J. B. Toxicities of certain larvicides to resistant and susceptible *Aedes aegypti* (L.). Bull. Wld. Hlth. Org. (in press).

METCALF, R. L., and GEORGHIOU, G. P. 1962. Cross-tolerance of dieldrin-resistant flies and mosquitoes to various cyclodiene insecticides. Bull. Wld. Hlth. Org. 27:251–256.

MULLA, M. S., METCALF, R. L., and KATS, G. 1964. Evaluation of new mosquito larvicides with notes on resistant strains. Mosquito News 24(3):312–319.

PILLAI, M. K. K., ABEDI, A. H., and BROWN, A. W. A. 1963. WARF antiresistant compounds as synergists against DDT-resistant *Aedes aegypti*. Mosquito News 23:112–117.

PILLAI, M. K. K., HENNESSEY, D. J., and BROWN, A. W. A. 1963. Deuterated analogues as remedial insecticides against DDT-resistant *Aedes aegypti*. Mosquito News 23:118–125.

SCHOOF, H. F., and JAKOB, W. L. 1964. Insecticides for use against *Aedes aegypti*. Mosquito News 24(3):309–311.

WORLD HEALTH ORGANIZATION. 1960. Insecticide resistance and vector control; tenth report of the expert committee on insecticides. WHO Tech. Rep. Series. No. 191, pp. 15–29.

THREE TECHNIQUES FOR LABELING *CULICOIDES* (DIPTERA: HELEIDAE) WITH RADIOACTIVE TRACERS BOTH IN THE LABORATORY AND IN THE FIELD

JOHN B. DAVIES

Mosquito-Sandfly Research Unit, Ministry of Health, Montego Bay, Jamaica

The use of radioactive substances for labeling insects for dispersal studies is becoming more widespread. Techniques have been developed for many insects, including mosquitoes (Provost, 1952) and blackflies (Fredeen *et al.*, 1953). A two-isotope technique was used by Lewis and Warloff (1964) to study the dispersal of mirids in plots of broom.

The smaller the insect, the more attractive does the possibility of using radioactive tracers become, since other labeling techniques involving dusts and dyes are impracticable.

As a preliminary to investigating the dispersal of *Culicoides furens* Poey, and *C. barbosai* Wirth and Blanton, from Jamaican swamps, the following three techniques have been developed. The larvae are introduced to sufficient concentration of P-32 for them to metabolize enough radioactive phosphorus to be detectable in the adult, from 9 days to 9 weeks later.

The three techniques, with examples, are described below:

DIRECT APPLICATION TO LARVAE IN THE LABORATORY. Larvae placed directly into solutions containing from 0.5 to 5 microcuries of P-32 per ml. will be adequately labeled after 24 hours. At the end of that time they should be removed and rinsed in distilled water to remove any active ions adhering to the cuticle.

The concentrated radiophosphorus, when received from the suppliers, usually has an activity of the order of 1 millicurie/ml. This should be diluted with a carrier solution of distilled water containing about 0.1 percent of inactive orthophosphate. Otherwise, a significant portion of the active ions will become adsorbed on the walls of vessels and pipettes.

Larvae to be checked are placed on pieces of card and fixed in place with adhesive cellulose tape. The card is then placed face down on a sheet of X-ray film (Kodak Industrial type AA serves well) and left in a cassette to expose for 6 days. When the film is developed a radioactive larva will be indicated by an exposed spot on the film.

Table 1 gives an example of *C. furens* larvae which were treated with 4 concentrations of P-32 in 3 batches. Batch 1 was exposed on film immediately, batch 2 was rinsed in water and then placed in

TABLE 1.—Numbers of *C. furens* recovered from 3 batches after immersion in P-32 solutions for 24 hrs. and held for 0, 7, 19 days (denominators), and numbers of radioactive specimens (numerators).

Batch	Days after treatment	Stages recovered	Concentrations μc/ml. 5	2.5	1.25	0.5	Control
1	0	larvae	9/9	8/8	10/10	8/8	0/9
2	7	larvae	5/5	6/6	5/5	5/5*	...
		pupae	2/2	3/3	1/1	...	...
		adults	...	...	1/1	...	...
3	19	larvae	3/3	1/1	...	...	...
		pupae	...	...	2/2	2/2†	...
		adults	1/1	4/4	1/2	1/1†	...
		pupal cases	1/3	1/1	...	0/2†	...

