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

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ABSTRACT. When posttreatment response to ultra low volume (ULV) application of insecticide has been followed for periods approaching a week, a damped oscillation in oviposition rates is observed, probably because females who have been recently blood-fed are more resistant to insecticides than their unfed siblings. We describe a simple model (ULVSIM) that incorporates physiologic changes in insecticide susceptibility and accounts for much of our field data. The model follows 30 cohorts over 30 days following insecticidal treatment. Multiple treatments or short-term residual activity can be evaluated. The model predicts that oviposition will follow a pattern of damped oscillations after an adulticidal treatment. The model gave a good fit to oviposition data obtained in 2 field trials of resmethrin for 7 to 9 days after treatment. It can be used to evaluate the effect of single and multiple treatments on the total female *Culex* population and on numbers of infected females surviving for different periods following an infective blood meal.

INTRODUCTION

Reiter et al. (1990) applied ultra low volume (ULV) resmethrin to control *Culex pipiens s.l.* mosquitoes in Memphis, Tennessee. They found that, after an initial rapid return to near pretreatment levels, oviposition exhibited a damped oscillation when followed for periods of about a week. Several workers have observed a rapid return to prespray levels following application of insecticides (Mitchell et al. 1969, Strickman 1979, Leiser et al. 1982), but did not monitor populations for sufficient time to observe any oscillatory behavior. Other workers have recorded, but not commented on, what appear to be similar oscillations following adulticidal control operations (Self et al. 1973, Pant et al. 1971). A possible explanation for the foregoing observations was first demonstrated by Hadaway and Barlow (1956). They found a cyclic change in insecticide susceptibility related to blood feeding and, presumably, to the gonotrophic cycle: recently blood-fed *Anopheles* and *Aedes* females were as much as twice as resistant as their unfed siblings to the insecticides tested.

In this article, we describe a simple model that incorporates temporal physiologic changes in insecticide susceptibility and accounts for much of the oscillatory behavior observed in the field.

METHODS

Model structure: The simulation model, titled ULVSIM, was originally written by one of us (CGM) using ACE-Calc®[2] spreadsheet software, running on a Franklin Ace 1200® microcomputer. The model has been expanded and adapted to run on Lotus 1-2-3®. The Lotus version is described here.

The model is simple in terms of the mathematics and the assumptions that it makes and it is deterministic. It consists of a 30 × 30 matrix of spreadsheet cells, each of which represents the number of individuals of a given cohort that are alive on a given day during the 30-day study period. All individuals are assumed to suffer the same natural mortality, and daily mortality rate is constant (exponential decay):

$N_t = N_o p^t$, where
N_t = Number alive on day t,
N_o = Number alive (emerging) on day O, and
p = Daily survival rate

A number of additional assumptions are made (Table 1). There is constant daily emergence of new adults into the population and there is no dispersal. All females that survive are successful in mating, finding and taking a blood meal, and ovipositing. All females in a given cohort feed and oviposit on the same day (or night). All females that oviposit successfully refeed on the same day (or night) that they oviposit. The length of the gonotrophic cycle is constant.

Daily emergence (N), natural daily survival rate (p), the day on which females take the initial blood meal (*b*, either day 2 or 3), the length of the gonotrophic cycle (*c*, either 3, 4 or 5 days) and the daily mortality (*k*) due to insec-

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[2] Use of trade names or commercial sources 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.

ticide (by day of the gonotrophic cycle) can be assigned by the user (Table 1). Mortality due to insecticide includes both the percent kill at each stage in blood digestion and the gonotrophic cycle and the number of days (up to 10) over which kill is obtained, enabling the effects of multiple treatments or short-term residual activity to be studied.

Stage-dependent mortality due to insecticide (k_i) is read from a user-supplied data list. Mortality in the period prior to the first blood meal (k_o) is assumed to be equal to that for other unfed individuals. Cohorts at different stages of the blood feeding and digestion cycle are assessed mortality according to the following scheme:

K_i , i=l. . .c, where
c = Length of the gonotrophic cycle, and
i = Day of the blood digestion and egg development cycle of a given cohort.

