Insecticide Resistance in Mosquitofish from Texas

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INTRODUCTION

The mosquitofish, Gambusia affinis (Baird and Girard) is a widespread topfeeding minnow that is sometimes used as a noninsecticide control agent for mosquitoes. The first case of insecticide resistance in feral vertebrates was the 1963 report of VINSON et al. (1963) of DDT resistance in a mosquito-fish population collected from the cotton producing Delta area of northwest Mississippi. Since that time, insecticide resistance in mosquitofish populations from that area has been studied extensively by FERGUSON and co-workers, e.g. FABACHER and FERGUSON (1970), FERGUSON (1970), FERGUSON and BINGHAM (1966), FERGUSON et al. (1967), FINLEY et al. (1970). They have found that resistance levels in the Mississippi populations are greatest to cyclodiene insecticides such as endrin, aldrin, dieldrin and toxaphene but less to DDT and organophosphates.

METHODS

Mosquitofish were collected from three sites near College Station, Texas. The sites varied in type of human activity taking place in adjacent areas and consequently, in the exposure pattern of each to insecticide contamination.

One site, hereafter designated "Old River", is in an extensive cotton growing area on the Brazos River flood plain, 14 miles southwest of College Station. The site is a semipermanent pond in an intermittent creek which is part of a drainage complex for several thousand acres of cotton. For many years insects in the adjacent area were controlled primarily with DDT and toxaphene. At present, toxaphene:methyl parathion combinations are most heavily used. Dosage rates are massive, frequently exceeding 20 pounds of insecticide per acre per year.

The second site, Bee Creek, is an intermittent stream draining a residential area of the town of College Station. The stream flows briefly after rainstorms. At other times, residual ponds are maintained by surface runoff from

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lawn irrigation in adjacent residential areas. Heaviest insecticide use is directed towards control of the chinch bug *Blissus leucopterus* (Say.). In the past, cyclodiene insecticides were heavily used for this purpose. Currently, organophosphates are most widely applied.

The third site is on the Navasota River, 15 miles east of College Station. The Navasota is a small stream draining mixed woodland-rangeland area and is not subject to extensive insecticide contamination.

Mosquitofish were seined from the three sites and held in aged tap-water in aquaria in the laboratory for several days before testing. For insecticide susceptibility tests, groups of 5 fish were placed in 250 ml water in pint glass jars. Appropriate amounts of insecticide in 0.5 ml acetone were added to each jar. Acetone only was added to controls.

Mortality was recorded 24 and 48 hours after exposure began. Death of the test fish was considered to be the absence of opercular movement. Individual assays were replicated 2 to 4 times, and LC_{50} and LC_{90} values were obtained by computer analysis of the test results at the Texas A&M Data Processing Center. Over 1,000 fish were tested.

FERGUSON (1968) suggested that resistant organisms possess an increased ability to store insecticides in their organ systems. Residue analysis of mosquitofish from the three sites supports his statement. For this part of the study, mosquitofish were collected from the three localities and samples were extracted according to a modified fat extraction method described in the FDA pesticide analytical manual #1 (1968).

The GLC, a Barber Coleman model 5360 Pesticide Analyzer, was equipped with an electron capture detector using tritium as the ionization source. The six foot long 1/4" I.D. coiled glass column was packed with 4% SE-30, 6% QF-1, on Anakrom ABS, 90/100 mesh. The operating temperatures were: injector - 230°C, column bath - 205°C, and detector heat - 215°C. Flow rate was 54 cm/min.; prepurified nitrogen was the carrier gas. The machine was set so that 0.4 ng of aldrin gave 1/2 F.S.D..

RESULTS AND DISCUSSION

Table 1 shows the toxicity (based on 48 hour exposures) of test insecticides to mosquitofish populations from the 3 sites.

The Navasota River population was consistently killed by the lowest concentrations of all toxicants and was arbitrarily designated as susceptible. However, in comparison with Mississippi susceptible populations reported earlier (CULLEY and FERGUSON, (1958)), the LC_{50} concentrations were 2 to 4 times as large, a finding which suggests that a gradual decrease in susceptibility to insecticides is widespread within the species.

