TOXICITY OF VARIOUS INSECTICIDES TO *CULICOIDES FURENS* LARVAE IN PUERTO RICO[1]

IRVING FOX, GISELA A. RIVERA AND CARMEN C. UMPIERRE
School of Medicine, School of Tropical Medicine, San Juan, Puerto Rico

Although the salt-marsh sand fly, *Culicoides furens* (Poey) has long been a pest in the general area of the International Airport, Isla Verde, Puerto Rico, control efforts have been desultory and undertaken mostly by hotel owners with the aid of commercial pest control operators. Nevertheless, insecticides have been used extensively there during government programs against malarial mosquitoes and *Aedes aegypti*, and *Culicoides* could be resistant to certain insecticides without specific control.

The present experiments were undertaken to determine (a) whether *Culicoides* larvae were resistant to some insecticides; and if so (b) whether other insecticides were more effective in laboratory tests even under conditions which might detoxify them.

MATERIALS AND METHODS. Topsoil samples were taken from the north shore of Torrecilla Lagoon, Boca de Cangrejos, Isla Verde, Puerto Rico. The larval habitat was a mangrove marsh located about one-fourth mile to the northeast of a light trap, which has been indicated on a published map (Light Trap No. 1, Yacht Club, in Fox and García-Moll, 1961). To obtain larvae we modified the technique of Bidlingmayer (1957). After dividing the sample into 250 ml. portions, we placed each portion in a 1-quart cardboard container, covered it with sand to about 2 inches above the soil, and added tap water to about one-half inch above the sand. The sample stood overnight. The next day we poured the water through an 80-mesh Tyler standard screen, removed the sand to a basin, stirred it in about one liter of tap water, and passed the wash water through the screen. Three more times we washed the sand and passed the wash water through the screen; then we washed the residue on the screen into a black tray where the whitish larvae were easily seen. Only larvae 4–5 mm long were used.

We knew no way to distinguish living *C. furens* larvae from other species which might be in the soil sample. It was assumed that all the larvae swimming in the characteristic *Culicoides* manner were *C. furens* for the following reasons: (1) The light trap near the site over a 5-year period (1961–1965) yielded 134,980 specimens of *Culicoides* of which more than 99 percent were *C. furens*; (2) many larvae were reared to adults and they were *C. furens*; and (3) the only species of *Culicoides* collected while biting nearby was *C. furens*.

Lindane, dieldrin, DDT, malathion, fenthion, and diazinon were World Health Organization standard solutions in ethanol. Abate, Bayer 39007 (Baygon), and naled were emulsifiable concentrates with the following percentages of active ingredients respectively, 43, 13.9, and 60. Endrin, chlordane, parathion, heptachlor, and coumaphos were acetone solutions of ESA Pesticide Reference Standards.

Two methods were used to make the toxicological experiments. The first method (Fig. 1) was as follows: Five larvae were exposed in 10 ml. of insecticide solution in a Syracuse Wheaton watch glass (67 mm outside diameter, 50 mm inside, and 10 mm deep, Arthur H. Thomas Co. Philadelphia, Pennsylvania) under three conditions. The first set-up involved simply insecticide solution in the watch glass, the second, insecticide solution plus about one-eighth teaspoon of mud from the habitat in the watch glass, and

[1] This investigation was supported by the Puerto Rico Department of Health, Graduate Research Training Grant 5 T1 AI-15 from the National Institute of Allergy and Infectious Diseases, and Research Grant GM 07152 from the National Institute of General Medical Sciences, U. S. Public Health Service.