The model removes one degree of freedom each for N, p, c and b, unless these parameters are estimated independently of the observed field data to which they are being compared. One additional degree is lost for each day in the gonotrophic cycle for which kill data are empirically fitted. Thus, for a 4-day gonotrophic cycle with N, p, c, b and the 4 insecticidal kill rates all estimated from the field data, 8 df are used by the model and 1 more by the chi-square test, so that a minimum of 10 days of field observation are required for chi-square with 1 df.

Operation of the model: The model is formulated as an age-specific life table (Begon and Mortimer 1986) (Fig. 1). It is not a complete life table, as the immature stages are ignored. Briefly, the life table tracks groups of individuals (cohorts) in different age classes and physiologic states through time. The daily status of each cohort can be visualized as moving diagonally across (older) and down (later) through the cells of a large grid. New adult females (A) emerge (E) and enter the population each day. Males are not counted in the model. A certain proportion of each cohort (P_{ij}) survives through the day and the remainder ($1\text{-}P_{ij}$) die due to natural causes. Insecticide-induced mortality (k), if any, also reduces the number of surviving adults. The number of adults in the next day/age class is thus diminished by both natural and insecticidal mortality. The number of survivors in each day/age class is used to calculate the number of individuals that are ready to oviposit. Summary statistics are then calculated and expressed both as total numbers and as proportions relative to the status of the population in the absence of insecticidal control (Table 1). Population reduction due to control is given both in numbers of females and numbers of ovipositions each day for the first 14 days as well as for the entire 30 days. Percent reduction of individual cohorts is also provided in order to evaluate the effects of control on young vs. old cohorts.

RESULTS

General behavior of the model: The age distribution of females in the absence of insecticidal control is shown in Fig. 2A. Figure 2B shows the proportion of individuals in each phase of the gonotrophic cycle. The effect of single ULV treatments on ovipositing and total females, assuming equal mortality for all females regardless

Table 1. Characteristics of the ULVSIM spreadsheet model of mosquito population changes in response to adulticiding.

Model assumptions
Constant emergence, no immigration or dispersal
Constant daily survival rate
First blood meal always on same day after emergence
All surviving females feed successfully
Gonotrophic cycle has constant length
All survivors oviposit successfully
Oviposition and refeeding occur on the same day (or night)
Variable parameters
Daily emergence
Daily natural survival rate
Length of gonotrophic cycle (3 to 5 days)
Day of first blood meal following emergence (2 or 3 days)
Percent kill from insecticide for each day in gonotrophic cycle
Number of days of kill or treatment (0 to 9 days)
Model output
Number of surviving females by age group (cohort)
Number of surviving females by day posttreatment
Number of egg rafts deposited by day posttreatment
Insecticide-induced mortality (%) by cohort
Insecticide-induced mortality (%) by day posttreatment

