered with screened lids. Total mortality for all stages as well as individual larval, pupal, larval-pupal and pupal-adult intermediates and abnormal adult mortalities were recorded at each 24-hr observation period. The 1d-p line for total mortality through the adult stage for each mosquito species was determined by the computer program for probit analysis described by Daum (1970). Where sufficient data were not collected for the computer to establish dosage-mortality regression lines, the regression lines were eye-fitted.

RESULTS AND DISCUSSION. The observed morphogenetic effects of the different compounds were characterized as: (1)

Mon-0585—dead unmelanized pupae; (2) Altosid®, R-20458, TH-6040 and H-24108—fully formed dead adults with hard dark cuticles, with appendages that remained within the pupal case and abdomens that extended to the position normally held during ecdysis; (3) All five compounds—dead larval-pupal and pupal-adult intermediates. (These were either partially molted larval-pupal forms or pupae-adult forms with wing-like structures and mouthparts protruded from the pupal case); and (4) All five compounds—abnormal adults.

The highest concentration of developmental inhibitor used in the dosage-mortality tests was used to calculate the percentage mortality that occurred during different stages of mosquito development. These data revealed that some 3rd instar larvae of certain species probably received enough developmental inhibitor to result in death at time of molting from 3rd to 4th instar. This type of activity was also observed by Mulder and Gijswijt (1973). Their histological examinations of *A. aegypti* larvae revealed that failure to successfully molt to the next larval instar could be ascribed to a defeat in the process of cuticle deposition caused by treatment with TH-6040. The stage of development in which mortality occurred varied from species to species depending on the type of developmental inhibitors used.

The stage of development-mortality means for all species treated with Mon-0585 were pupae—53%, larvae—43% and larvae-pupae intermediates—3%. Larval mortality was most evident in *P. confinnis* (95%), *P. ferox* and *P. varipes* (80%). Pupal mortality was 100% in *A. aegypti*, *A. sollicitans*, and *C. p. quinquefasciatus*. The highest percentage of intermediates (13%) occurred among Mon-0585-treated *C. salinarius* larvae.

When all species are considered collectively, Altosid® killed 74% of the mosquitoes as pupae, 19% as larvae, 2% as intermediates and 2% as abnormal adults. Larval mortality ranged from none in *A. sollicitans* and *P. confinnis* to 55% in *An.*

TABLE 1. Susceptibility of selected mosquito species to Mon-0585.

Species	PPM			Slope
	LC-50	LC-90	95% C.L. ^a	
<i>A. aegypti</i>	0.111	0.242	0.089-0.132	3.7820
<i>A. sollicitans</i>	0.014	0.138	0.002-0.028	1.2950
<i>A. taeniorhynchus</i>	0.143	0.437	0.108-0.179	2.6329
<i>A. triseriatus</i> ^b	0.530	1.600
<i>An. quadrimaculatus</i>	0.374	1.824	0.248-0.508	1.8613
<i>C. p. quinquefasciatus</i>	0.051	0.210	0.037-0.065	2.0916
<i>C. salinarius</i>	0.102	0.237	0.081-0.123	3.5224
<i>C. tarsalis</i>	0.188	0.552	0.135-0.236	2.7389
<i>Cu. inornata</i>	0.179	0.535	0.125-0.228	2.7256
<i>P. confinnis</i> ^b	0.070	0.150
<i>P. ferox</i>	0.053	0.334	0.006-0.104	1.6000
<i>P. varipes</i>	0.053	0.334	0.006-0.104	1.6000

^a Confidence Limits shown for LC₅₀ only.^b Eye fitted line.

TABLE 2. Susceptibility of selected mosquito species to Altosid®.

