LABORATORY AND FIELD INVESTIGATIONS IN CZECHOSLOVAKIA WITH FENITROTHION, PIRIMIPHOS-METHYL, TEMEPHOS AND OTHER ORGANOPHOSPHOROUS LARVICIDES APPLIED AS SPRAYS FOR CONTROL OF *CULEX PIPIENS MOLESTUS* FORSKAL AND *AEDES CANTANS* MEIGEN

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ABSTRACT. Eighteen organophosphorous (o-p) insecticides and DDT, as either emulsifiable concentrate or wettable powder formulations, were evaluated for larvicidal activity under laboratory conditions simulating natural breeding sites in Czechoslovakia. All 18 compounds were tested against *Culex pipiens molestus*, and 8 o-p's and DDT against *Aedes cantans*. Temephos and chlorpyrifos were the most toxic to *C. p. molestus* larvae (LC_{100} 0.002 ppm). Bromophos, pirimiphos-methyl and coumaphos were also highly toxic (LC_{100} 0.01 ppm). Among 8 o-p's tested under field conditions (flood plain forest), temephos (LC_{100} 0.005 ppm), chlorpyrifos and bromophos (LC_{100} 0.01 ppm) and pirimiphos-methyl and fenitrothion (LC_{100} 0.02 ppm) had the highest larvicidal action against young 4th-instar larvae of *Ae. cantans*.

The initial larvicidal activity of o-p compounds was reduced by the presence of decaying organic material. However, mud and water plants exerted only a negligible influence upon initial kill by fenitrothion, pirimiphosmethyl and temephos.

The LC_{100} range for temephos, pirimiphos-methyl and fenitrothion against all larval instars of *Ae. cantans* was 0.005–0.02, 0.02–0.05 and 0.02–0.1 ppm, respectively. Pupae showed low sensitivity (LC_{100} 0.5 ppm) to these compounds.

The residual effectiveness of fenitrothion, pirimiphos-methyl and temephos applied at doses giving close to 100% kill of *Ae. cantans* larvae was 3, 5 and 3–7 days, respectively. Under laboratory conditions simulating natural breeding sites, the residual effectiveness approached these values when water temperatures were 20°C. Presence of water plants greatly reduced residual effectiveness.

When fenitrothion, pirimiphos-methyl and temephos were used at doses giving close to 100% control of the young 4th-instar *Ae. cantans* larvae, most nontarget organisms survived (*Rhynchelmis* spp. [a water worm], Mollusca [water snails], *Asellus aquaticus* [an isopod], *Hydrachna* spp. [water mites], *Gerris lacustris* [water striders] and Dytiscidae [predaceous diving beetles]). Only with *Chirocephalopsis grubii* (a phyllopod) was mortality 100%. Trichoptera (caddis fly) larvae were very sensitive to fenitrothion and pirimiphos-methyl, as were *Cyclops* spp. (copepods) and *Daphnia* spp. (water fleas) to temephos and pirimiphos-methyl. The latter insecticide was also highly toxic to Ostracoda.

INTRODUCTION

In spring or after summer floods great numbers of *Aedes* mosquitoes appear in low-lying areas of Czechoslovakia and are a nuisance to inhabitants and domestic animals, even at considerable distances from the breeding sites (Trpiš, 1962, Palička, 1967, Hájková and Minãr 1970, Rettich 1971a).

Except where breeding sites are extremely widespread, *Aedes* mosquitoes are most effectively controlled in the larval stages. In addition, larval control of *Culex pipiens molestus*, an increasing nuisance pest in urban areas, is more economical than adult control by residual wall spraying in houses.

Recently chlorinated hydrocarbons were replaced in mosquito control programs by pesticides in other chemical groups, especially by certain organophosphorous (o-p) insecticides. The o-p compounds selected are safer to humans and to the environment and can effectively replace the chlorinated hydrocarbons (Rettich 1971b).

