

APPARENT INFLUENCE OF THE STAGE OF BLOOD MEAL DIGESTION ON THE EFFICACY OF GROUND APPLIED ULV AEROSOLS FOR THE CONTROL OF URBAN *CULEX* MOSQUITOES.

II. LABORATORY EVIDENCE

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ABSTRACT. The susceptibility of adult *Culex pipiens s.l.*, *Culiseta melanura* and *Aedes aegypti* to insecticide aerosols in wind tunnel exposures varied with time, depending on the stage of blood meal digestion. Greater than 2-fold differences were observed in the concentrations of malathion and synergized resmethrin required to kill test mosquitoes, depending on whether they had been given a blood meal and, if they had, the length of time following the blood meal. The period of lowest susceptibility varied from 24 h after feeding in *Ae. aegypti* to 72 h in *Cs. melanura*. The greatest variability occurred during the period when undigested blood was present. Data from tests with a malathion-tolerant strain of *Cx. pipiens s.l.* suggested little change in susceptibility regardless of blood feeding and the associated weight changes that occur from ingestion of blood.

INTRODUCTION

Several studies have reported a rapid return of the mosquito population to prespray densities following vehicle-mounted ultra low volume (ULV) application of insecticides for adult mosquito control (Strickman 1979, Fox 1980, Leiser et al. 1982). These results raise questions about the efficacy of ULV adulticiding as a technique for control of mosquito-borne diseases. Reported here is a possible reason for the apparent ineffectiveness of some ULV treatments and some implications for mosquito-borne disease control strategies.

During 1983 and 1984, four field trials of ULV synergized resmethrin for control of *Culex* vectors of St. Louis encephalitis were conducted in Memphis, Tennessee. The effectiveness of each treatment on *Culex* populations was monitored by counting the number of egg rafts found each day in oviposition pans (Reiter 1986). Following each of the spray applications, a similar pattern in the numbers of egg rafts appeared: on the first day following each application, they were substantially reduced, followed by a 1- or 2-day rebound and then a second reduction on the third or fourth day. The similarity of these oscillations suggested that the pattern was not due to weather factors, immigration or random fluctuations. Shelter of blood-fed and gravid females in refuges seemed unlikely because female *Culex* of all trophic stages had been observed leaving resting sites every evening and few specimens were encountered in those sites at the time the spray was applied (Reiter et al. 1990). In addition,

physiological and calendar age studies of truck-trap collections indicated that all but the very youngest individuals were represented in the flying population (Moore et al. 1986).

A possible explanation is that insecticide susceptibility among females varies according to the stage of blood meal digestion. Hadaway and Barlow (1956) applied DDT to *Anopheles stephensi* Liston and *Aedes aegypti* (Linn.) at different times after a blood meal and found twice as much DDT was required to kill the blood-filled females. The LD₅₀ returned to normal within 48 h after the blood meal. Their data clearly showed that the change in susceptibility was associated with time following the blood meal and not with the weight change due to blood feeding. Rawlings et al. (1981) studied the effects of body weight and trophic status on susceptibility of adult *An. culicifacies* Giles to dieldrin. While they did show a correlation of susceptibility with body weight of both unfed females and males, their data from blooded females is controversial because their methods depended on lengths of exposure to the insecticide, which varied from 15 min to as long as 16 h, and this was during the period when rapid changes in susceptibility would have been occurring.

The present study was designed to assess the effect of the stage of blood meal digestion on the susceptibility of colonized adult *Culex* and other species to aerosols of insecticides. It was postulated that temporal changes in susceptibility could account for the oviposition patterns that followed each of the field applications we had observed in Memphis. The null hypothesis was that no significant difference existed in susceptibility levels at different times following the blood meal.

