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ANOPHELES CULICIFACIES: THE EFFECTS OF ADULT BODY WEIGHT AND TROPHIC STATUS ON DIELDRIN LT_{50} DETERMINATIONS

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ABSTRACT. The live weight of adult *Anopheles culicifacies* was affected by variation in rearing conditions and using either unfed or blood fed females for insecticide tests. Mosquitoes homozygous resistant to dieldrin were the main genotype used on 4% dieldrin treated papers for exposure times from 5 to 16 hours. LT_{50} determinations showed significant heterogeneity but clearly heavier blood fed adults showed less susceptibility to dieldrin. All the laboratory reared adult sizes could be found in the field as shown by collections from one village during 1976. The implication of such weight and trophic status variation is discussed with respect to discriminating dosages and the degree of resistance involved.

INTRODUCTION

Insecticide tests with WHO test kits are generally used to detect the presence and measure the frequency of resistance genes in mosquito populations. However, as pointed out by Davidson and Zahar (1973), the results may be affected not only by genetic factors but also by environmental factors influencing the size and physiological status of the mosquitoes used for testing. Such variability might be particularly important when laboratory rearing of the mosquitoes for testing is not feasible and when wild collected specimens must therefore be used. Great variability in the size and other characteristics of wild mosquitoes is to be expected in areas such as Punjab when ambient temperatures may vary more than 40°C during the year.

The objectives of the present study were to describe the effects in the laboratory of variation in body-size and trophic status on dieldrin LT_{50} and try to extrapolate these findings to field conditions by monitoring seasonal changes in adult female body-size.

MATERIALS AND METHODS

STRAINS. Colonies of homozygous susceptible (SS) and resistant (RR) *An. culicifacies* (Giles) were selected respectively, from Sattoki and Multan, Punjab Province, parent strains colonized originally by Ainsley (1976) and studied genetically for the inheritance of resistance to dieldrin by Sakai et al. (1979). Dieldrin resistance in *An. culicifacies* was found to be semi-dominant, with exposure to 0.4% for 1 hr killing all SS and to 4.0% for 2 hr killing all SS and heterozygotes (RS), but not the RR.

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LABORATORY METHODS. Adult size in the RR stock was altered by varying the rearing temperature and density of the larvae. To produce small adults larvae were reared at 32°C under crowded conditions, while to produce large adults larvae were reared at 26°C under uncrowded conditions. Liver powder and well water in enamel trays were used for larval rearing. Larval-pupal separation was done manually. Newly emerged adults were sexed, counted, weighed in batches of 20–30 and then transferred to a WHO test-kit recovery tube. RR females and males were exposed to 4% dieldrin at a temperature of 28°C for selected times and then held in recovery tubes for a minimum of 5 hours. The remaining females were sorted into small, medium and large size classes, aged for 3 days and then offered a restrained laboratory mouse as a blood source. The following morning fully engorged females were counted, weighed, and tested the same way as newly emerged adults. Females succumbing to handling injuries were removed from the tubes prior to testing. Exposure times for the RR adults ranged between 5 and 16 hours.

Unweighed RS and SS adults were also tested. Unfed and blood-fed RS ♀♀ were exposed to 4% dieldrin for 15 to 120 min and to 0.4% dieldrin for 3 to 10 hr. Even 15 min exposure to 0.4% dieldrin killed all blood fed SS adults. All LT_{50} values were calculated from computed log-time—probit mortality regression lines.

FIELD METHODS. Temporal changes in adult body size as indicated by wing-length were determined for female *An. culicifacies* and, for comparison, *An. stephensi* (Liston) collected resting inside a cattle shed near the village of Sattoki during 1976–77. The length of the wing from its insertion at the mesothorax to its distal margin, exclusive of the fringe scales, was measured for samples of up to 25 females per species per week at 20X using an ocular micrometer. To ensure that wing-length was representative of body size, wing-length and live-weight was determined individually for 25 newly emerged, unfed females of each species reared in the insectary at various densities.

RESULTS

LABORATORY LT_{50} DETERMINATIONS. There was significant heterogeneity about the log time–probit mortality regression lines, and the LT_{50} values in Table 1 are therefore only approximations. However, it is clear that heavier RR adults exhibited decreased susceptibility to dieldrin (Table 1). The lightest, unfed females showed a high percentage mortality at all exposure times, but the extrapolated LT_{50} of 3.5 hr was greater than the 2 hr exposure time used to discriminate RR from RS females. The values for the remaining size classes progressively increased to 9 hr for the heaviest unfed females tested. Although males were consistently lighter than females in each size class, their LT_{50} values approached the range of LT_{50} values observed for heavier females.