TABLE 1

48 Hour LC_{50} and LC_{90} Values of Three Populations of Mosquitofish with Levels of Resistance when Compared to a Susceptible Population

SITE	INSECTICIDE	LC_{50} (ppb)	LC_{90} (ppb)	RESISTANCE LEVELS vs NAVASOTA SUSCEPTIBLES LC_{50}	LC_{90}
Navasota	Aldrin	145	237		
	Toxaphene	31	63		
	DDT	43	77		
Bee Creek	Aldrin	296	463	2.0x	2.0x
	Toxaphene	212	425	6.8x	6.8x
	DDT	313	555	7.3x	7.2x
Old River	Aldrin	642	1445	4.4x	6.1x
	Toxaphene	301	612	9.7x	9.7x
	DDT	528	1282	12.3x	16.6x

The Old River population was most resistant to all test insecticides. However, the pattern of resistance exhibited in the Texas populations differed from that found in the Mississippi populations. Compared with Mississippi populations, the Old River population proved more resistant to DDT (12-fold vs. 4-fold) and less resistant to toxaphene and aldrin (10-fold and 4-fold vs. 376-fold and 71-fold, respectively).

The Bee Creek population showed a lower level of resistance than the Old River strain to all test insecticides (2- to 7-fold), but the pattern of resistance proved to be similar to both strains; i.e. greatest with DDT and least with cyclodienes. It is of interest that Bee Creek is apparently free of agricultural pollution. The stream rises on the Texas A&M University campus and, after flowing through a residential area, drains eventually into the Navasota River. Thus, we consider it highly unlikely that a population of *Gambusia* selected by exposure to agricultural use of pesticides could be present in the stream. Rather, the population must be native and must have been selected with insecticides *in situ*.

Table 2 shows that the highly DDT-resistant mosquitofish from Old River contained the greatest amounts of DDT and its metabolites. Total residues in the Old River population averaged greater than 50 ppm. on a whole body basis. The urban (Bee Creek) population of mosquitofish, which exhibited lower levels of resistance to insecticides, contained low levels of p,p'DDE only. The susceptible population of mosquitofish showed only minute residues of p,p'DDE.

TABLE 2

Residue Levels (ppm) of p,p'DDT and its Metabolites in Three Populations of Mosquitofish

SITE	p,p'DDE	p,p'DDD	p,p'DDT
Navasota	<0.1	N.D.	N.D.
Bee Creek	<1.0	N.D.	N.D.
Old River			
Low	13.6	5.3	2.4
High	55.9	26.8	24.1
Mean	24.7	16.5	14.8

N.D. = Not detected in the sample

Although resistance to toxaphene and aldrin was evident, GLC analysis revealed no residues of either compound in the samples analyzed. A possible explanation as to why toxaphene was not detected in the samples analyzed is that no rainfall of any consequence occurred during the sampling period. Thus, toxaphene may not have been introduced into the sample site at this time, for runoff of pesticides into aquatic eco-systems is probably the major means of contamination in this area. Therefore, we may have sampled a population of mosquitofish whose ancestors were exposed to toxaphene; the ancestors passing on the capacity for resistance but not the contamination.

Resistance to aldrin probably came about as a result of cross-resistance from toxaphene selection. FERGUSON (1968) reported high cross-resistance to all cyclodiene insecticides in toxaphene-endrin selected populations in Mississippi.

The results suggest that resistant populations of mosquitofish are more common than previously suspected, especially with the discovery of resistance as a result of urban contamination. Resistance to insecticides in non-target organisms will continue to occur, at least as long as persistent pesticides are used. Those organisms that can adapt will continue to live in highly contaminated areas, and those that cannot will in all probability be eliminated.

SUMMARY

The present report from Texas confirms the Mississippi findings of insecticide resistance in *Gambusia*. In addition, we report evidence that urban, as well as agricultural, uses of insecticide may result in resistant *Gambusia* populations. Further, we observed a resistance pattern that is distinctly different from that found in Mississippi.

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