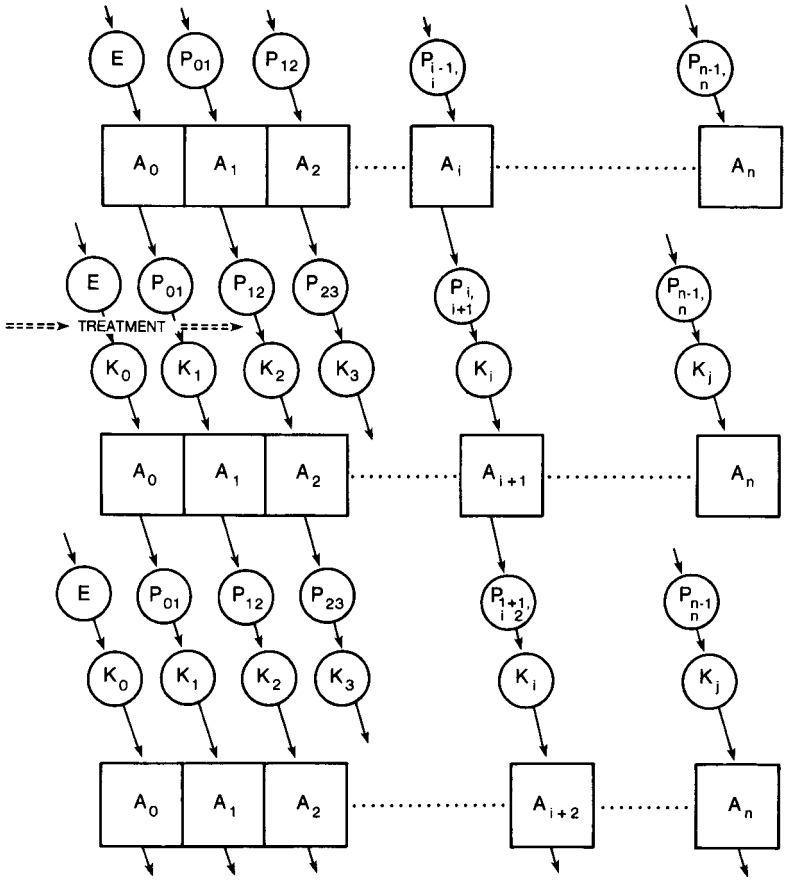

Fig. 1. Diagrammatic representation of the logic and sequence of calculations in the ULVSIM model. E = emerging females; A_i = number of adult females i days old; $P_{i,j}$ = survival probability between days i and $i+1$; k_i = mortality due to insecticide.

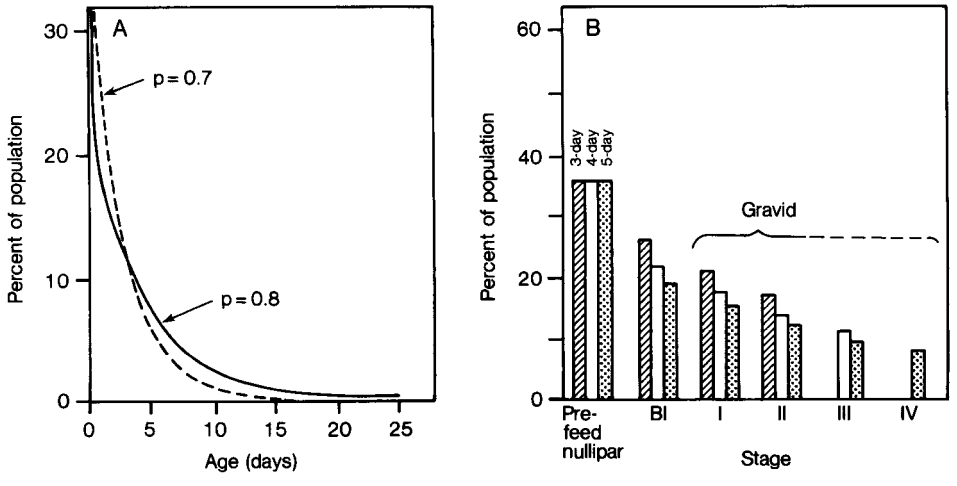

Fig. 2. Characteristics of the untreated population in the ULVSIM model: A, Age distribution of females at daily survival rates (p) of 0.7 and 0.8; B, Gonotrophic composition of populations with 3-, 4- and 5-day gonotrophic cycles. B1 = blooded females, I. . .IV = number of days spent in gravid state. Females are considered gravid if their ovaries contain developing or developed eggs.

of gonotrophic status, is shown in Fig. 3. Although the total number of females in the population followed a smooth curve in returning to normal after treatment, there was an irregular, stair-step pattern in the return of oviposition to pretreatment levels.