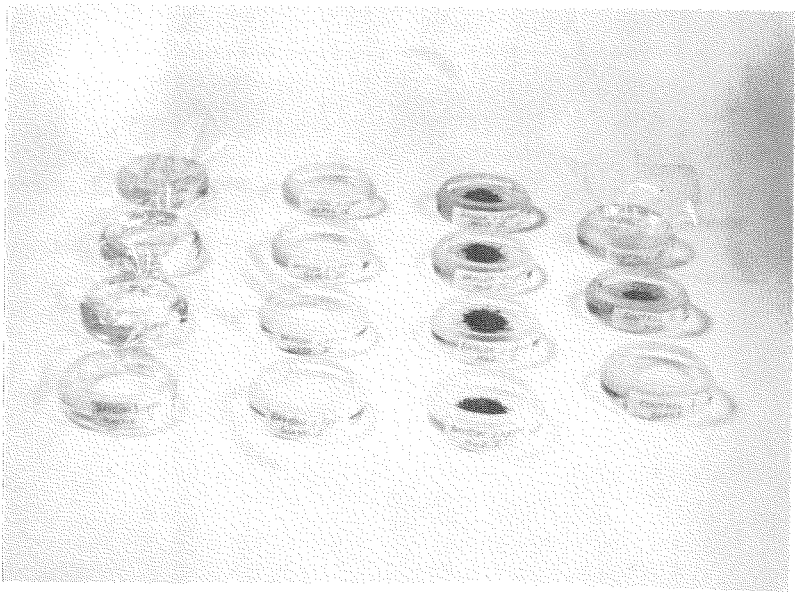

FIG. 1.—Method for testing insecticides against *Culicoides* larvae in plastic, glass, and glass plus mud.

the third, insecticide solution added after the watch glass was covered with a piece of polyethylene plastic (11 x 11 cm). Four replicates of each set-up plus appropriate controls were made at a time. The larvae were exposed for 24 hours when a reading was made.

After the 24-hour exposure period the chemical solution was decanted and replaced with tap water for a 24-hour recovery period. Results were recorded as 24- and 48-hour percent mortalities. A larva was designated "living" if it swam with the characteristic whiplash movement; "moribund" if it moved somewhat when poked but could not swim; and "dead" if it could not move. Often larvae which appeared moribund at 24 hours recovered at 48 hours sufficiently to be classified as living. The 48-hour percent mortality is shown in Table 1. After each use the watch glasses were washed with detergent and heated in an oven at more than 100° C. for 1 hour. The second method was that of Smith *et al.* (1959) and involved two replicates of 25 larvae each exposed in 100 ml. of insecticide solution in a beaker (250 ml.) for 24 hours, after which the reading was made.

RESULTS. Table 1 gives the concentration-mortality data for each insecticide but comparisons of one with another should be based on the formulation and source. In glass without mud the least toxic of the WHO insecticides—DDT, lindane, dieldrin, diazinon, malathion and fenthion—was DDT and the most effective was fenthion. Baygon did very poorly, and of the other two emulsifiable concentrates Abate was better than naled. The worst of the ESA standards (chlordane, coumaphos, endrin and parathion) was chlordane, but parathion was the best and both endrin and coumaphos killed 100 percent at 0.5 p.p.m.

Mud detoxified all the insecticides. DDT, which at 2.5 p.p.m. in glass killed 100 percent, lost practically all its potency when mud was added to the solution. But the most effective insecticides in glass alone (Abate, fenthion, and parathion) were detoxified the least by mud. Plastic also reduced greatly the activity of the insecticides, in some cases more so than mud, and

TABLE 1.—Percent mortality of *Culicoides* larvae after exposure to various insecticides in Syracuse watch glasses comparing effects of glass alone, glass plus mud, and plastic. (Dead plus moribund, average of 4 replicates).*

