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[1]

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ABSTRACT. The impact of ULV resmethrin on urban *Culex* mosquitoes was evaluated in 4 field trials by monitoring daily oviposition rate. A well-defined oscillation of effect, with a period corresponding to the duration of the gonotrophic cycle, was observed. We postulate that this oscillation arises from changes in susceptibility following blood feeding and/or behavioral factors. The data indicate that a single treatment with ULV may be inadequate for the effective control of vector mosquitoes.

INTRODUCTION

Urban *Culex* species, particularly *Culex pipiens* (Linn.) *s.l.* are a target for vehicle-dispensed ultra-low volume insecticidal control (ULV) because of their role as vectors of the St. Louis encephalitis (SLE) virus. Ultra low volume is considered the control method of choice during SLE epidemics (Breeland et al. 1980). A number of urban health authorities routinely monitor zoonotic transmission to determine when and where to apply ULV adulticides in order to prevent epidemic transmission (Bowen and Francy 1980).

Despite the importance ascribed to the method, only 3 articles on its impact on wild *Culex* populations have been published. Strickman (1979) monitored *Cx. pipiens* and *Cx. restuans* Theobald populations by daily counts of egg rafts laid in pails of alfalfa infusion that were maintained in a "suitably foul condition" by the occasional addition of more alfalfa. In 3 trials of ULV malathion he compared the number of egg rafts laid per day in 4 pails: 2 in treated and 2 in untreated areas. His data, albeit based on a small number of sampling sites, indicated mean reductions of the oviposition rate of 52, 47 and 31.3% on the first 3 nights after treatment, although only the reduction on the night of treatment was statistically significant. Leiser et al. (1982) conducted a similar small-scale evaluation of ULV malathion in northern Indiana. They noted a small reduction in the oviposition rate of *Cx. restuans* and *Cx. pipiens* after 2 of 3 applications. No figures for these reductions were given, but the authors concluded that ULV malathion was not a consistently effective method for urban *Culex* control. The third study was by Geery et al. (1983), who made a retrospective examination of 5 years of collection data from 10 light traps in northern Illinois. Their analysis of 296 ULV malathion treatments in the vicinities of the traps indicated a mean reduction of 27% (t-test, $P < 0.001$) of the *Culex* mosquitoes collected on the first 3 days after treatment. No details were given of the size of the treated "sections" around the traps, nor of the age or sex of mosquitoes that were collected.

Clearly none of these trials represents an exhaustive evaluation of the efficacy of ULV for urban *Culex* control, but it is notable that in each case it was concluded that ULV treatments did not result in a satisfactory reduction of the mosquito population. The remainder of published studies involve field bioassay of caged, laboratory-reared insects. We consider it unlikely that such tests can provide a useful simulation of the impact of a treatment on unrestricted mosquitoes in the natural habitat.

The adoption of simple standardized procedures for the surveillance of urban SLE vectors by egg raft collection (Reiter 1986), and the development of the CDC gravid mosquito trap (Reiter 1983, Reiter et al. 1986) enabled us to monitor oviposition rates on a daily basis at a large number of sites, and thus determine the impact of vehicle-dispensed ULV on the wild *Culex* population. We consider oviposition rate to be an acceptable indicator of the active vector population because each egg raft represents the culmination of a single gonotrophic cycle.

Vehicle-dispensed ULV malathion had been used for control of urban *Culex* by the Memphis and Shelby County Health Department since the early 1970s, and was extensively used in the SLE epidemics of 1974–76. Resistance to malathion in local *Cx. pipiens* was demonstrated by laboratory and field trials in 1976 (Moseley et al. 1977), and studies in 1981 demonstrated that it was present throughout the city (Brogdon and Reiter, unpublished data). Nevertheless, we de-

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[1] The 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.

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cided to evaluate malathion in our initial trial (June 1982) because it was still in routine use. Not unexpectedly, we failed to demonstrate an impact on *Cx. pipiens*, although there was some evidence of a reduction in the oviposition rate of *Cx. restuans*, a fully susceptible species. We chose resmethrin for subsequent trials because the *Cx. pipiens* population was known to be susceptible to this insecticide. In this article we describe 4 trials of ULV resmethrin, conducted in 1983 and 1984.