Females more than doubled their body-weight after imbibing a replete blood meal and concomitantly their LT_{50} for dieldrin increased by a factor of 5 for the lightest class and by a factor of 2 for the remaining classes (Table 1). Consistent with these results was the decrease in susceptibility to 4% dieldrin of RS females as a result of blood feeding (Table 2).

TEMPORAL CHANGES IN BODY-SIZE. Mean body-size, as indicated by wing-length, was inversely correlated with water temperature measured at one breeding site (*An. culicifacies*, $r = -0.87$, $n = 44$, *An. stephensi*, $r = -0.89$, $n = 41$, $p < .01$) (Fig. 1). The wings of overwintering females were almost twice as long as the wings of females emerging during the hot, dry pre-monsoon season. Wing-length was considered a valid index of female body-size for both species, and the coefficients of body-weight (m) regressed as a linear function of wing-length (l) significantly differed from 0 when tested by analysis of variance (*An. culicifacies:* $m = -1.243 + 0.700\ 1$, $r^2 = 0.788$; *An. stephensi:* m =

Table 1. Number of *Anopheles culicifacies* dead/number tested for each weight category and time of exposure to 4% dieldrin and the calculated LT_{50} for each category of the homozygous resistant genotype.

Status and weight of mosquitoes	Time of exposure (in hours)											LT_{50}
	5	6	6½	7	8	9	10	12	13	14	16	
Unfed ♀ < 0.9 mg	43	100		97	42							3.5
	56	124		115	42							
Unfed ♀ 0.9 – 1.09 mg	8	18	22	48	38	50	25					7.0
	56	63	71	126	50	50	25					
Unfed ♀ 1.1 – 1.3 mg	0	24	19	42	41	23	111	25		27		8.0
	25	123	72	179	87	35	129	30		27		
Unfed ♀ > 1.3 mg		9		10	9	19		27				9.0
		28		62	24	49		29				
Blood fed ♀ < 1.9 mg				0	2		3	3	16	27	16	16.2
				20	16		26	97	88	73	24	
Blood fed ♀ 1.9 – 2.09 mg				0	0	1		6	1	5	17	19.0
				20	39	18		53	51	55	43	
Blood fed ♀ 2.1 – 2.29 mg					0	0	2	7		5	26	19.3
					20	20	87	58		76	79	
Blood fed ♀ 2.3 – 2.49 mg							0	4	3	10	16	24.2
							44	23	30	82	90	
Blood fed ♀ > 2.5 mg							1	0	1	3	7	22.9
							19	54	57	25	47	
Unfed ♂ < 0.8 mg	32	47		7	84	25						3.6
	45	67		8	96	29						
Unfed ♂ 0.8 –1.0 mg	3	20	3	54	48	47	22	12				8.1
	57	131	25	100	105	59	30	26				
Unfed ♂ > 1.0 mg	1	3	8	9	7	17	29	28				9.1
	32	50	50	85	38	30	51	29				

Table 2. Number dead/number tested for each concentration of dieldrin and time of exposure and the calculated LT_{50} for each condition of heterozygous and homozygous susceptible female *Anopheles culicifacies*.

Status and weight of mosquitoes	Time of exposure (in hours)											LT_{50}
	0.25	0.33	0.5	0.75	1.0	1.17	2.0	3	4	5	6	10	
Unfed RS ♀	2		30	27	29								0.35
4% dieldrin	28		32	27	29								
Blood fed RS ♀		20	74		23	56	50						0.51
4% dieldrin		108	135		37	56	50						
Blood fed RS ♀								1	3	10	7	64	6.64
0.4% dieldrin								43	41	40	49	66	
Blood fed SS ♀	30		30	30	30								<0.25
0.4% dieldrin	30		30	30	30								

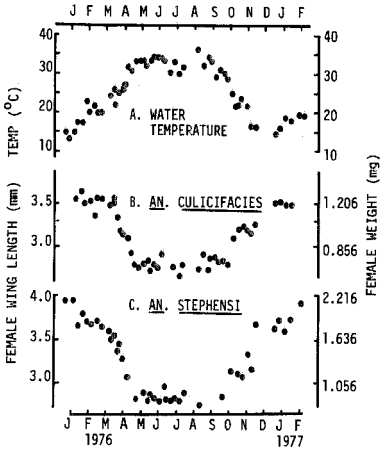

Fig. 1. Seasonal variation in water temperature at one breeding site and the wing-lengths of *Anopheles culicifacies* and *An. stephensi* collected near the village of Sattoki. Included on the right scale are the live-weight equivalents calculated from the fitted weight-wing length regression expressions.