Insecticide	Concentration ppm	Glass	Glass plus mud	Plastic
DDT	0.1	5	0	0
	0.5	50	0**	0**
	2.5	100	5**	10**
Lindane	0.1	0	0	0
	0.5	55	20**	30
	2.5	100	85	100
Dieldrin	0.1	55	10**	0**
	0.5	85	30**	0**
	2.5	100	100	100
Diazinon	0.1	45	0**	5**
	0.5	80	20**	25**
	2.5	100	100	100
Malathion	0.1	20	0	15
	0.5	85	50**	55**
	2.5	100	100	100
Fenthion	0.1	95	20**	55**
	0.5	100	85	100
	2.5	100	100	100
Baygon	0.1	0	0	0
	0.5	0	5	10
	2.5	35	15	40
Naled	0.1	31	0**	5**
	0.5	90	5**	55**
	2.5	100	100	100
Abate	0.1	90	75	45**
	0.5	100	75**	55**
	2.5	100	100	100
Chlordane	0.1	0	0	0
	0.5	50	20**	40**
	2.5	95	75	47**
Heptachlor	0.1	75	40**	25**
	0.5	85	50**	30**
	2.5	100	80	85
Coumaphos	0.1	15	5	10
	0.5	100	20**	35**
	2.5	100	100	100
Endrin	0.1	75	25**	10**
	0.5	100	85	65**
	2.5	100	100	100
Parathion	0.1	100	65**	85
	0.5	100	100	100
	2.5	100	100	100
126 Controls Mortality, 0%				

* 24 hours exposure in distilled water solution followed by a 24 hour recovery period in tap water (48 hour percent mortality).

** Significantly different at the 5-percent level when compared with glass (Two-by-Two Tables test of Owen, 1962).

at 0.5 p.p.m. only fenthion and parathion resulted in 100 percent mortality.

DISCUSSION. Smith *et al.* (1959) found that DDT, lindane, and dieldrin at 0.1 p.p.m. killed Florida larvae 100 percent. On this basis the results in Table 1 (glass) indicate that the Puerto Rican larvae are resistant, but questions may arise because the techniques differed. To obtain comparable data we tried their method using solutions of ESA standard insecticides in beakers. The results in Table 2 show that

TABLE 2.—Percent mortality of *Culicoides* larvae after 24 hours exposure to ESA standard insecticide solutions in beakers. (Dead plus moribund, 2 tests).*

Insecticide	0.01 ppm	0.1 ppm	1.0 ppm
DDT	0	68	100
	4	48	88
Lindane	0	52	100
	0	60	96
Dieldrin	8	84	92
	16	92	96
5 Controls Mortality, 0%			

* The method of Smith *et al.* (1959).

to achieve the same mortality which Smith *et al.* obtained we had to use about 10 times as much dieldrin and lindane at the 0.01–0.1 p.p.m. level. Further, DDT at 0.1 p.p.m. was much less effective against Puerto Rican larvae than it was reported to be against Florida larvae.

The results from these experiments demonstrate that resistance to certain compounds may be present even without large scale application of insecticides against *Culicoides* larvae. Laboratory tests before beginning control programs are therefore necessary. The test with watch glasses is somewhat simpler than that with beakers because less larvae are required and counting after exposure is easier. Since plastic obviously affects insecticides, it would be unwise to use disposable plastic containers to avoid washing glassware. Why mud detoxified the insecticides is a matter for present speculation and future research. The results with plastic suggest that microorganisms and organic matter, if at all responsible, were not entirely the cause.

SUMMARY. In laboratory tests against

Culicoides furens larvae, fenthion, Abate, and parathion were the most effective of 14 insecticides. The larvae were resistant to DDT, dieldrin, and lindane. Mud and plastic reduced the toxicity of all the insecticides.

Acknowledgments. The technical assistance of Ileana G. Bayona, Francisco Romero-Alemañ and Wigberto Trujillo is gratefully acknowledged. Thanks are also due summer medical student trainees Alicia G. Feliberti, Luis A. Escabí, and Juan R. Rodríguez.

References

Bidlingmayer, W. L. 1957. Studies on *Culicoides furens* (Poey) at Vero Beach. Mosq. News 17:292–294.

Fox, I., and Garcia-Moll, I. 1961. The *Culicoides* of the International Airport, Isla Verde, Puerto Rico as shown by light traps. Mosq. News 21:120–132.

Owen, D. B. 1962. Handbook of statistical tables. Addison-Wesley Publishing Company, Inc., Reading, Massachusetts. 580 pp.

Smith, C. N., Davis, A. N., Weidhaas, D. E., and Seabrook, E. L. 1959. Insecticide resistance in the salt-marsh sand fly *Culicoides furens*. J. Econ. Ent. 52:352–353.