METHODS

Treatment area: The treatment area was a 3-sided, 13-km^2 section of northeast Memphis, Tennessee, chosen for homogeneity of vegetation and housing type, and relative isolation in terms of urban *Culex* habitat. It was bordered to the northwest by a 4- to 6-lane highway and a treeless commercial and industrial area, to the northeast by a 6-lane expressway and undeveloped wooded country, and to the south by a 6-lane expressway. The average lot size was 450–700 m^2. Vegetation was mature, with hardwood trees up to 21 m high. Most houses were single story, separated from the sidewalk by unfenced, well-tended grass lawns. Yards had varying amounts of shrubbery and other low vegetation. Some backyards were bounded by wooden privacy fences, but in general the spaces between houses were fairly open. Untreated sites were not all contiguous, but were chosen for their apparent similarity to the treated zone in terms of plot size and vegetation.

Evaluation methods: Egg rafts were collected as described by Reiter (1986). In trials 1 and 2, adult *Culex* were collected with gravid mosquito traps (Reiter 1983). In the treatment zone, all collecting devices were placed 1–2 km from the periphery, to minimize the impact of immigration of mosquitoes from untreated areas. Five clusters of sampling sites were used within this evaluation area. Clusters were separated by at least 2 blocks, and sites within clusters were at least 4 houses apart. In trials 1 and 2, four oviposition pans and 2 gravid mosquito traps were set in each cluster. In trials 3 and 4, there were 5 oviposition pans per cluster; gravid traps were not used.

Insecticide application: Treatment began 30 min before astronomical sunset. Four Leco-HD fogging machines were used simultaneously, to ensure complete coverage within 2 h of sunset. A fifth machine was available in case of equipment failure. Resmethrin was applied as a solution of Scourge™ [3] in mineral oil, corresponding to 12% active ingredient (resmethrin) and 36% synergist (piperonyl butoxide). Delivery rate was 237 ml (8 fl oz) per min at a vehicle speed of 16 km (10 miles) per h, equivalent to 6.97 g ha^{-1} (0.0062 lb acre^{-1}) of active ingredient (based on a swath width of 90 m (300 feet)). Nozzle pressure was 0.42 kg cm^{-2} (6 lb inch^{-2}). Droplet size was determined for all machines before every treatment by measuring 200 droplets on each of 2 samples obtained by waving teflon-coated slides 8 m from the machines. In all cases mass median diameter (MMD) was close to 10 μm. A navigator was provided for the driver of the machine covering the monitoring portion of the treatment area to ensure complete coverage.

[3] Provided by Penick & Co. (now known as Penick-Bio UCLAF Corporation), 1050 Wall Street West, Lyndhurst, NJ 07071.

RESULTS

Dates of trials and weather conditions at the time of treatments are summarized in Table 1. In 1983, temperatures were above normal throughout the trials. There was very little air movement at the time of insecticide application in trials 1, 2 and 3; such conditions are fairly typical for the time of year in the area.

More than 95% of egg rafts collected were *Cx. pipiens*; the remainder were *Cx. restuans*. As the susceptibility of both species to resmethrin was similar (CDC, by wind tunnel test, unpublished data), results given here are not separated by species.

Mean numbers of egg rafts per 24-h period for all 4 trials are given in Table 2, and mean numbers of female *Culex* per trap are given in Table 3. In all 4 trials there was a marked reduction in the mean numbers of egg rafts (and adults) collected in the treated area on the day of the treatment (T), and oviposition rates remained lower for the rest of the evaluation period (T + 1, T + 2, etc.). Fluctuations in the untreated areas, mainly attributable to rainfall, did not match the posttreatment pattern in the treated area.

To correct for nontreatment variation, the percent change in the number of egg rafts collected at each site on each day, relative to the number collected on the day before treatment, was calculated, and daily means of these percent changes were derived for treated and untreated areas. The ratios (treated area/untreated area) of these daily mean percent changes are presented in Table 4 as the corrected mean percent change for the day of treatment (T) and for 3 subsequent days (T + 3) in each of the trials.

In all 4 trials, oviposition rates fell by 74–84% on the night of treatment (T), but on the 2 subsequent nights, (T + 1) and (T + 2), they were higher than on night (T). On night (T + 3), however, oviposition returned to a lower rate

Table 1. Dates of trials and weather conditions at the time of treatment.

Trial no.	Date begun	Date ended	Conditions during treatment: Temperature °C	Relative humidity (%)	Wind and sky
1	Aug. 9, 1983	Aug. 14, 1983	33.3	82	calm, clear
2	Aug. 28, 1983	Sep. 2, 1983	32.8	85	calm, clear
3	July 31, 1984	Aug. 9, 1984	26.7	90	calm, cloudy
4	Aug. 20, 1984	Aug. 31, 1984	29.4	85	SW (<5 kph) some cloud

Table 2. Mean number of egg rafts per oviposition pan.

