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LABORATORY EVALUATION OF TWO ORGANOPHOSPHORUS LARVICIDES AGAINST PUPAE OF *CULEX RESTUANS* THEOBALD AND INFLUENCE ON ADULT LONGEVITY

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INTRODUCTION. The availability of a compound which would kill both larvae and pupae would be of significant aid in mosquito control operations since it would make it possible to reduce the numbers of pupae as well as of larvae and adults of existing mosquito populations. None of the organophosphorus larvicides are known to kill pupae at larvicidal rates (Mulla, 1966). The characteristic quiescence of mosquito pupae affords considerable protection against larvicides in solution. This study was undertaken to determine the amount of protection and the factors influencing the degree of protection provided mosquito pupae against organophosphorus larvicides. Specific objectives were to determine the percent pupal mortality at different concentrations of Abate® (0,0,0′0′-tetramethyl 0,0′-thiodi-p-phenylene phosphorothioate) and Dursban® (0,0-diethyl 0–3,5,6-trichloro-2-pyridyl phosphorothioate) on mosquito pupae of different ages and to ascertain the influence on longevity of adults that emerged from pupae exposed to the larvicides.

MATERIALS AND METHODS. Laboratory tests were conducted against *Culex restuans* Theobald pupae. The pupal stage of this species lasts approximately 50 hours under laboratory conditions. Due to the large numbers of larvae needed to provide pupae for testing, plots established in the field were relied upon to furnish the test specimens. Ideal oviposition sites for *C. restuans* were produced in the field plots by adding one cup of Wayne Rabbit Ration,[3] produced by Allied Mills, Chicago, Illinois, to approximately 8 cubic feet of water every 2 weeks. Larvae were collected from the field plots and were brought to the laboratory where they were placed in white enamel trays and fed on Wayne Rabbit Ration.

The larvicides were evaluated against different age groups of pupae. The five age groups tested were (1) pupae 0–1 hour, (2) pupae 0–2 hours, (3) pupae 0–5 hours, (4) pupae 0–24 hours, and (5) pupae greater than 24 hours old. The pupae were separated into age groups by hand picking all pupae from the enamel trays at a given hour, (hour 0). All larvae that pupated from hour 0 to to hour X (hour X representing the age group desired) were picked and tested. For example, if a group 0–24 hours of age was desired, hour X was 24 hours. When a group greater than 24 hours old was desired, those pupae picked at 24 hours were held over an additional 24 hours prior to testing.

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[3] Mention of a proprietary product does not necessarily imply endorsement of this product by the United States Army.

Pupae obtained for one series of tests were separated by using the ice water method for harvesting mosquito pupae as described by Hazard (1967). Erratic results and high pupal and adult mortality in the controls discouraged further use of this method for collecting pupae.

The test procedure consisted of mixing formulations (w/w) of the insecticides in distilled water and making serial dilutions to obtain the desired dosage in 1000 milliliters of water. Eighty milliliters of treated water were poured into each of three 100 milliliter glass beakers and 50 pupae of a known age group were then added to each beaker to comprise one treatment. The three beakers were placed inside a screened, metal framed, 10 x 10 x 14 inch cage in order to obtain data on adult longevity as well as pupal mortality. A screen funnel was inverted over each beaker to prevent adults from returning to the water surface after emergence. Cotton pads saturated with a 4 percent sugar water solution were provided as a food source to emerging adults. A total of 22 tests were conducted in an insectary maintained at 75° F. and approximately 60 percent relative humidity.

Seventy-two hours after each treatment was begun, the dead pupae were counted, collected, and preserved in 95 percent ethyl alcohol. The 72-hour period provided sufficient time for emergence or for the pupae to succumb to the larvicides. Dead pupae were examined microscopically to determine if they had an obvious split along the dorsal longitudinal midline of the cephalothorax. This character indicated that the initial stage of emergence had begun. The number of dead adults was also recorded at the end of the 72-hour period.

RESULTS AND DISCUSSION. Test results indicated that the age of pupae and/or the length of exposure time to the larvicides had a direct effect on pupal mortality. The highest pupal mortality was present in the younger age groups of pupae. The mortality data for different concentrations of Abate and Dursban against variable age groups of *C. restuans* pupae are presented in Table 1. These data indicated that the greatest pupal mortality was obtained with lower concentrations of Dursban than with Abate. Dursban at 0.5 p.p.m. gave 100 percent pupal mortality against all age groups. For all age groups tested, the total kill of pupae and

TABLE 1.—Laboratory evaluation of Abate and Dursban against variable age groups of *Culex restuans* Theobald pupae.

Material	Concentration in p.p.m.	Age group in hours[a]	Percent mortality for 72 hour period		
			Pupae	Adults	Total
ABATE	2.5	<24[b]	72	28	100
	0.5	>24[b]	22	56	78
	0.5	<24[b]	62	39	100
	0.05	>24[b]	5	39	44
	0.05	< 1	92	8	100
DURSBAN	2.5	>24	90	10	100
	2.5	<24	100	..	100
	0.5	>24[b]	100	..	100
	0.5	<24[b]	100	..	100
	0.5	< 5	100	..	100
	0.05	>24	21	79	100
	0.05	<24	75	25	100
	0.025	>24	2	91	93
	0.025	< 2	32	67	99
CONTROL	0.0	<24	..	4	4
	0.0	Random	..	2	2

[a] Age group as given was the pupal age in hours prior to exposure to the larvicides.
[b] Average results from two replicates.

emerged adults averaged 89 percent for Abate at 0.5 p.p.m. and 96 percent for Dursban at 0.025 p.p.m. These data indicated a rate increase of 500 fold for Abate and 16 fold for Dursban over the larvicidal rates found by Lofgren, *et al.* (1967) to give an LC_{90} with larvae of *Culex pipiens quinquefasciatus* Say.