Species	PPM			Slope
	LC-50	LC-90	95% C.L. ^a	
<i>A. aegypti</i>	0.1532	0.7799	0.109-0.207	1.8137
<i>A. sollicitans</i>	0.00001	0.0013	0.0001-0.003	0.5626
<i>A. taeniorhynchus</i>	0.0146	0.1121	0.0001-0.083	1.4493
<i>A. triseriatus</i>	0.0201	0.6472	0.010-0.037	0.8507
<i>An. quadrimaculatus</i>	0.0138	0.4964	0.0-0.238	0.8244
<i>C. p. quinquefasciatus</i>	0.0026	0.1936	0.0009-0.005	0.6847
<i>C. salinarius</i>	0.0593	1.2388	0.033-0.108	0.9710
<i>C. tarsalis</i>	0.0006	0.0325	0.0001-0.002	0.7498
<i>Cu. inornata</i>	0.0191	1.6357	0.009-0.038	0.6637
<i>P. confinnis</i>	0.0071	0.1007	0.0003-0.012	1.1166
<i>P. ferox</i>	0.0001	0.0007	0.000002-0.0001	1.1375
<i>P. varipes</i> ^b	0.0002	0.0020

^a Confidence Limits shown for LC₅₀ only.^b Eye fitted line.

quadrimaculatus. All *A. sollicitans* and *P. confinnis* died as pupae. *P. ferox*, *P. varipes* and *An. quadrimaculatus* contained the highest percentage of intermediates (5%). *A. quadrimaculatus* contained the highest number of abnormal adults (10%).

Stauffer-R-20458 caused an average of 75% and 12% mortality to pupae and larvae, respectively, when all species of mosquitoes tested were considered; 1% died as intermediates, and 8% died as abnormal adults. Larval mortality of individual species ranged from none in *A. sollicitans*, *A. taeniorhynchus*, *C. p. quinquefasciatus*, *C. tarsalis* and *P. confinnis* to

47% in *A. triseriatus*. Five percent of the *P. ferox* died as intermediates. *A. taeniorhynchus* (30%) and *P. confinnis* (35%) had the highest number that died as abnormal adults.

For all species tested, TH-6040 caused 96% larval mortality. *C. salinarius* had a 50% larval mortality and a 50% pupal mortality.

The average larval mortality for all species caused by Hercules-24108 treatment was 69%. Larval mortality of 100% was observed for *C. salinarius*, *C. tarsalis*, *Cu. inornata*, *A. triseriatus* and *P. ferox*. This compound caused 100% mortality to *A.*

solicitans pupae. Thirty percent of the *A. taeniorhynchus* larvae tested died as intermediates and 2% of the *A. aegypti* died as abnormal adults.

Tables 1-5 show the susceptibility of selected mosquito species to each compound. Comparison of the data determined for the 5 developmental inhibitors showed that all 12 selected mosquito species were more susceptible to TH-6040 (Table 4) than they were to the other compounds tested in this study.

The LC₅₀ was used to compare the

susceptibility of the various mosquito species to each developmental inhibitor.

A. sollicitans, *P. ferox*, *P. varipes*, *C. p. quinquefasciatus*, and *P. confinnis* were the most susceptible to Mon-0585 of the species tested, having LC₅₀ values of 0.014, 0.053, 0.051 and 0.070 ppm, respectively (Table 1). The LC₅₀'s of *An. quadrimaculatus* and *A. triseriatus* to Mon-0585 were 0.374 and 0.530 ppm, respectively.

A. sollicitans, *P. ferox*, *P. varipes* and *C. tarsalis* were the most susceptible to Altosid® of the species tested having LC₅₀

TABLE 3. Susceptibility of selected mosquito species to R-20458.

Species	PPM			Slope
	LC-50	LC-90	95% C.L. ^a	
<i>A. aegypti</i>	0.1117	0.5428	0.079-0.152	1.8672
<i>A. sollicitans</i>	0.0001	0.0019	0.0001-0.005	0.5877
<i>A. taeniorhynchus</i> ^b	0.0140	0.0600
<i>A. triseriatus</i>	0.0136	51.7581	0.002-0.054	0.3581
<i>An. quadrimaculatus</i>	0.0077	0.1385	0.004-0.014	1.0236
<i>C. p. quinquefasciatus</i>	0.0131	0.1141	0.007-0.021	1.3643
<i>C. salinarius</i>	0.0959	0.8753	0.058-0.156	1.3346
<i>C. tarsalis</i>	0.0041	0.1002	0.002-0.008	0.9289
<i>Cu. inornata</i>	0.0070	0.4866	0.003-0.013	0.6973
<i>P. confinnis</i>	0.1809	1.8010	0.0004-12260.7	1.2842
<i>P. ferox</i>	0.0055	0.1964	0.002-0.012	0.8288
<i>P. varipes</i>	0.0001	0.0017

^a Confidence Limits shown for LC₅₀ only.