Data from ULV trials conducted by Reiter et al. (1990) in Memphis, Tennessee, were matched against the predictions of the model. In 4 field trials, conducted in July and August of 1983 and 1984, resmethrin was employed for control of *Culex pipiens* complex populations. The trials differed somewhat in percent reduction in oviposition, but the basic pattern of damped oscillations was the same in each case. Unfortunately, only one of the trials contained enough observations to provide data for analysis. The August 1984 trial (Fig. 4) could be matched fairly closely by empirically selecting mortality rates for each day of the gonotrophic cycle. A goodness-of-fit chi-square test shows, however, that the predictions of the model and field observations differ significantly ($0.05 > P > 0.025$, df = 4). The high chi-square value was due to the data for day 8, when oviposition in the field was much lower than predicted by the model.

Use of laboratory-derived mortality data: We used data obtained by Eliason et al. (1990) on mortality in Memphis *Cx. pipiens s.l.* exposed to ULV resmethrin at different stages in the gonotrophic cycle (Fig. 5). They obtained results similar to those of Hadaway and Barlow (1956), and their data clearly show that the effect of gonotrophic status on survival became more pronounced as the dose was lowered.

Two of the Memphis field trials, July and August 1984, were sufficiently long to provide data sets, since the wind tunnel mortalities were determined independently. Predictions of ULV-SIM incorporating wind tunnel mortality data

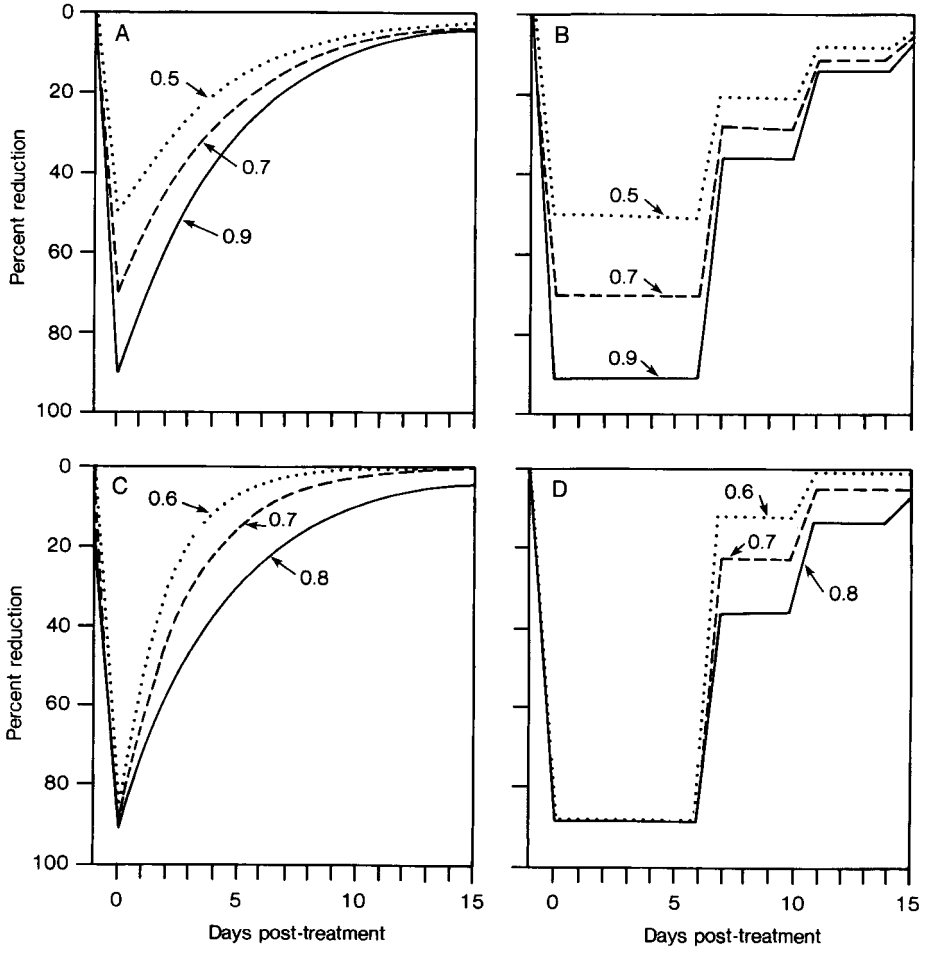

Fig. 3. Effect of constant (independent of blood meal and ovarian stage) insecticide-induced mortality on total adults (A, C) and oviposition (B, D). A and B show the effect of varying percent kill (k = 0.5, 0.7, 0.9) with constant survival (p = 0.8); C and D show the effect of varying survival (p = 0.6, 0.7, 0.8) with constant kill (k = 0.9). Gonotrophic cycle (c) = 4 days, first blood meal (b) on day 2.