Data obtained from microscopic examination of dead pupae to determine the percentage of specimens that were in the initial stage of emergence prior to death are presented in Table 2. Data are presented only from those treatments which had greater than 60 percent pupal mortality. The split cephalothorax indicated that the adult mosquito was preparing to emerge. For all specimens with a split cephalothorax, the adult was observed to be fully developed within the pupal skin, thereby indicating that the pupa had survived through its stage of transformation.

An average of 37 percent of all pupae tested in the two age groups 0–24 hours and greater than 24 hours old was determined to have survived to the initial stage of emergence prior to succumbing to the larvicides. These data indicated that the pupal stage may have been relatively immune to the toxic effects of the organophosphorus larvicides until metamorphosis to the adult was complete and the adult was preparing to or was in the process of emerging.

SUMMARY. Laboratory tests were conducted on field-collected pupae of *Culex restuans* Theobald to determine pupal kill and adult die-off after 72-hour exposure to Dursban and Abate at concentrations of 2.5, 0.5, 0.05, and 0.025 p.p.m. The pupae were separated into five age groups; namely, 0–1 hour, 0–2 hours, 0–5 hours, 0–24 hours, and greater than 24 hours old. Fifty pupae from one age group added to 80 milliliters of treated water in each of three beakers comprised one treatment. At the end of the 72-hour exposure time, a count was made of the dead adults and the dead pupae. The dorsal longitudinal mid-line of the cephalothorax of all dead pupae was examined to determine if there was a split indicating that the initial stage of emergence had begun.

The age of pupae and/or length of exposure time to the larvicides had a direct effect on pupal mortality. For both larvicides tested the younger age group of pupae demonstrated the highest pupal mortality. Dursban was shown to give total pupal kill at 0.5 p.p.m. while Abate did not give total pupae kill at any of the concentrations tested. For all age groups tested the total kill of pupae and emerged adults averaged 89 percent for Abate at 0.5 p.p.m. and 96 percent for Dursban at 0.025 p.p.m. An average of 37 percent of all pupae tested in the two age groups 0–24 hours and greater than 24 hours old

TABLE 2.—Results from microscopic examination of dead *Culex restuans* Theobald pupae to determine the percentage of specimens that were in the initial stage of emergence prior to succumbing to the organophosphorus larvicides.

Material	Concentration in p.p.m.	Age group of pupae[a]	Percent pupae w/split in cephalothorax[b]	Percent pupae w/o split in cephalothorax
ABATE	0.5	<24	36	64
	0.05	< 1	12	88
DURSBAN	2.5	>24	30	70
	2.5	<24	46	54
	0.5	>24	38	62
	0.5	<24	36	64
	0.5	< 5	46	54
	0.05	<24	38	62

[a] Age group as given was the pupal age in hours prior to exposure to the larvicides.
[b] Initial stage of emergence was signaled by the presence of a split along the dorsal longitudinal mid-line of the cephalothorax.

was determined to have survived to the initial stage of emergence prior to succumbing to the larvicides. Test results indicated that up to the initial stage of emergence these pupae may have been protected from the toxic effects of the larvicides in solution.

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BLOOD MEALS AND EGG PRODUCTION OF *CULISETA ALASKAENSIS* (LUDLOW) IN CAPTIVITY (DIPTERA:CULICIDAE)

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INTRODUCTION. A laboratory investigation of the feeding capabilities of wild caught Alaskan mosquitoes was undertaken to determine their potential as biologic vectors of pathogens among animals. At least two blood meals would be required, with the interval between meals sufficient for multiplication of the pathogen in the mosquito, thus number of blood meals taken by individual mosquitoes, interval between meals, and interval between first and last meals were determined. Information was also obtained on: number of blood meals taken to produce each batch of eggs and interval between batches; number of eggs in each batch and total number of eggs and batches produced by each individual; lifetime of female in captivity; and interval between oviposition and hatching.

CONDITIONS AND EQUIPMENT. There was no natural light in the laboratory, which is underground. Overhead fluorescent lights were all turned on by day and one-quarter were on at night in an attempt to simulate Alaskan summer conditions. Specimens were kept on shelves in low wooden cabinets, open on the front, so they received only indirect artificial light except during twice-daily examinations. Laboratory temperature remained fairly constant at 65° F., rising occasionally to 71° for brief periods. Fresh air was provided by a fan in the intake duct of the air-conditioning system.

Cages for individual specimens were made from 3½ ounce Lily Tulip water cups #450, 5½ cm deep, and 6 cm across the mouth. These paper cups are plaited and rimmed. A hole 18mm in diameter was cut in the center of the bottom, and another hole 7mm in diameter midway on the side, using #10 and #3 cork borers. The mouth of the cup was covered with a 10 cm square of nylon stocking held in place with a rubber band. The side hole was plugged with absorbent cotton for use as a sugar sop. Early in the season a willow catkin was substituted for the sugar sop in a few cages.

Fifteen to 18 cages were placed in white porcelain pans (41 x 25 x 6 cm) containing water 1 cm deep.

PROCEDURE. Each mosquito caught in the field, either by netting or after landing, was brought to the laboratory in a plastic culture tube with pin holes in the