# MALATHION TOLERANCE VS. RESISTANCE IN *CULEX QUINQUEFASCIATUS*

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ABSTRACT. Some reports of insecticide resistance in *Culex quinquefasciatus* have resulted from the misinterpretation of WHO insecticide susceptibility test data due to attempts to draw conclusions based on an insufficient number of collections and to a misunderstanding of the significance of laboratory test results. Because of the wide variability in insecticidal susceptibility of different collections of this species, and the need to be able to anticipate and quickly recognize resistance when it occurs, a correlated laboratory-field evaluation model is proposed. The results of such paired tests from a two-county area demonstrated that two of the most malathion-tolerant strains were not malathion-resistant under field conditions.

From time to time, during recent years in particular, we have heard undocumented reports of *Cx. quinquefasciatus* resistance to one insecticide or another in Texas and elsewhere. Moreover, it is clear from the data presented in some of these unpublished reports that the issue is usually one of insecticidal tolerance and not resistance. This kind of misinterpretation is not only picked up by, if not originally announced to, the news media, but even worse, may be extrapolated to an entire state or section of the country. The dissemination of such information places mosquito control districts in the unfortunate position of seeming incapable and inadequate to deal with problems they created. This is an issue of particular concern since *Cx. quinquefasciatus* is a major, urban, encephalitis vector.

Mistakes of some investigators in the interpretation of WHO insecticide susceptibility test data have centered around attempts to draw conclusions based on data from an insufficient number of collections, and misinterpretations of the significance of the test results. In the first instance, it has been established that there is considerable variability in the insecticidal susceptibility of specimens of *Cx. quinquefasciatus* collected from different areas (Palmisano et al. 1976, Micks and Rougeau 1977). Thus data from an isolated collection or two can be very misleading. In the second instance, an error which is still being made by some workers is that of inferring resistance directly from the results of WHO insecticide susceptibility tests on a given collection of specimens. It should be emphasized that this test was never designed to evaluate the effectiveness of insecticides in the field. Only field tests, preferably correlated with those in the laboratory, can determine field efficacy of an insecticide. Accordingly, we took the position a number of years ago that whereas the results of WHO tests may certainly indicate increased tolerance to a given insecticide, and thus serve as an early warning signal, resistance on the other hand must be substantiated by significant evidence of control failures.

The present study reports results of a series of correlated laboratory-field tests as an evaluation model for differentiating between insecticide-tolerance and true resistance.

## MATERIALS AND METHODS

LABORATORY EVALUATIONS. During 1978 and 1979, dozens of collections of *Cx. quinquefasciatus* larvae, usually third and fourth instar, were obtained from Galveston and Brazoria Counties and brought into the laboratory for insectici-

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dal susceptibility testing using the WHO larval and adult test kits. However, since the focus of this report is adult mosquitoes, insecticidal susceptibility data on larvae are not included here. Suffice it to say that larval susceptibility patterns corresponded with those of adults. All adult tests were conducted with specimens of the $F_1$ generation reared from field-collected material to insure maximal uniformity of specimens. Each test utilized from 3 to 5 replicates with 25 adult females per tube. Only malathion test papers (0.5, 3.2 and 5.0%) were used, and all data are based on standard 1-hr exposures.

The test methods described above were also used with the UTMB strain of *Cx. quinquefasciatus* which has been reared continuously in this laboratory for more than 20 years and is used by many investigators as a standard insecticidally-susceptible strain. A number of tests of this strain were set up in parallel with field-collected strains from time to time, using both larvae and adults. Such comparisons were most useful in determining levels of insecticidal susceptibility.