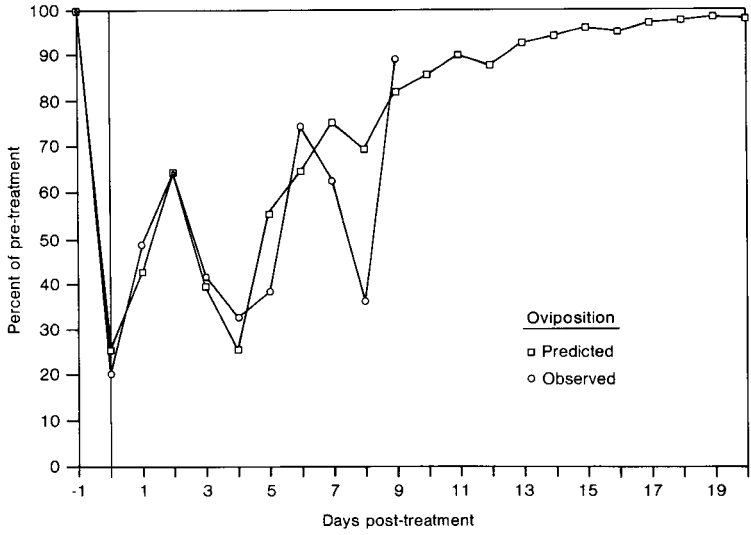

Fig. 4. Matching of predicted daily oviposition and observed oviposition in the field by empirically fitting mortality values (k_i) into ULVSIM. Observed data are from August 1984, field trial in Memphis, TN (Reiter et al. 1990).

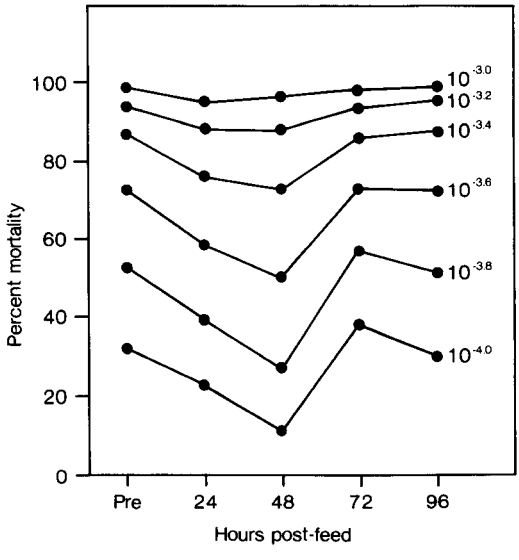

Fig. 5. Changes in mortality in relation to blood meal digestion and egg development. Memphis *Culex pipiens* (*s.l.*) were exposed to resmethrin aerosols at the indicated concentrations (v/v, in acetone), at different times following a blood meal. Data from wind tunnel study of Eliason et al. (1990).

