SELECTION FOR RESISTANCE TO MALATHION IN *ANOPHELES STEPHENSI MYSORENSIS*

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ABSTRACT. During June–July, 1974, at Kushkak Village, Mamasani, Kazeroun, it was observed that 3.2% malathion, the concentration that killed 100% of adult *An. stephensi mysorensis* at the beginning of malathion spraying in southern Iran, killed only about 94% of mosquitoes tested at 1 hr exposure and 24 hr recovery. Specimens that were alive after 1 hr exposure were reared in the laboratory, and their offspring were exposed for 60 minutes to 3.2% malathion-impregnated paper using WHO methods. Each subsequent generation was exposed 15 minutes longer than were their parents. It was observed that after 5 generations the mortality rate decreased to 46%. Parallel to this study, changes in in LT50's were investigated. Wild caught *An. stephensi mysorensis* had an LT50 of about 14 minutes. After 5 generations it increased about 5-fold more than that of the F_1.

During the past two decades, southern Iran has been subjected to insecticide application inside houses for malaria control and, to some extent, for agricultural pest control. The area has been under malathion and DDT spraying twice a year since 1967. In this area, *Anopheles stephensi mysorensis* is resistant to DDT and dieldrin and susceptible to malathion (Manouchehri et al., 1974).

During June-July, 1974, at Kushkak village, Mamasani, Kazeroun, it was observed that 3.2% malathion, the concentration that killed 100% of adult *A. stephensi mysorensis* at the beginning of malathion spraying in southern Iran, failed to kill 100% of the mosquitoes tested; the mortality was about 94% of mosquitoes tested.

Because our malaria eradication program in southern Iran relies on malathion spraying for the control of *A. stephensi mysorensis*, it was suggested that a study be undertaken to determine the ability of *A. stephensi* to develop resistance to this insecticide. The only anopheline that has developed resistance to organo-phosphorous compounds at the present time is *A. albimanus* in South America (Georghiou, 1972; Georghiou et al., 1972).

MATERIAL AND METHODS. In Kushkak village, Mamasani, Kazeroun, southern Iran, it was observed after malathion spraying in June, 1974, that the density of *A. stephensi* was about 600/room. Most of the mosquitoes were found resting on hanging cloths. Freshly-fed mosquitoes were collected early in the morning, and susceptibility tests were performed by the method developed by the World Health Organization (1970). As mentioned by Manouchehri et al., 1974, houses in the area have been treated with malathion twice a year since 1967 at the rate of 2 g/m^2. Organo-phosphorous compounds have also been used for the control of agricultural pests on cotton plantations and sugar beet fields. This includes the heavy application of several insecticides such as parathion, Sumithion, methyl parathion, Trichlorphon and others at 2-week intervals for at least 5 months (June–October), which is coincident with the seasonal activity of *A. stephensi* in this area.

The mosquitoes were tested against 3.2% malathion and the Lt50's were estimated by plotting the dosage mortality lines. These mosquitoes were considered as the F_1 generation in these experiments.

Specimens that were alive after 1 hr exposure to 3.2% malathion and 24 hr recovery were reared at the Kazeroun Medical Research Station. Avoiding any contamination of the area with the resistant strain, about 500 adults were transferred to our laboratory in Teheran, which is out of the range of distribution of *A. stephensi*. This strain was put under treatment by exposing freshly-fed mosquitoes to malathion-impregnated paper, rearing the survivors, and then exposing the offspring to 3.2% malathion. The mosquitoes were first exposed to malathion-impregnated paper for 60 minutes, and each subsequent generation was exposed 15 minutes longer than were their parents. The Lt50 for each generation was estimated by plotting the dosage mortality lines. The larvae were fed with Gerber (Merck Company, U.S.A.) and the adults were fed on a guinea pig. During this study the insectary temperature was kept at 26±1° C and the relative humidity at 80–85%.

RESULTS AND DISCUSSION. Previous studies in southern Iran, including the Kazeroun area, showed that the discriminating concentration that killed 100% of freshly-fed females of *A. stephensi mysorensis* was 3.2% malathion (Manouchehri et al. 1974). In July, 1974, at Kushkak village, Mamasani, Kazeroun, where organo-phosphorous insecticides are being used almost every 2 weeks for agricultural pest control, it was observed that this concentration failed to kill 100% of mosquitoes tested; the mortality was 94%.

The offspring of survivors of 1 hr exposure to 3.2% malathion were reared and tested within 24 hr after their emergence using the same concentration and exposure period as in the parent test. After 5 generations and 5 exposures to the same concentration and with the same exposure period, the mortality rate decreased to 46%.

Parallel to this study, changes in Lt50's were investigated. As Table 1 shows, after the exposure of wild caught *A. stephensi mysorensis* to 3.2% malathion with exposure times of 15, 30, 45 and 60 minutes, the LT50 was about 14 minutes. After 5 generations in our laboratory, the tolerance of *A. stephensi mysorensis* increased about 5-fold more than that of F_1. Our conclusion is that resistant individuals appear to be present in the field population, and *A. stephensi* is capable of developing resistance to malathion.

If this species were to develop resistance to malathion as it did in the laboratory, there is the possibility that our malaria program would suffer another setback as it did when *A. stephensi* became resistant to chlorinated insecticides in 1957. To prevent such a setback, it is suggested that new insecticides as well as other methods of controlling the *A. stephensi* population should be considered, in order to have an effective measure or measures at hand at the proper time.

A similar study was carried out on *A.*

Table 1. Results of selection for resistance to malathion in *Anopheles stephensi mysorensis* by exposing freshly-fed mosquitoes to 3.2% concentration.

	% Mortality after following minutes exposure, 24 hrs recovery								
Generation	Control	15	30	45	60	75	90	105	Lt50
Wild caught freshly-fed mosquitoes F_1	2 (99)	54.5 (132)	64.2 (109)	76.4 (102)	94 (100)	..	..	..	14
F_2	2 (98)	25.5 (94)	54.3 (81)	62 (79)	95.1 (83)	..	..	..	27
F_3	2.3 (42)	2.7 (36)	17.5 (40)	27.7 (36)	43.5 (39)	78.5 (42)	..	..	52
F_4	1 (95)	..	10 (100)	21.9 (105)	55.1 (107)	56.3 (119)	86.2 (102)	..	58
F_5	1.1 (87)	..	..	21.2 (80)	46.1 (91)	60.2 (98)	79 (86)	79.7 (133)	64

The figures in parentheses represent the number of mosquitoes tested.

albimanus larvae by Ariaratnam and Georghiou in 1971. They showed a 2.9-fold increase in LC50 in F_3. After 8 months, in February, 1971, development of resistance to carbamate and organophosphorous compounds in the field population of *A. albimanus* was observed (Georghiou et al., 1972). A genetic study as well as a study of cross-resistance to carbamate and other organo-phosphorous compounds is underway, and the results will be presented in a separate paper.

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XV INTERNATIONAL CONGRESS OF ENTOMOLOGY

Washington, D. C.
August 19–27, 1976

The second and final announcement of the Congress was distributed in late spring. Copies may be obtained from the Secretary-General, Box 151, College Park, Md. 20740. The Congress is sponsored by the Entomological Society of America and the National Academy of Sciences. Dr. Donald E. Weidhaas is in charge of the program for the section on Medical and Veterinary Entomology.