FIELD EVALUATIONS. All field tests were conducted with adults reared from the highly malathion-tolerant collections from Galveston and Brazoria Counties, the rationale being that mortality in such adults under normal operating conditions would provide good evidence of control failures (resistance) if present. The adult females were tested in cylindrical screen-wire cages with 20–25 specimens/cage staked approximately 5 ft. above ground level, 20 ft. apart (Galveston Co.) or 50 ft. apart (Brazoria Co.), perpendicular to the path of the fogging units. A LECO ULV Fog Generator Model HD was used for all tests in Galveston County and a London Aire ULV Aerosol Generator for those in Brazoria County. The truck-mounted units dispersed 91% malathion at a rate of 3 fl. oz/acre, the regularly used dosage. The wind direction was approximately that of the line of stakes. At vehicle speeds of 5 mph, the test runs were up to ⅓ mile long. Approximately 30 min. after exposure, the cages were removed and returned to the headquarters building where they were maintained at 76°F. A mortality count was made 24 hr later. Specimens in control cages placed upwind of the treated area were similarly exposed and handled. Cotton pads moistened with sugar-water were kept on the cages as a food source during holding periods.

## RESULTS AND DISCUSSION

A comparison of the malathion susceptibility of several collections of *Cx. quinquefasciatus* from Brazoria County tested as adults, and the susceptible UTMB strain are shown in Table 1. A wide range of tolerance is demonstrated with the Brazoria specimens being almost as susceptible as the UTMB strain, and the Angleton material being highly tolerant. The Danbury strain was used in subsequent field evaluations since it was the most malathion-tolerant strain available during the period when field tests were conducted. Whereas it might be tempting to conclude that these 1978–79 findings indicate that this species is becoming progressively resistant, data (Table 2) from a lengthy series of Galveston County collections emphasize the lack of such a trend and convey the fallacy inherent in attempts to extrapolate findings from one or two populations. Interestingly, the two collections from the Q-11 sector in 1978 and 1979 are remarkably similar and yet very different from the H-20 strain in terms of response to malathion. The lat-

Table 1. Malathion susceptibility of adult *Culex quinquefasciatus* from Brazoria County.

| | | % Mortality | | | |
|---|---|---|---|---|---|
| | | Malathion Test Papers | | | Control |
| Locality | Year | 0.5% | 3.2% | 5.0% | Papers |
| UTMB | 1978 | 6 | 100 | 100 | 0 |
| Brazoria | 1978 | 20 | 86 | 98 | 0 |
| Danbury | 1979 | 6 | 30 | 45 | 2 |
| Angleton | 1979 | 5 | 11 | 10 | 5 |

Table 2. Malathion susceptibility of adult *Culex quinquefasciatus* from Galveston County.

| Locality | Year | % Mortality: Malathion Test Papers 0.5% | 3.2% | 5.0% | Control Papers |
|---|---|---|---|---|---|
| UTMB | 1978 | 6 | 100 | 100 | 0 |
| Q-11 | 1978 | 13 | 87 | 95 | 12 |
| J-17 | 1978 | 7 | 63 | 79 | 3 |
| S-19 | 1978 | 3 | 57 | 68 | 0 |
| O-19 | 1978 | 3 | 41 | 57 | 0 |
| H-20 | 1978 | 3 | 21 | 36 | 3 |
| O-8 | 1979 | 1 | 37 | 52 | 0 |
| Q-11 | 1979 | 2 | 81 | 85 | 2 |

ter strain was selected for use in field experiments since it demonstrated the highest tolerance to malathion.

Three field tests were conducted with the Danbury strain of *Cx. quinquefasciatus* using a London Aire sprayer during a higher than desired wind speed averaging 9 mph (Table 3). Cage mortality following the first run ranged from 0–90. Twenty-four hour mortalities for the 2nd field test were higher, reaching 100% in the 1st 2 cages. Still better results were obtained with the 3rd evaluation with good control in cages no. 2 through 6.

The other field evaluations were done in Galveston County with the H-20 strain, which so far has exhibited the highest malathion tolerance of any Galveston County collections of this species. The windspeed averaged 3.5 mph, and a LECO sprayer was used. Twenty-four hour mortalities following the first run were 100% in all but 3 cages (Table 4). In the 2nd test, complete kills were obtained with all cages.