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MATERIALS AND METHODS

Insecticides used in the tests were malathion² and synergized resmethrin (Scourge[®]). Laboratory aerosol exposures were made in a model D-2 wind tunnel (American Biological Supply Co., Baltimore, MD).⁴ Mosquitoes used were from *Cx. pipiens s.l.* insectary colonies (Centers for Disease Control, Fort Collins, CO), originating from specimens obtained in Memphis in 1976 and replenished with field specimens in 1981 and 1985. The malathion-tolerant colony of *Cx. pipiens s.l.* was from specimens obtained from the University of California at Riverside. The *Ae. aegypti* colony was started from specimens obtained in San Antonio, Texas in 1984, and the *Culiseta melanura* (Coq.) colony was started in 1985 from specimens obtained from Yale University.

Female mosquitoes to be tested were placed (25–30 each) in 0.5-pint (237-ml) ice cream cartons, the tops and bottoms of which had been replaced with fine-mesh nylon tulle. The procedure used was modified from that of Mount et al. (1976); test cartons with mosquitoes were placed in the wind tunnel and exposed to an aerosol of 0.1 ml of insecticide diluted in acetone. Five or 6 concentrations (2-fold dilutions of the greatest concentration) were used in each run, beginning with the lowest concentration. With resmethrin the greatest concentration was usually 0.036% w/v and with malathion the greatest was 10.72% for the tolerant strain and 1.34% for the susceptible strain. A control (using acetone alone) was included at the beginning and the end of each run. Each test was repeated at least 4 times except for the exposures of *Cs. melanura* which included only a single run due to lack of specimens. The tunnel was kept closed with the fan running for at least 10 sec following injection of the test solution to ensure clearing of the aerosol before the cartons were removed. The mosquitoes in each carton were anesthetized with CO₂ immediately on removal from the wind tunnel and then transferred to disposable 100- × 15-cm petri dishes. Sugar was provided by placing several drops of a 10% solution on filter paper that lined the bottom of the petri

dish. Specimens were held for 24 h before mortality was determined.

With sublethal concentrations of resmethrin, specimens were frequently observed that had lost from one to all of their legs and many were still able to fly and appeared to be otherwise unharmed. Khoo and Sutherland (1981) reported the occurrence of this phenomenon in tests with bioresmethrin and other pyrethroids on 8 different mosquito species. They observed that some individuals having only 2 unilateral legs were able to obtain a blood meal from a restrained guinea pig. In the current study specimens that had lost at least 3 legs were arbitrarily designated as dead on the assumption that they would have difficulty surviving or obtaining a blood meal under field conditions.

Determination of the LC₅₀ values was accomplished using a probit analysis computer program (Daum 1970). Statistical analysis of the LC₅₀ values for *Cx. pipiens s.l.* was done using the student *t*-test or analysis of variance at the 1% level of significance.

RESULTS

Figure 1 shows the LC₅₀ values with 95% confidence levels for 3 genera of mosquitoes exposed to synergized resmethrin in the wind tunnel. The LC₅₀ for nonblooded, female *Cx. pipiens s.l.* was 0.014% (w/v). There was a substantial increase in the LC₅₀ (a decrease in mortality) following the blood meal and by 48 h, the LC₅₀ reached 0.025% or nearly double the concentration required to kill the nonblooded mosquitoes. Between 48 and 72 h, the LC₅₀ returned to a level similar to that for nonblooded females and, during the following 24 h, there was little change, whether or not the mosquitoes were allowed to oviposit. A similar pattern was observed during the second gonotrophic cycle. Using the student *t*-test at the 1% level of significance, the null hypothesis (that there was no difference in the values of the groups tested) was rejected. Analysis of variance showed that the values at 24 and 48 h were significantly different than values from unfed, 72- and 96-h groups and from each other. Specimens exposed to either insecticide oviposited earlier than did untreated controls. Females that had been exposed to the aerosols 72 h after the blood meal often had oviposited egg rafts on the moist filter paper lining the bottoms of the petri dishes by the end of the 24-h observation period. In untreated controls, egg rafts were not observed until 96 h after feeding. Egg hatch and larval development appeared to be normal in both groups.

