STUDIES ON THE USE OF FLUORESCENT DYES FOR MARKING *ANOPHELES QUADRIMACULATUS* SAY

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Introduction

In order to study the flight range of mosquitoes under natural conditions, a number of workers have tried staining larvae or marking adults with metallic dyes in the form of solutions or dusts. Recently fluorescent compounds have been successfully used to mark *Anopheles* mosquitoes (Zukel, 1945). By the use of this method, a large number of specimens can be readily marked and recognized when subsequently collected in a matter of a few minutes. No details have been given concerning the toxic effect caused by the marking and how long the marking would last. The present paper is a report of a seven-week study on the use of fluorescent dyes for staining and marking the larvae and adults of *Anopheles quadrimaculatus* during my visit in the Health and Safety Department, Tennessee Valley Authority, Wilson Dam, Alabama.

Materials and Methods

Anthracene, rhodamine B, and fluorescein were used for staining larvae as well as for adult marking. For the larvae, 20 or 50 early fourth instar larvae as a lot were stained in a crystallizing dish, thus giving a 24 to 48 hour staining period before pupation. Since rhodamine B is not readily dissolved and anthracene is not dissolved at all in water, they were first allowed to dissolve in a few ml. of acetone in the later part of the experiment. This gave a complete solution of rhodamine B and a dispersible suspension of anthracene in water. Stained larvae were kept in the insectary at a constant temperature of about 76° F. and a relative humidity of about 70. Regular ground dog food was offered until the time of pupation was reached.

Stained larvae were allowed to emerge into lantern globes and killed either by heat or cooling or by chloroform mosquito catching tubes. Care was taken to disturb the emerged mosquito as little as possible. Killed specimens were examined under an ultra-violet light which will turn anthracene, rhodamine B, and fluorescein into blue, red, and green fluorescent colors, respectively. The light was obtained from a high-pressure mercury vapor lamp with its ballast transformer and two Corning glass filters, #5840 red-ultra and #4308 light shade blue green. Dissection was made when it appeared necessary.

For the adult marking 1–2 day old mosquitoes were released into a quart ice cream carton and covered at both ends with bobbinet cloth. They were heavily sprayed with a home-made atomizer consisting of a 200 ml. flask fitted with a rubber bulb and a smoothly curved outlet tube. One part of anthracene was mixed with six parts of gum arabic, whereas the other two were mixed with four parts. Marked specimens were transferred into clean holding screen cages which had a diameter of 8 inches and a length of 16 inches and kept in the insectary. Only sugar solution was given during the test period. Dead mosquitoes were taken out and examined every day.

Results

Larvae Staining

The results obtained from staining larvae in vitro were not satisfactory. They are summarized in Table 1. The per

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TABLE 1. Mortalities of Larvae Stained in Vitro with Fluorescent Dyes.

Fluorochrome	Dilution	Larvae Used	Larvae Emerged	Percentage of Mortality (D–C/T–C x 100)
	Lot No. 1			
Check (no dye)	200 ml. of water	50	16	
Anthracene	1:4,000 (50 mg. in 200 ml. of water)	50	0	100
Rhodamine B	"	50	0	100
Fluorescein	"	50	0	100
Anthracene	1:8,000 (25 mg. in 200 ml. of water)	50	4	75
Rhodamine B	"	50	0	100
Fluorescein	"	50	9	43.8
	Lot. No. 2			
Check (without acetone)	100 ml. of water	20	13	
Check (with acetone)	100 ml. of water	20	6	53.8
Anthracene	1:8,000 (12.5 mg. in 100 ml. of water)	20	4*	69.2
Anthracene	1:10,000 (10 mg. in 100 ml. of water)	20	7**	46.1
Rhodamine B	1:10,000 (10 mg. in 100 ml. of water)	20	0	100

* 1 specimen dead upon emergence.
** 2 specimens dead upon emergence.

centage of mortality was calculated by the formula D–C/T–C x 100, where T was the total number used in the test, D was the number of dead in the test, and C was the number of dead in the check.

One adult from a larva marked with anthracene at 1:8,000 showed a definite blue fluorescence; one adult from a larva marked with fluorescein showed a doubtful trace of green fluorescence; all other adults gave no evidence of the fluorochromes with which the larvae were marked.

Adult Marking

The results of marking adults with the three selected fluorochromes are summarized in Table 2. It will be noted that a different number of adults were used in each of the tests. This was due to the fact that it was difficult to count exactly the number of adults which were released into the ice cream carton. The adults used were about equally distributed between the two sexes, and, although the females in general survived for a greater period than the males, this did not appear to be a factor in the final results.

