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OCCURRENCE OF *ANOPHELES BARBERI* IN MASSACHUSETTS

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The distribution of *Anopheles barberi* Coquillett, a tree hole breeding mosquito, includes 30 states of the midwestern and eastern United States, the District of Columbia, and southern Ontario and Quebec (Darsie and Ward 1981, Zavortink 1969). The northeastern limit of this species' distribution in the United States is held to be eastern New York. This note reports the occurrence of *An. barberi* in Massachusetts, which is the first record of this species in Massachusetts and New England.

On August 13, 1982 at about 1400 hr a single female *An. barberi* was captured while it attempted to bite the author. The mosquito was collected on the University of Massachusetts campus in Amherst, Hampshire County. Subsequent collection attempts at this site yielded no more *An. barberi*. I have not previously encountered this species, despite recent extensive collections in western Massachusetts of mosquito larvae from tree holes and discarded tires, and adults from forested areas with treeholes.

This collection record brings to 46 the total number of mosquito species known to occur in Massachusetts (Darsie and Ward 1981). The specimen has been deposited in the University of Massachusetts insect museum.

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TIME/CONCENTRATION IMPACT OF THE *SIMULIUM* LARVICIDE, ABATE, AND ITS RELEVANCE TO PRACTICAL CONTROL PROGRAMS

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INTRODUCTION

For the last seven years research has been conducted in this laboratory on the reactions of *Simulium* larvae, and select non-target macroinvertebrates to larvicides used practically or experimentally in the World Health Organization Onchocerciasis Control Program (OCP). In the absence of any corresponding laboratory phase of evaluation in that program, it was hoped that the tests with European stream fauna would establish some general principles of use to that project.

The laboratory technique of choice for *Simulium* larvae was the miniature simulated stream, well designed for assessing the effect of the short, 10–15 min, field application rates. A second technique, the rapid through-flow system, was used for studying a range of non-target macroinvertebrates, usually for standard exposures of 1 hr. This also provided an alternative method of testing *Simulium* larvae along with non-targets in the same vessel (Muirhead-Thomson 1981).

In the course of this investigation two findings provide an essential introduction to the present communication. (1) The first series of experiments using Abate® (temephos) [Procida 200 EC (emulsifiable

concentrate)] showed that in the miniature simulated stream, larvae of *Simulium ornatum* and *S. equinum* had a consistently high survival rate after time/concentrations similar to those used for effective control in the OCP. In that program, current practice is to apply the effective larvicide, Abate, at concentrations of 0.05 ppm for 10 min in the rainy season, and 0.1 ppm for 10 min in the dry season; this short intensive application being carried out by both helicopters and fixed-wing aircraft (World Health Organization 1980a, 1980b, 1982; Stiles and Quelennec 1977, Davies et al. 1978, Philippon et al. 1976; Quillevere et al. 1976a, 1976b).

In this laboratory it was found that at a standard exposure of 15 min, concentrations of Abate in excess of 0.4 ppm were required to produce mortalities (after 24 hr) of 90% or more (Table 1). (2) In a more recent series of tests using 1 hr exposures in the rapid through-flow vessel, observations of particular relevance to the present communication were made (Muirhead-Thomson 1981). With progressively decreasing concentrations of Abate, unexpected high mortalities were still being recorded at concentration levels much lower than would have been expected on the basis of the previous 15 min exposures. In view of the implications of these two sets of experiments it became imperative to compare the effects of the two different exposure periods under exactly similar experimental conditions, namely the more precise miniature simulated-stream.

EXPERIMENTAL PROCEDURE

Over a period of several months in 1981, larval collections brought in from the field at the beginning of each week were distributed in four test vessels, which in turn were converted into four identical miniature simulated streams. In two of these, larvae were exposed for the longer periods, 1 hr in one series and 2 hr in another, while the larvae in the third were exposed for the shorter period of 15 min. The fourth simulated stream provided the control. In each of these weekly experiments, time of exposure × concentration was the same, 0.1 ppm for 15 min, for example, being matched with 0.025 ppm for 1 hr, etc. One essential condition of this strict comparison was provided by maintaining control mortality at or near zero during the 3–4 days duration of the test. This was doubly important in that the nature of this simulated stream technique, with its careful set-up and preparation, made it quite impracticable to carry out large numbers of replicates with fixed numbers of larvae. Results had to be based on comparatively few tests carried out with very large numbers of larvae, the numbers varying according to the availability of test material from field sources. In these tests, freshly made-up dilutions of the Abate EC formulation were run through each of the experimental channels at an average rate of 80 liters/hr or approximately 1.3 liters/min.