Figure 1 also shows the responses of *Cs. me-*

² Sample provided by American Cyanamid Company, Princeton, NJ 08540.

³ Sample provided by Penick & Co. (Now known as Penick-Bio-UCLAF Corporation), Lyndhurst, NJ 07071.

⁴ The use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

lanura and *Ae. aegypti*. The peak tolerance level in *Cs. melanura* occurred at or near 72 h after the blood meal. Peak tolerance in *Ae. aegypti* occurred 24 h after the blood meal.

The responses to malathion of a malathion-susceptible and a malathion-tolerant strain of *Cx. pipiens s.l.* were compared (Fig. 2). Mortality of the susceptible strain appeared to change with time following the blood meal, in the same pattern as had been observed with resmethrin. However, with the malathion-tolerant strain there appeared to be little change over time. Unfortunately the confidence limits in these tests with malathion were broad and overlapping so no firm conclusions can be drawn.

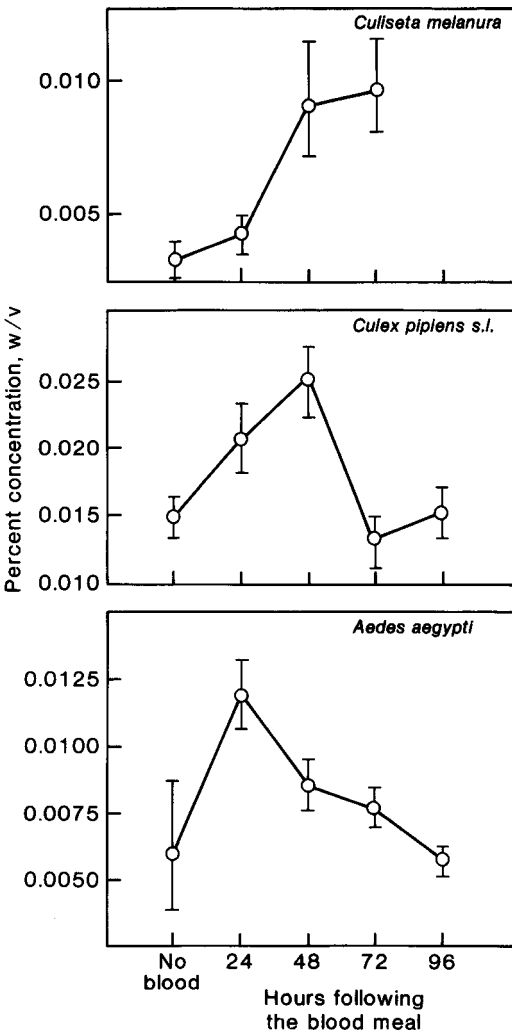


Fig. 1. LC₅₀ values for synergized resmethrin following a blood meal in 3 genera of mosquitoes. The bars represent 95% confidence levels for the probit analysis.

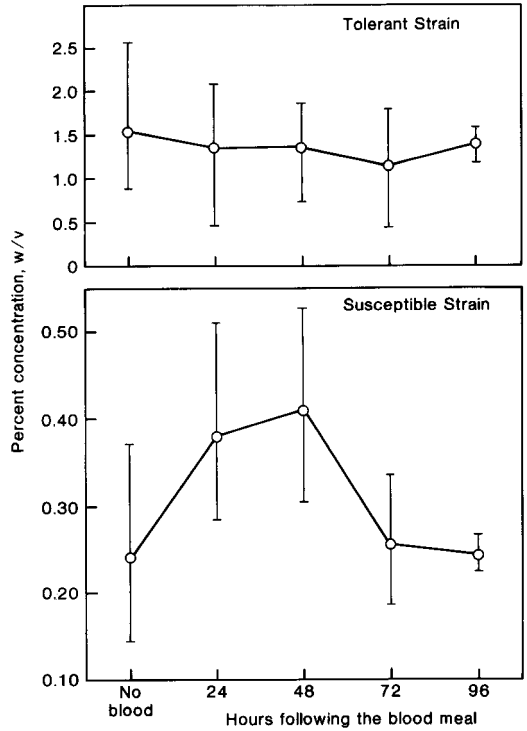


Fig. 2. LC₅₀ values for malathion following a blood meal in strains of *Culex pipiens s.l.* The bars represent 95% confidence levels for the probit analysis.