Granular formulations of o-p larvicides

such as bromophos-ethyl, fenitrothion and temephos have already been successfully applied in the Elbe lowlands for control of *Aedes cantans* (Rettich and Privora 1972, 1973, 1975). The purpose of the present study was to evaluate fenitrothion, pirimiphos-methyl, temephos and some other o-p compounds for larvicidal effectiveness when applied as sprays. Initial larvicidal action against *Ae. cantans* and *Cx. pipiens molestus*, residual effectiveness, and effects on nontarget organisms in the treated water were recorded.

MATERIALS AND METHODS

Emulsifiable concentrate (EC) and wettable powder (WP) formulations of the insecticides tested and dosages used in both laboratory and field trials are presented in table 1.

LABORATORY TESTS. A slightly modified standard WHO test (WHO 1963) was used to estimate the initial toxicity of insecticide preparations. In summary, 950 ml boiled tap water was measured into several 1-liter glass jars. Then, using a pipette, water emulsions or suspensions of insecticide preparations were added in 5 concentrations to form a geometric series with a quotient of 2. Each concentration was replicated. Fifteen minutes after thorough mixing, 30 *Cx. p. molestus* or *Ae. cantans* larvae in 50 ml water were added to each jar. A second set of test jars

Table 1. Laboratory studies of susceptibility of *Culex pipiens molestus* and *Aedes cantans* larvae to various insecticides added to tap water or in tap water containing decaying leaves, mud and water plants.

Insecticide and formulation	Lethal concentration (ppm a.i.), LC_{50}/LC_{90} At 24 hours	At 48 hours
Culex pipiens molestus		
	Plain tap water	
Chlorpyriphos 50% EC	0.0005 / 0.0008	0.0002 / 0.0004
Temephos 50% EC	0.0007 / 0.0017	0.0005 / 0.0012
Bromophos 25% WP	0.0016 / 0.0035	0.0008 / 0.0024
Pirimiphos-methyl 50% EC	0.0042 / 0.0112	0.0028 / 0.0093
Fenitrothion 50% EC	0.0078 / 0.0148	0.0048 / 0.0099
DDT (standard) 30% EC	0.0086 / 0.0282	0.0072 / 0.0190
Malathion 30% EC	0.0303 / 0.0935	0.0240 / 0.0758
	Decaying leaves added	
Chlorpyriphos 50% EC	0.0012 / 0.0030	0.0015 / 0.0024
Temephos 50% EC	0.0019 / 0.0043	0.0008 / 0.0034
Pirimiphos-methyl 50% EC	0.0047 / 0.0146	0.0036 / 0.0142
Bromophos 25% WP	0.0101 / 0.0465	0.0061 / 0.0249
Fenitrothion 50% EC	0.0170 / 0.0774	0.0110 / 0.0390
	Mud added	
Temephos 50% EC	0.0008 / 0.0017	0.0005 / 0.0010
Pirimiphos-methyl 50% EC	0.0038 / 0.0081	0.0031 / 0.0071
Fenitrothion 50% EC	0.0070 / 0.0205	0.0050 / 0.0135
	Water plants added	
Temephos 50% EC	0.0006 / 0.0015	0.0005 / 0.0011
Pirimiphos-methyl 50% EC	0.0048 / 0.0116	0.0039 / 0.0106
Fenitrothion 50% EC	0.0136 / 0.0346	0.0054 / 0.0178
Aedes cantans		
	Plain tap water	
Temephos 50% EC	0.0010 / 0.0021	0.0009 / 0.0018
Pirimiphos-methyl 50% EC	0.0020 / 0.0040	0.0016 / 0.0029
Fenitrothion 50% EC	0.0037 / 0.0061	0.0016 / 0.0044
DDT (standard) 30% EC	0.0050 / 0.0135	0.0042 / 0.0102

was similarly prepared, using water containing decaying leaves (100 ml/jar), mud (50 ml/jar) and water plants (*Myriophyllum* and *Elodea*). Water temperature fluctuated between 20°C and 22°C during the tests. After 24 and 48 hr, larval mortality was recorded and the LC_{50} and LC_{90} values were calculated.