These results verify and extend our previous findings (Micks and Rougeau 1977) that collections of *Cx. quinquefasciatus* from different areas even in close proximity to one another exhibit a wide range of responses to malathion. More importantly, they demonstrate that some of the most tolerant strains are still not resistant to regular field applications of the insecticide. As a matter of fact, the control actually obtained is probably of a higher order than indicated by some of the field test results since these tests were conducted with uniformly young (3–5 days of age), vigorous specimens. Regular field populations are known to consist of individuals which are variable in age, nutritional status, etc., and higher mortalities would be expected.

Our findings indicate the pitfalls inherent in insufficient population sampling in determining insecticidal susceptibility, or in routinely associating long term use of a particular insecticide with resistance. Galveston and Brazoria County Mosquito Control Districts have been using ULV

Table 3. Susceptibility of malathion-tolerant *Culex quinquefasciatus* (Danbury) from Brazoria County in field tests with ULV malathion.

| Cage No. | Test No. 1 % Mortality | Test No. 2 % Mortality | Test No. 3 % Mortality |
|---|---|---|---|
| 1 | 0 | 100 | 43 |
| 2 | 83 | 100 | 100 |
| 3 | 80 | 70 | 100 |
| 4 | 90 | 54 | 90 |
| 5 | 57 | 70 | 76 |
| 6 | 56 | 70 | 80 |
| 7 | 18 | 38 | 45 |
| 8 | 55 | 50 | 54 |
| 9 | 60 | 18 | 53 |
| 10 | 38 | 46 | 64 |
| Controls | 0 | 8 | 7 |

Table 4. Susceptibility of malathion-tolerant *Culex quinquefasciastus* (H-20) from Galveston County in field tests with ULV malathion.

| Cage No. | Test No. 1 % Mortality | Test No. 2 % Mortality |
|---|---|---|
| 1 | 100 | 100 |
| 2 | 100 | 100 |
| 3 | 100 | 100 |
| 4 | 100 | 100 |
| 5 | 95 | 100 |
| 6 | 100 | 100 |
| 7 | 100 | 100 |
| 8 | 100 | 100 |
| 9 | 65 | 100 |
| 10 | 60 | 100 |
| Controls | 0 | 0 |

malathion continuously as an adulticide for 17 years without control failures. Surprisingly, the *Cx. pipiens* complex population in Memphis, Tennessee, was reported to be resistant to malathion after only 8 seasons of use (Moseley et al. 1977). Susceptibility monitoring continues as an integral part of the control programs of both Texas counties.

We hope that this correlated laboratory-field approach to testing mosquito populations will serve as a practical resistance evaluation model for other control programs too, and that pronouncements concerning the presence of insecticidal resistance will be based on data which documents actual control failures.

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# STUDIES OF MALE OFFSPRING FROM OVERWINTERING *CULEX PIPIENS* COMPLEX MOSQUITOES

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ABSTRACT. Female overwintering *Culex pipiens* complex mosquitoes were collected in February and March, 1979, in Memphis, Tennessee. Following shipment to Fort Collins, Colorado, and acclimation to laboratory conditions, the DV/D ratio of male progeny of these mosquitoes were determined. Based on the interpretation of DV/D ratios, approximately 50% were intermediate forms, 40% *p. pipiens*, and 10% *p. quinquefasciatus*. The data indicate that in the Memphis area, a small proportion of the overwintering population will be *quinquefasciatus*-like, even though *quinquefasciatus* has not been shown to hibernate.

## INTRODUCTION

Recent renewed interest in the *Culex pipiens* complex has led to the listing of *Cx. pipiens* Linnaeus and *Cx. quinquefasciatus* Say as distinct species (Knight 1978). This revision was based on studies of the complex in Southeast Asia (Sirivanakarn 1976), Australia (Miles 1976), and South Africa (Jupp 1978). Within the U.S.A., the status of the complex remains perplexing, and the only known reliable method of distinguishing *pipiens pipiens* and *pipiens quinquefasciatus* adults is by the DV/D ratio of the male genitalia (Sundararaman 1949). An intergradation zone was outlined where both *pipiens* and *quinquefasciatus* occur and where intermediates and various mixtures of the three are likely to be found (Barr 1957).