^b Eye fitted line.

TABLE 4. Susceptibility of selected mosquito species to TH-6040.

Species	PPM			Slope
	LC-50	LC-90	95% C.L. ^a	
<i>A. aegypti</i>	0.000124	0.000706	0.000069-0.00019	1.7035
<i>A. sollicitans</i>	0.0000005	0.000036	0.0-0.000003	0.6906
<i>A. taeniorhynchus</i>	0.000003	0.000045	0.000002-0.00001	1.0707
<i>A. triseriatus</i>	0.000040	0.000718	0.00002-0.0001	1.0256
<i>An. quadrimaculatus</i>	0.000001	0.000086	0.0-0.000001	0.6744
<i>C. p. quinquefasciatus</i>	0.000075	0.000064	0.00005-0.0001	1.3767
<i>C. salinarius</i>	0.000011	0.000121	0.000004-0.00002	1.2368
<i>C. tarsalis</i>	0.000039	0.001049	0.00002-0.0001	0.8982
<i>Cu. inornata</i>	0.000078	0.001639	0.00003-0.0002	0.9712
<i>P. confinnis</i>	0.000083	0.003833	0.00003-0.0002	0.7703
<i>P. ferox</i> ^b	0.000007	0.000072
<i>P. varipes</i>	0.000001	0.000069	0.00000001-0.000005	0.7023

^a Confidence Limits shown for LC₅₀ only.

^b Eye fitted line.

TABLE 5. Susceptibility of selected mosquito species to Hercules-24108.

Species	PPM			Slope
	LC-50	LC-90	95% C.L. ^a	
<i>A. aegypti</i>	0.1890	0.4519	0.159-0.223	3.3853
<i>A. sollicitans</i> ^b	0.0004	0.0070
<i>A. taeniorhynchus</i> ^b	0.0008	0.0068
<i>A. triseriatus</i>	0.0950	0.6385	0.059-0.131	1.5491
<i>An. quadrimaculatus</i>	0.0294	0.2777	0.002-0.063	1.3149
<i>C. p. quinquefasciatus</i>	0.0107	0.2043	0.005-0.019	1.0031
<i>C. salinarius</i>	0.0042	0.0929	0.002-0.008	0.9546
<i>C. tarsalis</i>	0.0084	0.2148	0.004-0.015	0.9129
<i>Cu. inornata</i>	0.0143	0.1902	0.006-0.027	1.1415
<i>P. confinnis</i> ^b	0.0086	0.0620
<i>P. ferox</i>	0.1600	0.3500
<i>P. varipes</i> ^b	0.0006	0.0052

^a Confidence Limits shown for LC₅₀ only.

^b Eye fitted line.

values of 0.00001, 0.0001, 0.0002 and 0.0006 ppm, respectively (Table 2). *Aedes aegypti* required a concentration of 0.1532 ppm to cause a LC₅₀ mortality.

A. sollicitans and *P. varipes* were the most susceptible to R-20458 of the species tested, both having LC₅₀ values of 0.0001 ppm (Table 3). *P. confinnis* required a concentration of 0.1809 ppm to cause to a LC₅₀ level of mortality.

A. sollicitans had a LC₅₀ value of 0.000005 ppm for the TH-6040 compound while it was 0.000124 ppm for *A. aegypti* (Table 4).

P. varipes and *A. taeniorhynchus* were the most susceptible to Hercules-24108, having LC₅₀ values of 0.0006 and 0.0008 ppm, respectively (Table 5). *A. aegypti* required a 0.1890 ppm concentration of Hercules-24108 to cause a LC₅₀ level of mortality.

The toxicological response to the various compounds varied to such a degree among species within genera that no generalizations concerning the comparative susceptibility of genera could be made. These dosage-mortality data indicated that the susceptibility of each mosquito species to these compounds must be evaluated individually and with consideration of the type of compound used. Some species differences in susceptibility varied as much as 10,000 times between compounds. *A. sollicitans* was highly susceptible to all 5

of the compounds tested.

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