In laboratory tests under simulated natural breeding conditions the larvicidal action of the 22 insecticide formulations listed in table 2 was evaluated for control of *Cx. p. molestus* larvae. In these tests the containers used were 10-liter aquariums containing a 2- to 3-cm deep layer of decaying leaves. Water emulsions or suspensions of insecticides were pipetted into the aquariums 24 hr after setting them up, and each insecticide was tested at several concentrations to form a geometric series with a quotient of 2. A total of 30 *Cx. p. molestus* larvae was added to each aquarium, larval mortality was determined 3 days later and from these results the minimum LC_{100} dose was calculated.

The residual effectiveness of the insecticides was studied in the laboratory using water containing decaying organic material; water temperatures were 5°C and 20°C. Similar studies were conducted using tap water containing the water plants *Elodea* and *Myrophylium*. At regular intervals 0.5-liter water samples were removed to another container to which 20 *Cx. p. molestus* larvae were added. The insecticide was considered ineffective when at least 20% of the larvae survived 3 days exposure at 20–22°C.

All tests were compared with untreated control groups. When mortality in the controls was above 20%, the test was repeated.

FIELD TESTS. Small field tests were conducted in the Elbe region in a flood-plain forest near Podebrady in test pools containing a bottom cover of leaves but otherwise almost free of any aquatic vegetation. The water in the pools during field testing (February-May) was relatively clean and neutral to slightly acid (pH 6.4–7).

In the Elbe region mosquito larvae ap-

Table 2. Minimum LC_{100} values of test insecticides against 4th-instar *Culex pipiens molestus* larvae under laboratory conditions simulating natural breeding sites and against young 4th-instar *Aedes cantans* larvae under field conditions.

Insecticide and formulation	Minimum LC_{100} (ppm a.i.) *Culex pipiens molestus* (laboratory)	*Aedes cantans* (field)
Temephos 20% EC	0.002	—
50% EC	0.002	0.005
Chlorpyrifos 40% EC	0.002	0.01
Bromophos 25% WP	0.01	0.01
Pirimiphos-methyl 50% EC	0.01	0.02
Coumaphos 25% WP	0.01	—
Fenitrothion 50% EC	0.02	0.02
Bromophos-ethyl 40% EC, 60% EC	0.02	—
Trichlormetaphos-3 50% EC	0.02	—
Tetrachlorvinphos 24% EC	0.05	—
Chlorfenvinphos 24% EC	0.02	—
Fenchlorfos 66% EC	0.05	—
Diazinon 60% EC	0.05	0.1
Malathion 35% EC	0.05	0.2
Dichlorvos 50% EC	0.05	—
Trichlorphon 25% EC	0.1	0.1
Crotoxyphos 24% EC	0.2	—
Naled 50% EC	0.5	—
Dimethoate 5% WP	0.5	—
DDT (standard) 30% EC, 50% WP	0.05	0.02

pear during March, and their development through the larval and pupal stages is completed by the beginning of May; *Ae. cantans* is the predominant mosquito species. Fenitrothion, pirimiphos-methyl and temephos were applied during the period of 1st, 2nd, 3rd and 4th larval instars and the pupal stage of *Ae. cantans;* other insecticides were applied when larvae were in the 4th instar.

Emulsion and suspension sprays containing 1% a.i. were prepared with the EC and WP formulations and applied with a hand sprayer. The total water volume in the breeding pools was estimated by multiplying the surface area by the mean depth. The size of smaller test pools ranged from 4–100 m^2, with mean depths of 10–40 cm. The insecticides were applied to larger pools with a Stihl SG-17 knapsack mistblower with an output of 0.5 liter/min.

The numbers of 3rd- and 4th-instar larvae and pupae were determined immediately before application and again on the 3rd day after by dipping larvae from 1–10 dm^2 water surface with a wire strainer. The number of 1st- and 2nd-instar larvae in 500 ml water taken from the edges of breeding pools were counted.