Trial	Day	Untreated area		Treated area	
		Pans	Mean ± SE	Pans	Mean ± SE
1	−2	20	13.4 ± 2.3	20	10.9 ± 2.4
	−1	19	20.1 ± 3.6	19	27.5 ± 2.9
	Treatment	20	26.4 ± 4.1	20	9.3 ± 1.6
	1	20	15.3 ± 2.1	20	8.0 ± 1.4
	2	19	41.3 ± 6.5	19	26.8 ± 2.5
	3	19	18.0 ± 1.7	19	11.2 ± 1.5
2	−2	19	21.7 ± 3.2	20	23.1 ± 2.5
	−1	19	16.2 ± 2.5	20	23.8 ± 2.8
	Treatment	19	10.8 ± 2.4	20	3.0 ± 0.5
	1	19	9.8 ± 1.5	20	6.0 ± 0.8
	2	18	23.1 ± 3.2	20	12.7 ± 5.1
	3	19	16.3 ± 1.8	20	5.1 ± 0.6
3	−2	24	28.1 ± 4.2	24	24.4 ± 3.1
	−1	25	20.2 ± 2.2	24	23.8 ± 3.9
	Treatment	25	15.7 ± 1.8	24	3.0 ± 0.5
	1	25	14.0 ± 1.9	24	9.5 ± 1.3
	2	23	9.7 ± 1.3	24	6.4 ± 0.9
	3	25	42.8 ± 3.9	24	17.3 ± 1.7
	4	Heavy rain, no data			
	5	25	32.8 ± 3.4	24	19.6 ± 2.2
	6	25	23.6 ± 2.6	24	16.0 ± 1.4
	7	25	33.8 ± 3.2	23	21.8 ± 2.8
4	−2	25	30.4 ± 3.5	23	38.0 ± 3.0
	−1	22	36.0 ± 3.6	21	36.9 ± 4.2
	Treatment	25	36.1 ± 4.4	24	7.4 ± 1.2
	1	21	54.7 ± 5.3	22	17.9 ± 2.4
	2	24	28.0 ± 3.3	24	22.7 ± 2.4
	3	23	30.4 ± 3.7	24	14.0 ± 1.9
	4	21	33.8 ± 4.7	24	10.3 ± 1.1
	5	19	30.5 ± 3.8	24	12.0 ± 1.7
	6	24	38.5 ± 5.4	23	24.8 ± 2.4
	7	22	36.6 ± 4.4	24	20.0 ± 2.3
	8	25	17.9 ± 2.5	23	9.8 ± 1.2
	9	23	29.5 ± 3.0	23	28.3 ± 2.4

than on night (T + 2), and in all trials except 4, the rate was lower than on night (T + 1).

Trials 1 and 2 were terminated on day (T + 3) but collections were extended for 6 more days in trials 3 and 4. Adverse weather, with numerous heavy thunder showers, plagued trial 3, limiting interpretation of the results, but weather was more acceptable during trial 4. Corrected mean percent change for 10 days posttreatment in trial 4 is illustrated in Fig. 1.

In addition to the cycle of decrease and then increase of mean percent reduction on days (T) through (T + 3), a second cycle is evident for days (T + 4) through (T + 7), and the beginning of a third may be apparent on days (T + 8) and (T + 9).

DISCUSSION

Resmethrin achieved an impressive reduction of the oviposition rate of *Culex* mosquitoes on the evening of spray, and this impact was discernable for at least 8 days after the application. Important features of the trial included "stand-

Table 3. Mean number of female *Culex* mosquitoes per gravid mosquito trap.

		Untreated area		Treated area	
Trial	Day	Traps	Mean ± SE	Traps	Mean ± SE
1	−2	7	198.5 ± 11.4	10	97.5 ± 7.6
	−1	9	350.7 ± 15.5	9	64.1 ± 13.5
	Treatment		no data		no data
	1	9	140.1 ± 8.9	9	24.1 ± 4.5
	2	9	159.1 ± 8.0	9	38.5 ± 2.5
	3	9	196.7 ± 10.0	8	27.0 ± 1.5
2	−2	7	251.3 ± 167.3	8	23.1 ± 2.5
	−1	7	239.1 ± 183.5	7	23.8 ± 2.8
	Treatment	9	93.6 ± 46.1	10	3.0 ± 0.5
	1	9	184.3 ± 63.9	8	6.0 ± 0.8
	2	9	153.2 ± 97.7	9	12.7 ± 5.1
	3	9	115.3 ± 45.8	6	5.1 ± 0.6

Table 4. Corrected mean percent change of egg rafts collected after treatment.