The tests were carried out over a period of several months in the summer when water temperatures in the laboratory remained fairly constant at 16 to 18°C. The composition of each batch of field-collected larvae with regard to the proportion of *S. ornatum* and *S. equinum* was checked by identification of pupal samples. The overall proportion of the two species was *S. ornatum* 58%, and *S. equinum* 42%. During the two most active months, June and July, the proportions of *S. ornatum* remained steady around 62–64%.

Table 1. Summary of the results of 17 experiments on the reactions of late instar *Simulium* larvae (*S. ornatum* and *S. equinum*) to serial dilutions of Abate (temephos) Procida 200 EC, as judged by mortality 24 hr after a 15 min exposure in a miniature simulated laboratory stream, 17±1°C (Muirhead-Thomson 1981).*

	Concentration of Abate (ppm)						
	1.0	0.5	0.4	0.3	0.2	0.1	0.05
Total larvae tested	453	1075	898	907	731	347	541
% mortality	99	90	68	45	29	32	2.4

* Controls: 5 experiments, 1292 larvae, 1.7% mortality.

RESULTS

The figures (Table 2) show that over a wide range of Abate concentrations, the longer exposures of 1 hr and 2 hr to low concentrations produced a much higher mortality than the short 15 min exposures to the corresponding higher concentration. This is particularly marked in the 0.1 ppm for 15 min level which resulted in a mortality of 6.6%, as compared to mortalities of 34 and 94% produced by the same absolute quantity of Abate applied for 1 hr (0.025 ppm) and 2 hr (0.0125 ppm) respectively.

DISCUSSION

Experience with the behavior of toxic chemicals applied to rivers and flowing waters has revealed that the initial heavy application produces a pattern of extension or 'attenuation' of the wave or 'slug' as it moves downstream (Bath et al. 1970). From the point of application downstream the concentration of chemical progressively decreases, while the length of the slug and its period of impact increases. In the case of *Simulium* larvicides this has been well demonstrated for methoxychlor, using both marker dyes and direct analysis (Wallace et al. 1973, Fredeen et al. 1975). Unfortunately, in the case of Abate as used in the OCP, there is still a lack of precise information on this vital point (Quelennec et al. 1977, WHO 1980b), although it is recognized that the carriage of the larvicide, and its effectiveness for long distances downstream from the point of application, are inevitably accompanied by a progressive decrease in concentration from the initial heavy dose.

Clearly, the relevance of these findings to the OCP will have to await a repetition of this type of laboratory simulated stream test on larvae of *S. damnosum* s.l. Making full allowance for the possibility, not yet demonstrated, that there may be significant differences between the susceptibility of different species of *Simulium* to Abate, and for the higher water temperatures, 26–30°C (Grunewald 1976) of the tropical rivers involved, there still remains the serious

Table 2. Summarized results of 22 experiments on the effect of different time/concentrations of Abate (Procida 200 EC) on late instar larvae of *Simulium ornatum* and *S. equinum* as judged by percentage mortality after 24 hr in clean water. Exposures in laboratory miniature simulated stream at 17±1°C. (Overall composition of samples, *S. ornatum* 58%, *S. equinum* 42%).*

	Periods of exposure		
	15 min	1 h	2 h
Concentration of Abate	0.4 ppm	0.1 ppm	0.05 ppm
Total larvae	487	1527	239
Number of tests	3	4	2
% mortality	30	93	99
Concentration	0.2 ppm	0.05 ppm	0.025 ppm
Total larvae	717	1094	278
Number of tests	2	3	2
% mortality	1.4	31	100
Concentration	0.1 ppm	0.025 ppm	0.0125 ppm
Total larvae	988	1143	535
Number of tests	2	2	2
% mortality	6.6	34	94

* Controls: 9 experiments, 4575 larvae, 0.36% mortality (range 0–1.4%).

implication that control or eradication of *Simulium* larval populations in the OCP is being brought about, not so much by the time/concentrations used in practical field control (0.05 to 0.1 ppm for 10 min), but by the increasingly longer exposure to a correspondingly lower concentration of larvicide downstream. In view of the fact that Abate is still being used in 80% of the OCP project area (WHO 1982) the findings in this communication are still pertinent.

