EFFECT OF RADIOPHOSPHORUS (P^{32}) ON LIGHT RESPONSE OF *AEDES AEGYPTI* (L.) LARVAE

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Radioisotopes have been used with great success in tracing particular elements. The need for effective markers in ecological studies has led to the use of radioactive materials as markers of individual animals in dispersal studies, measurement of population densities, and behavior examinations. Tagging with radioisotopes rather than conventional pigments and dyes has definite advantages. Pigments may rub off, fade, or be eliminated during molting. Examination of large groups of insects for specially colored individuals can be a tedious task. Radioisotopes administered internally either by feeding or injection provide an internal tag that will not rub off and that permits rapid counting of large populations.

There are several disadvantages to the use of radioisotopes. Toxicity to individuals can be a problem if a high level of radiation is present since it will not only reduce the number of labelled insects but also cause errors in interpretation of results. Another possible disadvantage is change in behavior of the labelled insect contrasted with the unlabelled one.

The recent monograph by O'Brien and Wolfe (1964) includes the most comprehensive review of work utilizing radioisotopes in entomology. Studies on foraging intensity of honey bees by Lee (1965) utilized P^{32} and I^{131}. A dose of 1 mc of P^{32} per colony (1 mc/2 quart solution) produced a significant effect on distribution of foragers from the hive. An effect was also shown with a 1.5 mc dose of I^{131}. Hassett and Jenkins (1949) reared larvae of *Aedes aegypti* (L.) in water containing 1 μc P^{32}/ml and produced adults having counts up to 10,000 per minute. Third and fourth instars were successfully tagged, younger larvae were too sensitive, and pupae absorbed too little phosphorus. The effects of tagging with P^{32} were studied by comparing stage, age, and P^{32} concentrations. Since concentrations of P^{32} greater than 1 μc/ml were harmful from the standpoint of life history, it was hypothesized that higher concentrations also affected behavior.

Although careful studies of life cycles, longevity, and reproduction have been made, there appear to be no significant reports on behavior of tagged mosquitoes. The purpose of this study was to determine if varying levels of radiophosphorus (P^{32}) had any effect on the light response of tagged *A. aegypti* larvae.

MATERIALS AND METHODS

Aedes aegypti larvae were chosen because they are characteristically negatively phototaxic. Prior to each of three replications, 400 mechanically separated third instar larvae were obtained from the laboratory colony at the USDA Laboratory for Insects Affecting Man and Animals, Gainesville, Florida. One hundred larvae were manually separated into a 400 ml glass beaker for each of the four treatments. Each beaker contained 250 ml. of distilled water.

The four treatments were H_2O, 0.1 μc P^{32}/ml, 5 μc P^{32}/ml, and 1 ml of 0.5 N H_3PO_4. The desired amount of P^{32} (P-32-P-Processed, Carrier Free, as PO_4 in weak HCl, a 0.5 N solution) was calculated and added to two treatments. The volumes added for the 0.1 μc/ml dosage were 25, 28, and 30 μ liters. Those for the 5 μc/ml dosage were 1250, 1370 and 1540 μ liters. Prior to each of the three replications, correction was made for radiodecay. The P^{32} was produced and assayed by Union Carbide which operates the Oak Ridge National Laboratory, Oak Ridge, Tenn., under contract to the Atomic Energy Commission. The larvae were kept in the radioactive solution for 24 hours. At the end of this period they were transferred to fresh water. Each group was fed about 25 mg of crushed dog food.