Residual effectiveness of larvicides in the field was determined by bioassay. Three-liter samples of treated pool water were put in glass jars and about 50 *Ae. cantans* larvae were added to each jar. The insecticide was considered ineffective when at least 20% of the larvae survived 3 days exposure.

Effects on some commonly occurring nontarget water organisms were also observed during these tests. In contrast to the mosquito tests, only visual observations were recorded; no actual counts were made.

RESULTS

Of 6 o-p pesticides tested in plain tap water, chlorpyrifos was the most toxic to 4th-instar *Cx. p. molestus* larvae (table 1). Temephos and bromophos ranked next as very toxic to this species. These 3 insecticides as well as pirimiphos-methyl and fenitrothion were more toxic than DDT, the standard. Malathion had significantly less larvicidal action than the other o-p compounds tested.

The larvicidal action of temephos, pirimiphos-methyl and fenitrothion for control of *Ae. cantans* larvae was also significantly higher than that of DDT. *Ae. cantans* larvae were more susceptible to fenitrothion, pirimiphos-methyl and DDT than *Cx. p. molestus;* however, *Ae. cantans* larvae were not quite as susceptible as *Cx. p. molestus* to temephos (table 1).

Of the 5 o-p compounds tested in tap water containing decomposing organic material, chlorpyrifos and temephos were significantly more toxic to *Cx. p. molestus* larvae than pirimiphos-methyl, bromophos and, especially, fenitrothion. LC_{50} and LC_{90} values of chlorpyrifos, temephos, bromophos and fenitrothion in these tests were 2 to 8 times higher than in plain tap water (table 1). Toxicity of pirimiphos-methyl in tap water and in water with decomposing organic material was approximately the same. Presence of mud or water plants did not influence the larvicidal action of temephos and only slightly reduced the action of fenitrothion and pirimiphos-methyl (table 1).

Of the 18 o-p compounds tested under laboratory conditions simulating natural breeding sites of mosquitoes, the most effective for control of 4th-instar *Cx. p. molestus* larvae were temephos and chlorpyrifos (table 2). Bromophos, pirimiphos-methyl and coumaphos ranked next as very toxic; less toxic were fenitrothion, bromophos-ethyl, trichlormetaphos-3 and chlorfenvinphos. Tetrachlorvinphos, fenchlorphos, malathion and dichlorvos had low larvicidal action, as did DDT. Particularly low larvicidal action was obtained with trichlorphon, crotoxyphos, naled and dimethoate.

The larvicidal action of the 8 o-p pesticides tested under field conditions in spring for control of young 4th-instar *Ae. cantans* larvae was similar to that obtained under laboratory conditions (table 2). Temephos again had the highest larvicidal activity, although the LC_{100} minimum dosage of 0.005 ppm was higher than the minimum LC_{100} for *Cx. p. molestus*. Next in rank as highly toxic, were chlorpyrifos, bromophos, fenitrothion and pirimiphos-methyl. DDT, used for comparison, was also highly toxic. Diazinon, trichlorphon and malathion were considerably less toxic.

LC_{95} and LC_{100} doses of fenitrothion, pirimiphos-methyl and temephos against all larval instars and pupae of *Ae. cantans* are summarized in table 3. The larvicidal action of temephos was approximately twice that of pirimiphos-methyl and fenitrothion. Susceptibility of pupae to all 3 insecticides was low.

In laboratory tests in the presence of decomposing organic material, residual effectiveness was longer at 5°C than at 20°C for temephos, fenitrothion, bromophos and DDT and only slightly longer for pirimiphos-methyl (table 4). At 20°C in the presence of decomposing organic material or water plants the o-p residual effectiveness approached that of the o-p's tested in the field in springtime when water temperatures generally are not above 15°C. Residual effectiveness was very short under field conditions at temperatures around 20°C in summer, with the higher concentrations of insecticides giving proportionally longer residuals than the lower. Pirimiphos-methyl, bromophos and, especially, temephos were more persistent under field conditions than fenitrothion. The residual effectiveness of DDT at 0.25 ppm (7 days) was usually shorter under laboratory conditions than that of the o-p compounds studied; under field conditions, however, the DDT residual was similar to bromophos and pirimiphos-methyl, longer than that of fenitrothion, but shorter than that of temephos. Residual effectiveness of temephos at 0.01 ppm and fenitrothion at 0.02 ppm in tap water at 5°C was more than 300 days.