While the conclusions reached in this preliminary report may appear to introduce a new dimension into the evaluation of Abate in *Simulium* control, a close parallel has already been established in the allied field of *Simulium* control by means of *Bacillus thuringiensis* var. *israelensis* (Frommer et al. 1981). In that work it was found, as with chemical larvicides, that the concentration of particulate formulation carrying the *B.t.i.* when applied to a flowing stream for a defined period of time, spreads out as it is carried downstream. This results in a reduction of the intended dosage in terms of concentration, but an increase in intended dosage in terms of time. The importance of this increasing time factor was demonstrated in stream tests which showed that while a 35 min exposure of a 3.10 ppm suspension of *B.t.i.* produced only a 25% reduction of *S. vittatum* larvae 24 hr following treatment, halving this concentration but doubling the exposure period, 1.55 ppm for 70 min, produced a 50–70% reduction of larvae along the entire 312 m of test stream.

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SEPARATION OF FIRST-INSTAR LARVAE OF FOUR FLORIDA *CULEX (CULEX)*[1]

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While conducting field studies on ovipositional activities of wastewater mosquitoes, we collected several thousand egg rafts from sites in central and southern Florida. The vast majority of these field-collected egg rafts had been deposited by females of the following species: *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx. salinarius* Coq. and *Cx. restuans* Theobald. These are the four most common *Culex (Culex)* that will be encountered in Florida where they occur throughout the state. Rather than identify these rafts from the morphological characteristics of the eggs, we have relied on an indirect approach based on the examination of first-instar larvae following their eclosion. Most mosquito identification keys for first-instar larvae use only morphological traits (Bohart 1954, Price 1960, Dodge 1966). However, we have found that the process of identifying first-instar larvae can be greatly facilitated if consideration is given to both morphological and behavioral traits. This report gives a brief description of our procedure for distinguishing live first-instar larvae of the four above-mentioned mosquito species. *Culex bahamensis* Dyar and Knab and *Cx. tarsalis* Coq., the two other members of the subgenus, *Culex* in Florida, have very restricted distributions within the state. Therefore, we have not included them in this study.

Field-collected egg rafts were isolated individually into short, flat-bottom vials (25 mm diam, 35 mm height, 10 ml capacity) containing ca. 5 ml of tap water. Approximately 0.5 mg of liver powder was added to each vial shortly after the larvae had hatched. Without removing the larvae from the vial, species identifications were made 12–24 hours later using a dissecting microscope at low magnification (15X to 25X).

In addition to the taxonomic characters described by Dodge (1966), we found several others which were useful for distinguishing the first-instar larvae of *Cx. nigripalpus, Cx. quinquefasciatus, Cx. salinarius* and *Cx. restuans*. For example, in *Cx. nigripalpus* larvae the abdomen appears to have a clear band in the middle because the fourth abdominal segment is much less pigmented than the adjacent segments. This pigmentation pattern is much more difficult to discern in preserved specimens than it is in live material. As larval development in *Cx. nigripalpus* proceeds to later stages similar levels of pigmentation are attained by the third, fourth and fifth abdominal segments. In contrast, first-instar larvae of the three other species of *Culex (Culex)* mosquitoes found in our study areas did not possess a readily distinguishable difference in the amount of pigmentation among abdominal segments.

Another diagnostic character for separating these species of *Culex* mosquitoes involves the dorsal brush (setae 2, 3–$\overline{X}$) of the anal segment (abdominal segment $\overline{X}$). Actually, many components of the dorsal brush are structurally very similar among the four species. For the first-instar larvae of each species, the dorsal brush consists of two long caudal setae (a single upper seta, Z–$\overline{X}$, and a single lower seta, 3–$\overline{X}$) on each side of the anal segment. The upper and lower caudal setae, which are about the same size, are often more than twice the length of the anal segment. There is, however, a major interspecific difference which can be used to distinguish *Cx. salinarius* from the three other species. When *Cx. salinarius* larvae come to the surface for air, they hold their upper and lower caudal setae separated by an angle of 30° to 45° in a plane parallel to the water's surface. This angle is usually more acute in *Cx. nigripalpus* and *Cx. restuans* larvae. Practically no lateral separation occurs between the upper and the lower caudal setae of *Cx. quinquefasciatus* larvae. Thus, when observed from above the upper caudal seta often obscures the view of the lower caudal seta.

Vibrations induced by handling the shell vials invariably caused the larvae to dive toward the bottom of the vial. Following this alarm reaction *Cx. quinquefasciatus* larvae normally spent at least one minute and often longer away from the surface, whereas larvae of the three other species usually returned to the surface in less than 20 seconds. Even when un-

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