		Collection night		
Trial no.	T (Treatment)	(T + 1)	(T + 2)	(T + 3)
1	−74	−62	−53	−70
2	−74	−43	−48	−71
3	−81	−38	−47	−60
4	−84	−72	−41	−64
Mean (all trials)	−78	−54	−47	−66

ardization" of the attractant medium, day-to-day monitoring of the wild mosquito population, and a treatment area large enough for the monitoring sites to be surrounded by a buffer zone.

The oviposition data for days (T) through (T + 3) in all the trials, and days (T + 4) through (T + 7) in trial 4 revealed a distinct 4-day oscillation. At temperatures prevailing during the trial, the gonotrophic cycle of the *Culex* population would also have been about 4 days, which leads us to suspect that the impact of the treatment may have been modified by gonotrophic stage. Two mechanisms for this modification seem plausible: behavioral and physiologic.

It is widely held that mosquitoes are relatively inactive for much of the period between feeding and oviposition. A cohort corresponding to 2–3 days of the gonotrophic cycle could have escaped the ULV aerosol if the resting sites were less accessible to the insecticide, giving rise to the observed cycle of effect. However, field observations and truck trap collections in Memphis (Reiter, unpublished data) had demonstrated that adult *Cx. pipiens* in all gonotrophic stages emerged from their resting sites every night, soon after astronomical sunset, so it would seem that all stages should have been exposed to the ULV aerosol.

An alternative explanation is based on changes in susceptibility that occur during the gonotrophic cycle: susceptibility decreases shortly after blood feeding, and gradually returns to the original level as the insect becomes gravid (Hadaway and Barlow 1956). Using mosquitoes from Memphis, we have demonstrated greater than 2-fold changes in the susceptibility of *Cx. pipiens* during the gonotrophic cycle (Eliason et al. 1990), and a simple computer model has shown that the 4-day cycle observed in the field trials could well be the result of such changes (Moore et al. 1990).

If variation in susceptibility is the source of the cyclic effect, this indicates that the insecticide is being delivered at a marginally effective application rate, and that an increased rate would correct the problem. If the effect is due to behavior, or if higher rates are not acceptable, then modifications of the spray regime might augment overall efficacy. For example, in trial 4, control over the first 6 posttreatment days averaged 67.7%, with a reduction of 41–84% on individual days. Simple extrapolation of the data suggests that a pair of treatments on successive nights could give 79–96% control on every day, with a mean kill over the whole period of 87.8%.

The impact of the treatments on the *Culex* population was far more encouraging than that indicated in previously published studies (Strickman 1979, Leiser et al. 1982, Geery et al. 1983). This may have been due to the insecticide used, but environmental factors such as vegetation may also have been favorable.

The significance of vector population levels in the dynamics of arbovirus transmission is not clear; therefore, the potential impact of routine ULV treatments on arbovirus transmission remains a matter for conjecture. What is certain, however, is that the true efficacy of such a widely used and costly control method deserves far greater attention than it has received in the past.

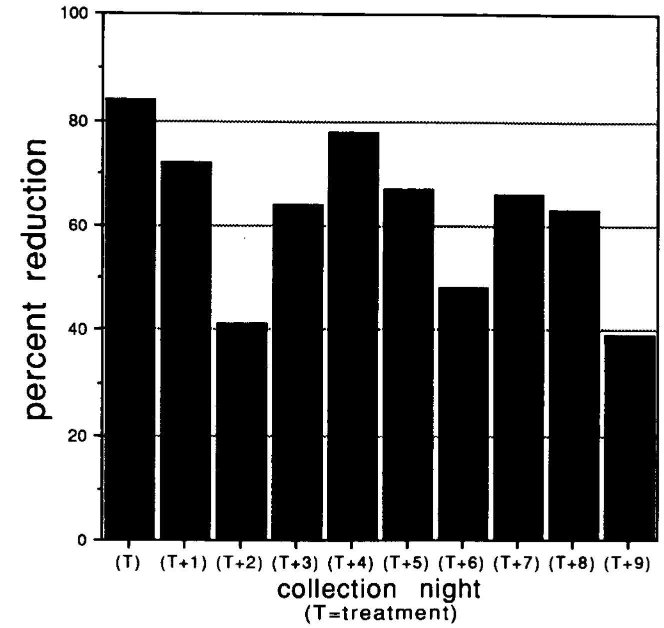

Fig. 1. Corrected mean percent change of egg rafts collected after treatment, fourth trial only.

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