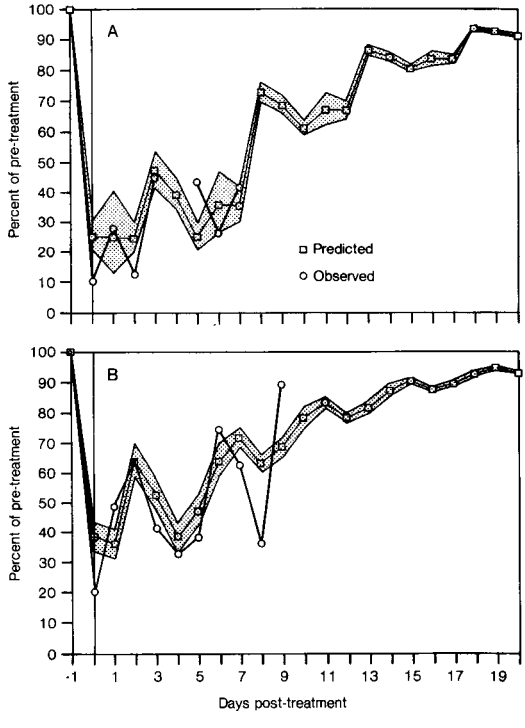

Fig. 6. Comparison of daily oviposition in a Memphis, TN, field data series to ULVSIM predictions using mortality data shown in Fig. 5. Observed and predicted oviposition (shaded region represents 95% C.L. estimates for wind tunnel mortality data): A. July, 1984 (n =33, p = 0.885, b = 2, c = 5, k values for 0.026% resmethrin, no collections on day 5 due to rain); B. August 1984 (n = 76, p = 0.801, b = 2, c = 5, k values for 0.019% resmethrin). The field data were detrended by using linear regression to account for changes in oviposition in untreated zones.

are shown in Fig. 6. In the case of the July field trial (Fig. 6A), the best fit was obtained by using mortality for a concentration of 0.026% resmethrin. The predicted and observed values are similar, and they do not differ significantly (chi-square = 9.42, df = 4, $0.10 > P > 0.05$). Oviposition was slightly lower than expected on days 0 and 2 and higher than expected on day 5. By excluding day 5 from the calculations, very close

agreement between model and data was obtained (chi-square = 5.51, df = 3, $P > 0.10$). There was a close but not significant fit to the data from the August trial (Fig. 6B), using mortality for 0.019% resmethrin (chi-square = 16.56, df = 7, $0.025 > P > 0.01$). The model failed to predict a large decrease in oviposition on day 8 and a moderate increase on day 9. By excluding day 8, however, good agreement was obtained between model and data (chi-square = 9.89, df = 6, $P > 0.10$).

Number of females surviving extrinsic incubation: Given a particular set of starting conditions and a treatment regime, we can predict the number of females that might survive long enough to transmit a pathogen to another host. Since, under our assumptions, females that successfully oviposit also successfully take the next blood meal, we know the number of females that feed after any given cycle. Table 2 compares the predicted numbers of survivors with and without treatment, assuming a treatment mortality equal to that obtained at 0.0234% resmethrin in the wind tunnel. Data on transmission rates were obtained from Chamberlain et al. (1959) for *Cx. quinquefasciatus* Say from Montgomery, Alabama. Under these conditions the percentage of females available for refeeding 8 or more days postinfection (third blood meal) was reduced by about 53%. There was a 64% reduction in the percentage of females feeding 4 or more times (surviving 16 days or longer following the first blood meal).

Effects of multiple treatments: The model can be used to examine the effects of multiple treatments over a 10-day period or to study the short-term residual effects of an adulticide. Figure 7 compares the predicted number of females surviving to feed following no treatment, a single treatment, or dual insecticide treatments separated by 1 to 7 days. Dual treatments, as expected, reduced populations more than single treatments. The differences between treatment intervals in the multiple treatments were not pronounced. Longer intervals between treatments produced slightly lower kills of females feeding 4 or more times, which might be significant in epidemic interruption, depending on the extrinsic incubation period of the pathogen in the mosquito. In contrast, longer intervals resulted in slightly better kill of younger age groups, which could be important in transovarially transmitted diseases. The additional mortality—measured by decrease in total bites—obtained from a second treatment was not a simple additive quantity. The added mortality ranged from less than 50% of the single-treatment value in the case of a 24-h separation, to nearly 95% of the single-treatment value in the 96-h separation between treatments.