$-2.423 + 1.160\ 1$, $r^2 = 0.712$, df = 1, 23, $p < 0.001$). All *An. culicifacies* females used in the laboratory tests were within the size ranges observed in nature.

DISCUSSION

Changes in adult body-size and trophic status do greatly affect longitudinal measurements of insecticidal LT_{50}'s. However, all SS females were killed by the minimum test time (15 min) on 0.4% dieldrin whereas 4 hr gave negligible mortality for RS, but none of these survived 2 hr on 4% dieldrin. Therefore it is apparent that although LT_{50} estimates are affected by physiological and rearing procedures, discriminating doses are not—in the case of such clear-cut intermediately dominant resistance as found for dieldrin in *An. culicifacies*. Where there is fail-safe discrimination between susceptible and resistant genotypes adult trophic status and body-size variation will not be a problem. There are two instances where such variation could become important: 1) where the degree of resistance is low and there is no well defined discriminating dose as in some DDT-resistances e.g. *An. sundaicus* (Davidson 1957); 2) serial exposures of susceptible adults in bioassay tests to assess the persistence of an insecticide. In each case small adults would give misleading data on the presence of resistance in the test populations and the effectiveness of the insecticide against wild populations of this species.

Our data show that such size variation does occur in the field in response to changes in water temperature. High water temperatures during June and July in Pakistan also correspond with dry conditions which restrict the number of breeding sites available. Intense competition within breeding sites and high water temperatures will create similar conditions to those we produced in the laboratory for varying body size in *An. culicifacies*. The apparent changes in susceptibility status of field populations of mosquitoes may reflect these differences in larval conditions and should be confirmed in the laboratory using uniformly reared mosquitoes of known age and trophic status. Even in the laboratory, care must be taken to ensure that sequential tests are not affected by variations in rearing.

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EFFECTS OF A NEW INSECT GROWTH REGULATOR, UC-62644, ON TARGET CHIRONOMIDAE AND SOME NONTARGET AQUATIC INVERTEBRATES[1]

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ABSTRACT. A new IGR, UC-62644, was bioassayed in the laboratory against 4th-instar larvae of *Glyptotendipes paripes* and *Chironomus decorus*. A 25% WP of this IGR was tested against midges in experimental ponds at 25, 50 and 100 g AI/ha (5.5–22.0 ppb), and in a golf course pond at 100 g AI/ha or 16.0 ppb. Impact of UC-62644 on nontarget invertebrates in the midge habitats was also studied.

The IGR caused 90% mortality of *G. paripes* and *C. decorus* at 3.1–5.7 ppb. In experimental ponds, the WP produced an excellent control of midges. Even the lowest dose induced 99% inhibition of total midge emergence, and control lasted for more than 4 wk. In the golf pond, 56–98% of the total emergence was suppressed for 4 wk. The treatments also caused significant mortality of midge larvae.

In experimental ponds, Rotifera, *Cyclops* spp., *Daphnia* spp., *Chaoborus* sp., *Baetis* sp., corixids, notonectids, and coleopterous larvae and adults were affected but most of these nontarget invertebrates recovered within 2–3 wk after treatment except for *Cyclops* spp. and possibly corixids and beetles. Rotifers, ostracods, and oligochaetes in golf pond were not affected but *Cyclops* spp. and *Hyalella azteca* (Saussure) were sensitive to the IGR.

UC-62644 is the most effective IGR thus far tested against chironomid midges and has moderate and temporary adverse effects on the nontarget aquatic invertebrates.

INTRODUCTION

In the past decade a number of insect growth regulators (IGRs) have been evaluated against aquatic chironomid midges (Ali and Mulla 1977a,b; Ali et al. 1978, Ali and Lord 1980a, Mulla and Darwazeh 1975, Mulla et al. 1974, 1976). These nonbiting midges pose a variety of nuisance and economic problems in many parts of the United States and abroad (Ali 1980).

Among the various IGRs tested against a number of chironomid species, methoprene, diflubenzuron, and Bay SIR-8514 have shown the most activity under laboratory and field conditions (Ali and Lord 1980a, Mulla and Darwazeh 1975, Mulla et al. 1974, 1976). These compounds are especially useful in areas where conventional chemicals are either ineffective due to build up of resistance

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