

The Effects of Combinations of Insecticides on Susceptible and Resistant Mosquito Fish¹

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Although the effects of two pesticides acting simultaneously upon non-target organisms has received little attention in laboratory investigations, animals living near certain types of croplands may be exposed to two or more insecticides regularly. In cotton-producing areas of the Mississippi Delta, most fields receive 10-15 insecticide applications a season, usually between June 1 and September 10. The most commonly used insecticides are endrin, DDT, toxaphene, and methyl parathion, applied either individually, in some pattern of rotation, and/or mixed in a variety of combinations. Adjacent fields may be on different spraying schedules and treated with different insecticides or insecticide combinations. Most insecticides are applied with airplanes, especially after midsummer when the cotton plants get tall. Where drift and runoff from such operations reach nearby bodies of water, mosquito fish (Gambusia affinis) and several other species of fish may exhibit high levels of resistance to a variety of pesticides (1, 2, 3, 4, 5).

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The present account compares the responses of a highly resistant population of mosquito fish from a heavily contaminated site and a susceptible population from an uncontaminated locality to simultaneous exposures to all possible paired combinations of endrin, DDT, toxaphene, and methyl parathion.

Materials and Methods

Resistant mosquito fish were obtained from a ditch that drains and bisects several large cotton fields near Belzoni, Mississippi. Susceptible fish were collected from a pond on non-agricultural land near State College, Mississippi. All fish were collected with a fine-meshed seine and held overnight in the laboratory prior to testing.

Technical grade samples of the four insecticides, provided by the manufacturers, were prepared as 1% solutions in acetone and diluted to desired test concentration with tapwater (pH 7.8, hardness 28 ppm). In the initial dilution, additional acetone was employed to facilitate dissolution of the insecticides, but the amount was regulated so as not to exceed 2 ml per liter of final test solution.

In one series of tests, samples of 50 mosquito fish were exposed for 36 hours in 20 liters of insecticide solution in 15-gallon aquaria. Four aquaria were required to test fish from one population against a pair of insecticides, e.g., an aquarium containing insecticide solution A, an aquarium containing

insecticide solution B, an aquarium containing a mixture of A and B at the same concentrations placed in the separate aquaria, and an aquarium of tapwater for controls. In this manner, samples from both susceptible and resistant populations were tested against endrin-DDT, endrin-toxaphene, endrin-methyl parathion, DDT-toxaphene, DDT-methyl parathion, and toxaphene-methyl parathion. Because of differences in tolerance of the two populations, it was necessary to use higher insecticide concentrations in tests of resistant fish, especially for endrin and toxaphene (Figs. 1 & 2). Mortality was recorded at 15- or 30-minute intervals early in the tests, followed by hourly observations or 6 hour checks, depending upon the progress of the test.

All tests were run at temperatures of $72 \pm 4^{\circ}\text{F}$, and fish were not fed.

Results and Discussion

In the aquarium tests, control mortality never exceeded one fish out of 50. In the treatments, the much higher concentrations used for the resistant population resulted in less mortality than was produced by lower concentrations used in tests of susceptible fish (Figs. 1 & 2). Whereas the combination of two insecticides produced higher mortality among resistant fish than did the individual insecticides, the combination scarcely exceeded the individual kills of toxaphene and endrin in the tests of susceptible fish. The relative positions of the