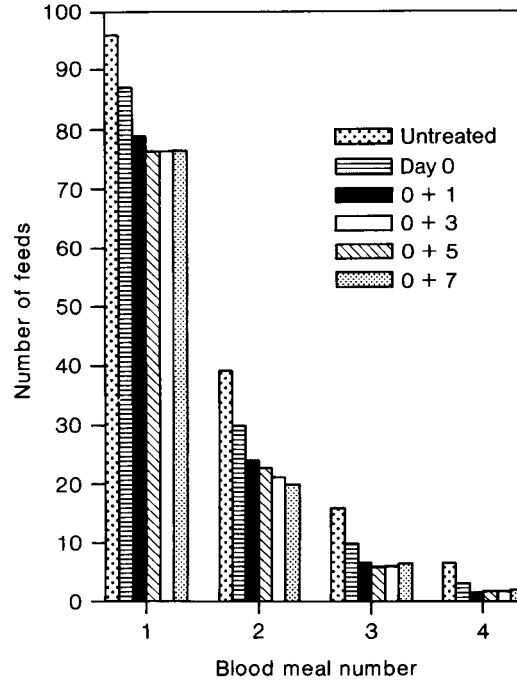

Fig. 7. Predicted effect of the number and timing of ULV treatments on the number of blood meals taken by surviving females ($n = 10$, p = 0.8, $b = 2$, $c = 4$, 0.019% resmethrin).

Table 2. Predicted effect of ULV treatment on the number of female *Culex pipiens* surviving to take multiple blood meals over a 14-day period.*

No. of blood meals	No treatment		Single treatment		Percent reduction
	Females feeding	% of total	Females feeding	% of total	
1	22,410	59.6	19,272	67.7	14.0
2	9,167	24.4	6,390	22.4	30.3
3	3,737	9.9	1,991	7.0	46.7
4	1,521	4.0	550	1.9	63.8
5	605	1.6	225	0.8	62.8
6	176	0.5	56	0.2	68.2
Total feeds	37,616	100.0	28,484	100.0	24.3

* Daily emergence = 2,340; survival rate = 0.80; gonotrophic cycle = 4 days; insecticide kill corresponding to 0.0234% resmethrin (Eliason et al. 1990)

DISCUSSION

The effect of survival rate on the model is mainly on the speed of return to pretreatment levels; populations with high natural mortality (low daily survival rates, p) return to prespray levels more rapidly than those with low natural mortality (high p) (Fig. 3, C and D). This is because populations with low p contain relatively more newly emerged individuals (Fig. 2A). Thus, the effect of a single treatment is more rapidly diluted by recruitment of new individuals to populations with low p in the days following treatment. The observation (Fig. 5) that gonotrophic cycle effects are most pronounced at low dosages suggests, as would be expected, that when we observe these patterns, the actual amount of insecticide reaching the mosquitoes may be relatively small.

It is important to note that the cyclic changes described here relate to blood feeding, oviposition, and related behavior, and not to the population as a whole. A behaviorally unbiased sampling method (i.e., one that collects all physiologic and behavioral stages equally) should not detect this cyclic pattern (Fig. 3, A and C).

The general patterns of both model and field observation are in correspondence. The ULVSIM model provides a statistically significant fit to one of the 2 sets of field data available to us and, when one day was eliminated, a significant fit was obtained for the second data set. The inability of ULVSIM to fit both data sets could be due to inadequacies in the model, to random changes in the field population, to inadequacies in design of the field trials (cf. Hurlbert 1984, Reiter et al. 1990) or all of these factors.

Inadequacies in the model stem from its simplifying assumptions and include the following areas:

1) The exponential decay formula may not always provide the best descriptions of mortality (Clements and Paterson 1981).

2) We know from other studies that not all females *do* successfully feed or oviposit in synchrony (Moore and Reiter, unpublished data).

3) Day-to-day variation in temperature, humidity, rainfall, wind, etc., will cause changes in blood feeding and oviposition intervals.

4) Variation in daily emergence will increase or decrease numbers of females entering the cohort of ovipositing females.