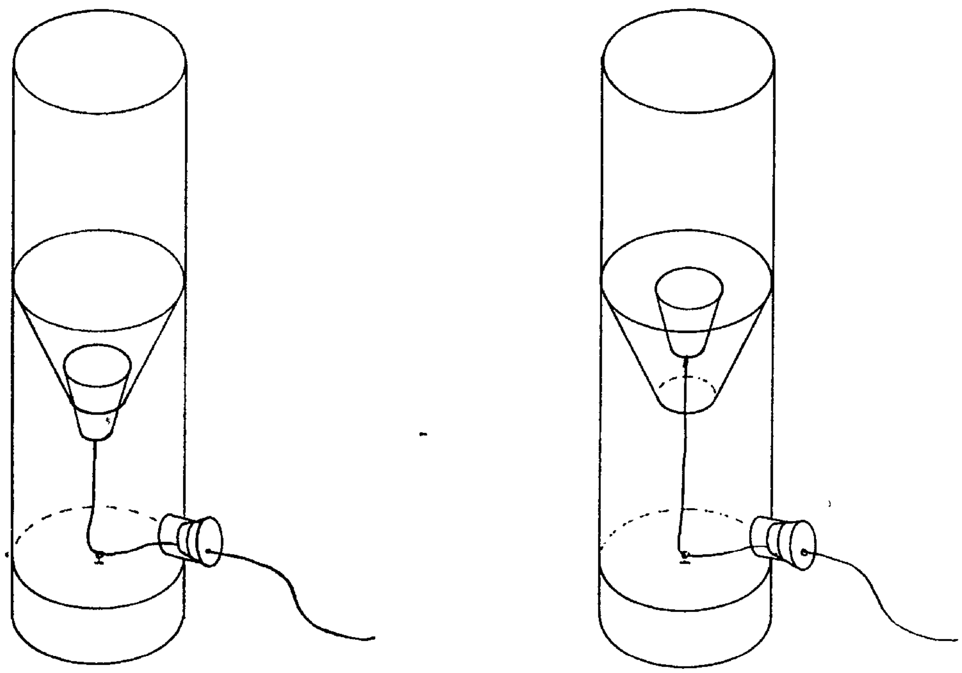

Fig. 1. Cylinder-funnel apparatus. A. Funnel plug in closed position. B. Funnel plug in open position.

For observing response to light, a cylinder equipped with a trap funnel was used (Fig. 1). The cylinder and funnel were made of cellulose acetate and were then mounted on a 6-inch plastic freezer dish. The cylinder was 18 inches deep and the bottom of the funnel was 12 inches from the top. The funnel plug was a truncated cone of plastic, the top of which was ringed with a ½-inch strip of sponge rubber to provide a good seal. The floating plug was manipulated with a string through the drain plug.

At the beginning of the observation the cylinder, which was lighted from below, was filled with water and the funnel was plugged. The larvae were poured from a beaker into the top of the cylinder. All of the treatments were tested in a random sequence. After the larvae quieted, the funnel plug was floated up and the bottom light turned out. A pause of a few seconds was allowed and the top light was turned on for 20 seconds. The funnel was again plugged at the end of this timing. Fig. 1 shows the plug positions during the test. Each treatment was conducted in a dark laboratory with the investigator well away from the apparatus. Water and mosquitoes from the top of the apparatus were siphoned off. The drain plug was pulled and the water and mosquitoes in the bottom of the apparatus were collected. The larvae from the top and bottom were counted.

For counting of radioactivity, eight larvae were taken from the top and bottom of each replication, placed in 2-inch aluminum planchets, and dried under infra-red lamps. A Packard Scaler-Ratemeter, Model 150, with a Nuclear Chicago Geiger-Mueller tube having a 1.4 mg/cm^2 mica window was used for counting. Counts were taken at one-half inch from the tube surface.

Results and Discussion

The data for the three replications are given in Table 1. The larvae, top and bottom, from each of the treatments, were counted for radioactive content to determine if there was any variation in the level at which they had absorbed the isotope. The paired t test on these data showed no significant difference between the radioactivities of the larvae in the top in relation to those in the bottom. Also tested was the dosage variation in tagging levels throughout the replications. No significant difference was found.

TABLE 1.—Larvae Observed in Top and Bottom for Each Replication.

	Check		H_3PO_4		.1 μc/ml		5 μc/ml	
Replication	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
1	30	70	22	78	41	59	67	33
2	29	71	27	73	47	53	65	35
3	30	70	29	71	43	57	69	31
Average	29.6	70.3	29.3	74	43.6	56.3	67	33

Comparison of the check in relation to the H_3PO_4 showed no significant difference between these treatments.

For analysis with respect to light response the data were tested by a chi-square with an orthogonal comparison of the treatments. The results of this analysis are given in Table 2.

TABLE 2.—Analysis in Relation to Various Levels of Radiophosphorus.

	d.f.	x^2	P
Check vs. others	1	23.40	.001
H_3PO_4 vs. others	1	70.89	.001
0.1 μc/ml vs. 5 μc/ml	1	33.23	.001

This study indicates that a concentration of radiophosphorus .1 μc/ml or greater, affects the light response of *A. aegypti* larvae. Since the effect of phosphorus alone was considered as well as radiophosphorus, the radioactivity must have caused the variation in response and not the phosphorus.

Since radioisotopes are being used so commonly as labelling tools, it is important to understand that their use might alter behavioral patterns and lead to unreliable information. A behavioral change in the larvae of *A. aegypti* does not mean there would be a behavioral change in the adult, but it does open the area to further study.

Tagging mosquitoes for the release-recapture method of population sampling requires that the adults merely be radioactive enough to be detected. This may allow use of concentrations of isotopes below the deleterious levels. Many tagging techniques require the use of very high concentrations of radioactive material. For this reason, the reactions of the specific insect to the chosen radioisotope should be thoroughly studied with relation to behavior and life history prior to any organized research use.

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