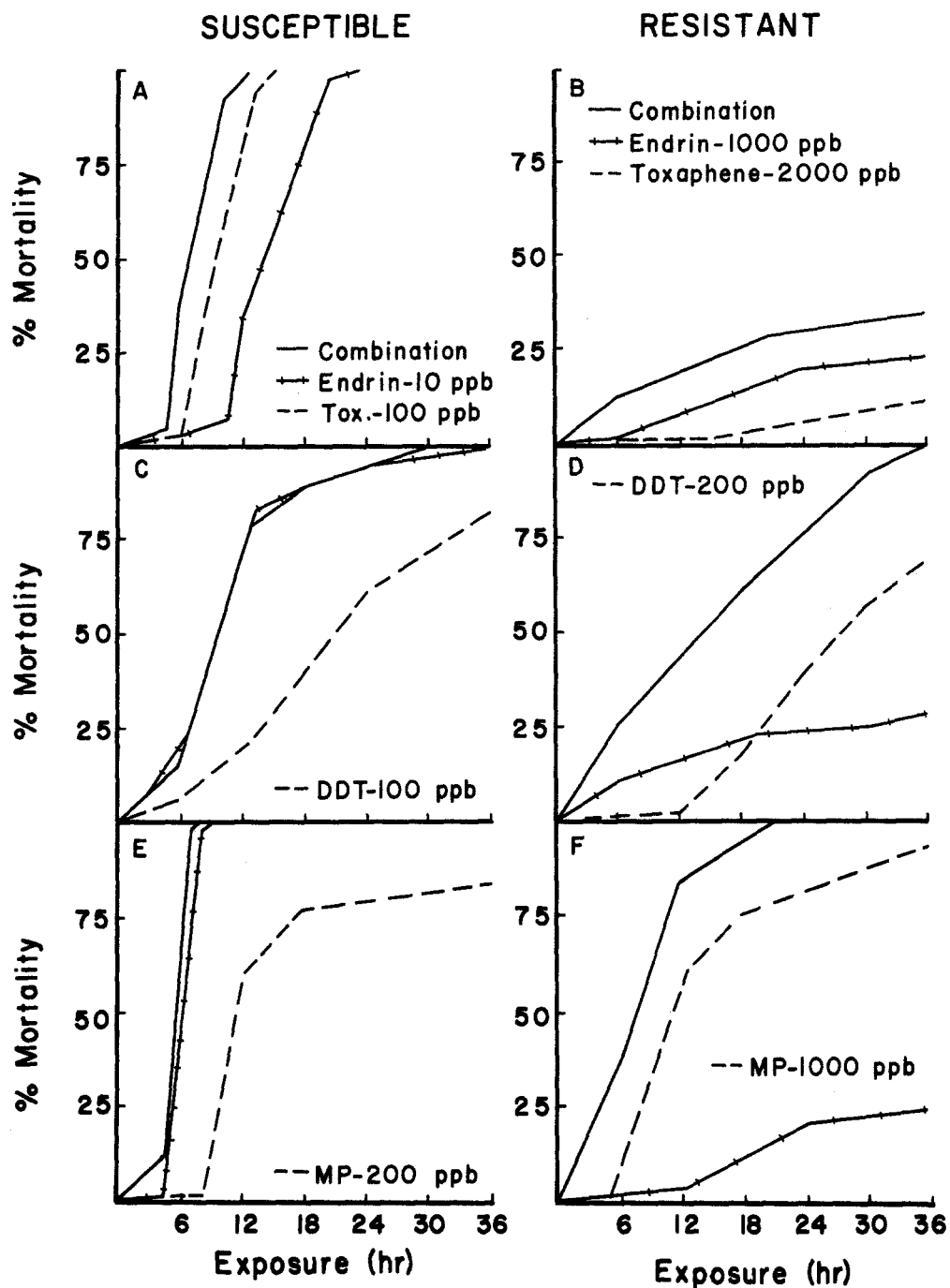


Figure 1. Mortality produced in samples of 50 mosquito fish from resistant and susceptible populations by two insecticides separately and in combination.