5) Changes in host abundance or behavior will also affect the proportion of females that successfully feed on a given day.

It seems likely that the lack of significant fit between model and the second field data set was due to environmental or population disturbances external to the insecticide treatment, that are not dealt with by the model. Such effects could probably be averaged out by using a very large data set, collected over several years—an unlikely occurrence, given the cost.

Despite the simplicity of the model and the use of several simplifying assumptions, the model approximates reality reasonably well. The existing field data are probably too crude to benefit from mathematically more elegant models containing more realistic assumptions.

The results of the multiple treatment simulations suggest that the technology of insecticide application may be more complicated than it is normally viewed. The adulticiding schedule that kills the largest number of mosquitoes may not be the best schedule for stopping disease transmission if most of the mortality is in individuals least likely to be infected. Such a counterintuitive proposal requires extensive testing, both in the field and in more sophisticated models.

The simulations described here suggest that we can learn a good deal from simple models. The absence of suitably collected field data is a major difficulty in extending and testing the predictions of this and other models. We strongly encourage those designing ULV trials aimed at answering questions raised in this study to consider the following 4 criteria: 1) the evaluation of ULV must be done directly on the wild population, and population measurements must be made on a 24-h basis; 2) proper experimental design must be used, and problems such as pseudoreplication (Hurlbert 1984) must be avoided; 3) data collections must be on as large scale as possible within time and budget constraints; and 4) data collection must continue as long as possible, but at least longer than the length of the gonotrophic cycle of the species of interest.

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REFERENCES CITED

Begon, M. and M. Mortimer. 1986. Population ecology. A unified study of animals and plants (2d. ed.). Blackwell Sci. Pubs., Oxford.

Chamberlain, R. W., W. D. Sudia and J. D. Gillett. 1959. St. Louis encephalitis in mosquitoes. Am. J. Hyg. 70:221–236.

Clements, A. N. and G. D. Paterson. 1981. The analysis of mortality and survival rates in wild populations of mosquitoes. J. Appl. Ecol. 18:373–399.

Eliason, D. A., E. G. Campos, C. G. Moore and P. Reiter. 1990. 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. J. Am. Mosq. Control Assoc. 6:371–375.

Hadaway, A. P. and F. Barlow. 1956. Effects of age, sex and feeding on the susceptibility of mosquitoes to insecticides. Ann. Trop. Med. Parasitol. 50:438–443.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecologic field experiments. Ecol. Monogr. 54:187–211.

Leiser, L. B., J. C. Beier and G. B. Craig. 1982. The efficacy of malathion ULV spraying for urban *Culex* control in South Bend, Indiana. Mosq. News 42:617–618.

Mitchell, C. J., R. O. Hayes, P. Holden, H. R. Hill and T. B. Hughes, Jr. 1969. Effects of ultra-low volume applications of malathion in Hale County, Texas. I. Western encephalitis virus activity in treated and untreated towns. J. Med. Entomol. 6:155–162.

Pant, C. P., G. A. Mount, S. Jatanasen and H. L. Mathis. 1971. Ultra-low-volume ground aerosols of technical malathion for the control of *Aedes aegypti* L. Bull. W.H.O. 45:805–817.

Reiter, P., D. A. Eliason, D. B. Francy, C. G. Moore and E. G. Campos. 1990. Apparent influence of the stage of blood meal digestion on the efficacy of ground applied ULV aerosols for the control of urban *Culex* mosquitoes. I. Field evidence. J. Am. Mosq. Control Assoc. 6:366–370.

Self, L. S., H. I. Ree, C. S. Lofgren, J. C. Shim, C. Y. Chow, H. K. Shin and K. H. Kim. 1973. Aerial applications of ultra-low-volume insecticides to control the vector of Japanese encephalitis in Korea. Bull. W.H.O. 49:353–357.

Strickman, D. 1979. Malathion as ground-applied ULV evaluated against natural populations of *Culex pipiens* and *Cx. restuans*. Mosq. News 39:64–67.