Discussion

The results of the staining tests with larvae indicate that the materials used are too toxic to be used for this purpose. However, the results with the second lot of mosquitoes (Table 1) indicate that the high mortalities obtained with the anthracene and rhodamine B specimens may have been due to acetone rather than to the fluorochromes themselves. It appears from the observation of the few adults which did emerge that the dyes were not effectively transferred from the larvae through the pupae to the adult stage. Thus, the over-all conclusion seems warranted that the three fluorochromes would not be satisfactory for the purpose for which they were tested.

The use of anthracene, rhodamine B, and fluorescein for marking adult mosquitoes appears much more promising.

TABLE 2. Results of the Use of Fluorescent Dyes to Mark *A. quadrimaculatus* Adults.

Period	Anthracene				Rhodamine B				Fluorescein				Check *
	Cumulative % Mortality	Effectiveness Pos.	Neg.	?	Cumulative % Mortality	Effectiveness Pos.	Neg.	?	Cumulative % Mortality	Effectiveness Pos.	Neg.	?	Cumulative % Mortality
1st Wk.	16.6	8	0	0	22.2	8	0	0	2.0	1	0	0	14.3
2nd Wk.	45.8	12	1	1	58.3	12	1	0	26.5	10	1	1	28.6
3rd Wk.	81.2	15	2	0	91.6	12	0	0	79.5	24	2	0	38.2
4th Wk.	93.7	5	1	0	97.2	2	0	0	91.8	5	1	0	52.4
5th Wk.	100.0	3	0	0	100.0	1	0	0	100.0	4	0	0	81.0
Totals		43	4	1		35	1	0		44	4	1	
Percentages		89.6	8.3	2.1		97.3	2.7	0		89.8	8.2	2.0	

* A total of 21 specimens were included in the check.

Each of the materials averaged approximately 90 per cent or more effective over the 5-week period during which the observations were made. There was some indication of significantly greater mortalities in the treated specimens than in the check lot. However, survival was sufficient to make it quite feasible to use these materials in flight dispersion and flight range studies.

Summary

Observations were carried out over a 5-week period to determine the feasibility of using three fluorochrome dusts, namely, anthracene, rhodamine B, and fluorescein, for marking larvae and adults of *A. quadrimaculatus*. Because of high mortalities and ineffectiveness the materials are not considered suitable for marking larvae. However, all three materials appear to be quite satisfactory for use in marking adult mosquitoes.

Reference

ZUKEL, JOHN W. Marking *Anopheles* Mosquitoes with Fluorescent Compounds. Science, August 10, 1945.

THE IDENTIFICATION OF *ANOPHELES BRADLEYI* LARVAE

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The larva of *Anopheles bradleyi* King was the first North American *Anopheles* larva to be differentiated. This was done by Smith (1) in 1904, in a key by which he separated *crucians* larvae from *punctipennis* and *maculipennis* by the following characters: antennae shorter, brownish; tracheal gills short. His *"maculipennis"* has since been found to be *quadrimaculatus,* and his *"crucians,"* reported to be rare in New Jersey except at Cape May, is undoubtedly the brackish water species described 35 years later as *Anopheles crucians bradleyi* King.

Numerous examples of *bradleyi* larvae have been received from Brunswick and Savannah, Georgia, and the antennae are invariably brownish, in striking contrast to all associated species, in which the brown (or yellow in pale larvae) antennae of younger instars become pale, with dark apices and sabres, in the fourth instar.

Two articles have recently been published in which distinguishing characters of *bradleyi* larvae have been proposed. Bickley (2) remarks upon the short anal gills of *bradleyi* and *atropos,* and Miles (3) found, in comparing *bradleyi* with *punctipennis* and *georgianus,* that *bradleyi* has stouter, dark-pigmented antennal spicules, and confirms the fact that the palmate hairs on abdominal segments 3 and 7 are usually reduced in size, with leaflets mostly slender and without notches or serrations on the margins. The brown color of *bradleyi* antennae has not been mentioned in the literature since this species was described, and Headlee (4) erroneously ascribes this character to *crucians* larvae. Since the brown antennae are readily distinguishable at very low magnifications, this character is here suggested as a means of easily recognizing fourth instar *bradleyi* larvae from the larvae of other common species at the time of collection.

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