The effects of fenitrothion, temephos and pirimiphos-methyl on non-target organisms is shown in table 5. *Chirocephalopsis grubbi* (a phyllopod) was the most susceptible organism; it was killed by the lowest test concentration of fenitrothion (0.01 ppm) and temephos (0.005 ppm). Very susceptible were Trichoptera (caddisfly) larvae, especially to fenitrothion. Fenitrothion was least toxic to *Cyclops* spp. (copepods); a part of these populations survived doses of 0.1 ppm. Temephos significantly reduced populations of *Cyclops*, even at 0.02 ppm, and primiphos-methyl killed some *Cyclops* at 0.1 ppm and all at 0.2 ppm. Mortality of *Daphnia* spp. (water fleas) was 100% with doses of 0.02 ppm pirimiphos-methyl, 0.1 ppm temephos and 0.2 ppm fenitrothion. The limiting doses for Dytiscidae adults (predaceous diving beetles) to the 3 insecticides varied between 0.1 and 0.2 ppm; the most toxic was pirimiphos-methyl, with fenitrothion the least toxic. *Asellus*

Table 3. Toxicity of temephos, pirimiphos-methyl and fenitrothion to preimaginal stages of *Aedes cantans* under field conditions.

Larval or pupal stage	Water temperature (°C)	LC_{95} / LC_{100} Temephos (ppm a.i.)[1]	Pirimiphos-methyl (ppm a.i.)	Fenitrothion (ppm a.i.)
Larval instar				
1st	2– 6	0.01 / 0.02	0.02 / 0.05	0.02 / 0.05
2nd	6–13	0.01 / 0.02	0.01 / 0.05	0.01 / 0.02
3rd	8–16	0.005 / 0.01	0.02 / 0.05	0.01 / 0.02
Young 4th	8–16	<0.005 / 0.005	0.01 / 0.02	0.01 / 0.02
Old 4th	10–20	0.005 / 0.01	0.02 / 0.05	0.08 / 0.1
Pupal	10–20	0.2 / 0.5	0.2 / 0.5	0.2 / 0.5

[1] Formulation used, 50% EC.

aquaticus, an isopod, was more resistant than Dytiscidae but susceptible to fenitrothion (LC_{100} 0.2 ppm). All *Hydrachna* spp. (water mites) survived 0.1 ppm temephos and pirimiphos-methyl and 0.2 ppm fenitrothion; Ostracoda survived even the high doses of fenitrothion and temephos (2.5 ppm) but some were killed by pirimiphos-methyl at doses as low as 0.05 ppm. *Rhynchelmis* ssp. (a water worm) and water snails were not killed, even by the highest concentrations of the larvicides tested.

DISCUSSION

The high larvicidal activity of temephos EC formulations (LC_{100} 0.005 ppm) obtained in this study conforms with our earlier results with a 1% granular formulation (Rettich and Privora 1973), where the LC_{100} for *Ae. cantans* and *Ae. cinereus* larvae was 0.007 ppm. The effectiveness of temephos against mosquito larvae has also been reported by other investigators, including von Windeguth et al. 1971 and Gassabi et al. 1973 (WHO, Unpublished) Krivcova (1970) obtained good results with fenitrothion and bromophos against *Aedes* spp. larvae in the colder climates of Jakutsk, USSR. Mulla et al. (1973) reported 100% control of *Cx. tarsalis* and *Cx. peus* larvae with pirimiphos-methyl at 0.05 lb/acre, which roughly corresponds to the dosage used in the Czechoslovakian studies.