DISCUSSION

The observed changes in susceptibility to insecticides associated with time after blood feeding were of sufficient magnitude and of the proper duration to explain the cyclic phenomena reported earlier following field applications of resmethrin against *Cx. pipiens s.l.* (Reiter et al. 1990). Most field populations of *Cx. pipiens s.l.* would be expected to consist of cohorts in different stages of blood meal digestion with as much as 2-fold differences in their susceptibility to malathion and resmethrin. Selective mortality would favor individuals that had taken blood 1 or 2 days before the spray application. After the spray application, unequal mortalities would be expected to result in cyclic oviposition patterns. The oviposition pattern would depend on the proportions of the original population that were in various stages of the gonotrophic cycle at the time of spraying. It follows that oviposition data collected to monitor space-spray applications must be interpreted with care. If the length of the gonotrophic cycle is 4 days, females that had just taken a blood meal when spray was applied would not be ready to oviposit for 4 days, and so on for each cohort at different stages of the cycle (cf. Moore et al. 1990). Accordingly,

oviposition sampling must continue for at least 4 days to represent the entire population of survivors. Other sampling methods, such as shelter collections, which may be less biased in selection of physiologically distinct cohorts, may give better estimates of the true impact of insecticide spraying. However, with *Cx. pipiens s.l.*, such methods are labor-intensive, and adequate numbers of such sites are difficult to locate.

If the data presented here are representative, we must reexamine our strategies for control of mosquito-borne diseases with the use of ULV aerosols. These data suggest that substantial numbers of females survive the brief presence of aerosols because of increased tolerance associated with having taken a recent blood meal. A second application, perhaps 2 days later, may be needed to kill remaining cohorts of females after they have digested their blood meals and are again more susceptible. Doubling the insecticide dosage might be another useful method when permitted by the label. Focks et al. (1987) reported reductions of 73 and 75%, respectively, in mean adult captures and oviposition rates of *Ae. aegypti* field populations, following 11 sequential applications of ULV malathion over a 5-day period. They estimated that a single application killed only 30% of females.

Variation in susceptibility associated with the stage of blood meal digestion among mosquitoes in genera tested (*Anopheles*, *Aedes*, *Culiseta* and *Culex*) suggests that this may be a general phenomenon in mosquitoes and perhaps in other hematophagous arthropods. Our data, along with those of Hadaway and Barlow (1956), show that although the period of least susceptibility following the blood meal varied between species, there was not an association with the period of greatest weight (often double the unfed weight) which immediately follows ingestion of the blood meal. The apparent lack of variation in susceptibility following a blood meal in the malathion-tolerant strain, if confirmed, would provide additional evidence that the increase in weight and volume from the blood was not a factor.

The differences in the susceptibility of *Ae. aegypti* and *An. stephensi* to DDT during the 2 days immediately following the blood meal (Hadaway and Barlow 1956), and in *Ae. aegypti*, *Cx. pipiens s.l.* and *Cs. melanura* to malathion and synergized resmethrin in the present study, argue against the practice of using only blooded specimens in susceptibility tests with wild mosquitoes (Brown 1986). Unless feeding times are taken into account when measuring susceptibility, the possibility exists that individuals may vary in insecticide susceptibility by as much as 2-fold and that the variability may be unrelated to the insecticide being tested. In both laboratory and field tests, the use of specimens in

which no blood is seen should result in decreased variability of results and allow greater precision with smaller numbers of specimens.

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