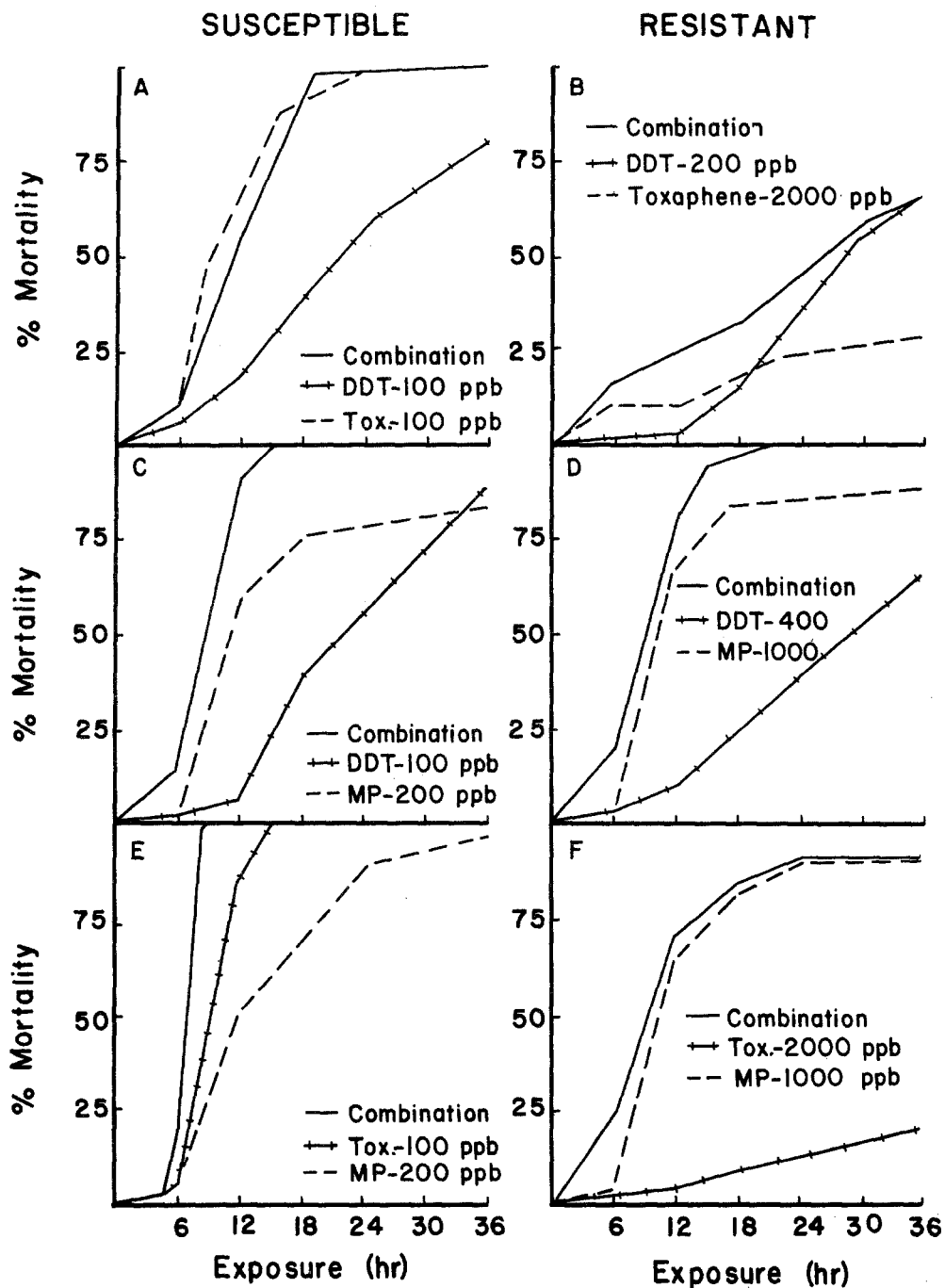


Figure 2. Mortality produced in samples of 50 mosquito fish from resistant and susceptible populations by two insecticides separately and in combination.

mortality curves for the individual insecticides (compared with the combination curve) are reversed in equivalent tests of susceptible and resistant populations, except in the DDT-methyl parathion tests (Fig. 2, C & D). In general, the results reflect the extreme levels of endrin and toxaphene resistance in the resistant population--i.e., endrin and toxaphene are relatively more toxic to the susceptible fish; DDT and methyl parathion are relatively more toxic to the resistant fish. The mortality curve for methyl parathion shows a consistent deflection after 12 hours, probably indicating rapid hydrolysis of the compound. The results failed to indicate additive effects wherein the combination mortality exceeded the sum of the mortalities produced by the individual insecticides.

The results of the jar tests agree with the findings of the tests run in aquaria, i.e., the combination of two insecticides produced higher mortality than did either insecticide alone. In all tests, the sum of the mortalities caused by individual insecticides exceeded that for the same insecticides in combination, hence, there were no additive effects. In tests of resistant fish involving the endrin-toxaphene, endrin-methyl parathion, the toxaphene-methyl parathion combinations, the mixture produced levels of mortality very close to the total mortality produced by the compounds separately. In general, higher mortality from mixtures probably indicates differences in the modes of action of the toxicants involved.

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