Table 4. Residual effectiveness (in days) of temephos, pirimiphos-methyl, fenitrothion and bromophos under laboratory and field conditions against *Culex pipiens molestus*.

Insecticide and formulation	Concentration (ppm)	Laboratory tests: Decaying leaves added 5°C	Laboratory tests: Decaying leaves added 20°C	Laboratory tests: Water plants[1] added 20°C	Field tests[2] Spring	Field tests[2] Summer
Temephos 50% EC	0.005	14	3	3	3	
	0.01	14	7	7	7	
	0.025	14	7	7–21	7	
	0.05	30	21	7–21	7	2
	0.1	60	30	14–21	30	2
	0.2	120	60	14–21	30	21
Pirimiphos-methyl 50% EC	0.025	7	7	7	5	
	0.05	7–14	7	10	5	
	0.1	14	14	10	14	
	0.2	21	30	21	14	
Fenitrothion 50% EC	0.025	7–14	1	3	3	
	0.05	14	3	7	3	
	0.1	14–30	3	7	7	2
	0.2	60	7	14	7	
	0.5	60	7	14	14	7
Bromophos 25% WP	0.025	3	3	3	—	
	0.05	30	3	3	7	
	0.1	30–60	7	7	14	
	0.2	60	7	14	14	
DDT 30% EC	0.25	7	7	7	14	

[1] *Elodea* and *Myrophylium* were used.
[2] The insecticide was considered ineffective when at least 20% of the larvae survived 3 days' exposure.

The susceptibility values of *Ae. cantans* to temephos, pirimiphos-methyl and fenitrothion and of *Cx. p. molestus* larvae to chlorpyrifos and temephos obtained in the laboratory in plain tap water do not correspond to our previous findings (Rettich 1977). The toxicity of EC formulations was higher than that of ethanol solutions. Also, the LC_{95} and LC_{100} values of the EC formulations of temephos, pirimiphos-methyl and fenitrothion from the field are higher than could be anticipated from laboratory tests, apparently due to low temperature and the high content of organic material present in the flood plain forest breeding-site water. In the case of testing for control of 1st and 2nd instars in the field where new larvae were additionally hatching, higher concentrations than those found effective in the laboratory (Rettich 1976) were necessary to increase the residual effect of insecticides. The low susceptibility of pupae to o-p's in our study is consistent with the findings of Roberts et al. 1969; Porter and Gojmerac 1969, Bhatnagar et al. 1969, Gaaboub et al. 1971 (WHO, Unpublished) and others.

Table 5. Effect of fenitrothion, temephos and pirimiphos-methyl on some nontarget organisms.

Insecticide concentration (ppm a.i.)	*Rhynchelmis* spp. (fresh water turtles)	Mollusca (water snails)	*Chironomid*... *Chirocephalopsis grubii* (a water mite)	*Cyclops* spp. (copepods)	*Daphnia* spp. (water fleas)	Ostracoda	*Asellus aquaticus* (an isopod)	*Hydrachna* spp. (water mites)	Trichoptera (caddis fly) larvae	*Gerris lacustris* (a water strider)	Dytiscidae[1] larvae	Dytiscidae[1] adults
Fenitrothion 50% EC												
0.01	+	+	−	+	+	+	+	+	x	+	+	+
0.02	+	+	−	+	+	+	+	+	x	+	+	+
0.05	+	+	−	+	+	+	+	+	−	+	+	+
0.1	+	+	−	+	x	+	x	+	−	x	−	+
0.2	+	+	−	+	−	+	−	+	−	−	−	x
0.5	+	+	−	x	−	+	−	x	−	−	−	−
1.0	+	+	−	x	−	+	−	x	−	−	−	−
2.5	+	+	−	x	−	+	−	x	−	−	−	−
Temephos 50% EC												
0.005	+	+	−	+	+	+	+	+	+	+	+	+
0.01	+	+	−	+	+	+	+	+	+	+	+	+
0.02	+	+	−	x	+	+	+	+	+	+	+	+
0.05	+	+	−	−	x	+	+	+	+	+	+	+
0.1	+	+	−	−	−	+	+	+	−	+	−	+
0.5	+	+	−	−	−	+	x	x	−	+	−	−
2.5	+	+	−	−	−	+	−	x	−	+	−	−
Pirimiphos-methyl 50% EC												
0.02	+	+		+	−	+	+	+	+	+	+	+
0.05	+	+		+	−	x	+	+	−	+	+	+
0.1	+	+		x	−	x	+	+	−	x	+	x
0.2	+	+		−	−	−	+		−	x		−
0.5	+	+		−	−	−			−	−		−

+=All organisms survived.
x=Some organisms survived.
−=All organisms killed.
[1] Predaceous diving beetle.

Length of residual effectiveness of insecticide preparations corresponds directly with the concentration used and indirectly with water temperature and pollution. In laboratory tests insecticides were effective for significantly longer periods at 5°C than at 20°C, with pirimiphos-methyl and, in particular, temephos effective longer than fenitrothion, bromophos or DDT. Presence of water plants also reduces residual effectiveness. In field tests a combination of these conditions was encountered; residual effectiveness during the spring was much longer than in the summer, probably because of warmer water temperatures and higher organic matter content of the water in summer.

The importance of water pollution on initial toxicity and residual activity of larvicides has been confirmed by many investigators, including Mulla 1963, Bransby-Williams 1965, Womeldorf et al. 1969 and Schaefer and Dupras 1969.

O-p larvicides must be selected and applied with care not only for efficacy against the species of mosquito to be controlled but also in relation to nontarget organisms in the aquatic environment. However, with some nontarget organisms such as *Chirocephalopsis grubii*, 100% kills may result, no matter which larvicide is used, while with other nontarget organisms, such as Trichoptera and *Cyclops* spp., the extent of kill will depend to a great extent on the o-p larvicide selected.

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LABORATORY OBSERVATIONS ON THE TIME OF MATING OF *ANOPHELES CULICIFACIES* GILES

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ABSTRACT. Under insectary conditions the Sattoki laboratory strain of *Anopheles culicifacies* emerged during late afternoon and early evening. Mating occurred rhythmically, primarily during the simulated dusk with a few matings occurring during dawn. Females were sexually mature by the dusk of their 2nd day of life, whereas males required an extra day to attain maximum maturity. Maximum male mating activity on day 3 was followed by a rest interval, after which mating activity was again renewed on day 7.

Knowledge of the reproductive behavior of the target species is a vital aspect of any genetic control research. In 1975 *Anopheles culicifacies* Giles, the primary vector of human malaria in the Indo-Pakistan subcontinent, was successfully colonized at our laboratory (Ainsley 1976), and recently several chromosomal aberrations have been induced (Baker et al. 1978). As a prelude to planned field experimentation, studies on the reproductive behavior of this species have been initiated to develop protocols for the manipulation and release of genetically engineered adults.

Previous studies on the swarming and mating behavior of *An. culicifacies* have been restricted to observations on an outdoor insectary population by Russell and Rao (1942) and unconfined wild populations by Reisen and Aslamkhan (1976) and Reisen et al. (1977). In Pakistan pairing was commonly observed at male swarms during winter, but was rarely seen during summer when swarms occurred later in relation to sunset, leading Reisen et al. (1977) to suggest that mating may be occurring elsewhere. However, casual observations under "midsummer" insectary conditions indicated that mating pairs were always formed in flight during periods of male activity.

The purpose of the present series of experiments was to describe the time of mating in a laboratory-adapted colony of *An. culicifacies* under summer day-length conditions to ensure that mating was not occurring at other times of the diel. Additional observations on the time of emergence and the age of males and females at sexual maturity were also included and are of considerable importance in devising release procedures.

METHODS AND MATERIALS

STRAIN. The Sattoki, Pakistan, strain of *An. culicifacies* originally colonized by