* Trace on film faint.
† Trace almost too faint to be observed.

damp sand for 7 days, while batch 3 was placed in damp sand for 19 days. At the end of these periods larvae, adults and pupae were collected from each batch and exposed on film. A control batch was run by allowing "clean" larvae to swim in the strongest solution for one minute; they were then rinsed and exposed on film to determine whether any radioactive material was carried over through the rinsing on the surface of the larvae.

With the exception of one unlabeled adult from batch 3, all stages recovered were sufficiently radioactive to register on the X-ray film. Two out of six empty pupal cases were labeled showing that the P-32 must have been fully metabolized and even incorporated in the cuticle. The lowest concentration, 0.5 μc/ml did not leave a satisfactory mark on the film after 7 and 19 days. All others were satisfactory, and even adults recovered 19 days after treatment were well labeled. None of the controls showed any radioactivity.

DIRECT APPLICATION TO MUD IN THE LABORATORY. Larvae contained in samples of mud brought in from the field can be labeled by spreading the mud into a layer about 1 inch deep in a photo-developing dish or a similar container. If 200 ml. of solution containing 50 μc of P-32 (0.25 μc/ml) is applied to each square foot of mud surface almost all the sand flies which emerge over the next nine weeks will be radioactive.

Table 2 gives an example of the numbers of adult *C. barbosai* recovered from mud in an 8" x 10" developing dish treated as above and exposed to X-ray film. The first labeled females were taken 9 days after treatment and the first males 2 days later. Since the pupal stage takes 3 to 4 days, it presumably takes 5 days for the P-32, which would almost certainly become adsorbed onto the mud particles, to pass through the food chain into the larvae. The experiment was abandoned after the 67th day when the activity in each insect had become so low that it could only be detected on the film with

TABLE 2.—Numbers of adult *C. barbosai* recovered from mud treated with P-32 in the laboratory.

Days after treatment	*C. barbosai* Recovered ♂	Recovered ♀	Labeled ♂	Labeled ♀
1	4	3	0	0
2	12	6	0	0
4	5	6	0	0
5	2	1	0	0
7	1	3	0	0
9	1	7	0	7
11	9	17	5	16
12	12	14	12	14
13	31	15	30	15
20	41	15	41	15
23	26	25	26	25
25	46	23	46	23
33	32	19	32	19
40	20	19	20	19
48	8	14	8	14
63	8	2	5	2
67	2	1	2	1

great difficulty, even after increasing the exposure time to 8 days. Between the 20th and the 48th days 100 percent labeling was achieved. Since P-32 has a half-life of 14.2 days the activity of the solution would have decayed to about 3.8 percent of its original level during the 67 days, so it must be assumed that the larvae progressively accumulate more and more radioactive material, compensating for the natural decay of the P-32.

DIRECT APPLICATION TO MUD IN THE FIELD. In a similar manner radiophosphorus can be applied direct to the larval habitats. Applied at the same rate of 50 μc/sq. ft. to the mud of a tidal mangrove swamp, about 50 percent of adults emerging from the treated area can be expected to be radioactive. It is this technique that will probably have the greatest application for dispersal studies.

The mud of a tidal mangrove swamp that was flooded daily to a depth of 3 inches and supported a reasonable population of *C. barbosai* was isolated by inserting an 18-inch-wide hoop of galvanized iron 6 inches into the mud, enclosing a circular area of 2 sq. ft. Four hundred ml. of solution containing 100 μc of P-32 was poured evenly over the mud and the hoop left in position for 4 days. The object of the hoop was to prevent tidal water from flushing the radiophosphorus out of the mud and to reduce the migration of larvae out of the area. The hoop was then removed and the area left for an additional 4 days. On the 8th day a circular emergence trap (A) 2 feet in diameter was placed over the treated area, and four similar traps, (B, C, D and E) placed around it in the form of a cross so that their inner edges touched the centre trap. Table 3 shows the numbers of labeled and unlabeled *C. barbosai* recovered from each trap.

Roughly 50 percent of adults recovered directly from the treated area were radioactive, and the proportion appeared to increase with time. Initially, apart from trap A, the only trap to catch labeled adults was trap D, situated on the opposite side of A from the sea. These specimens presumably resulted from larvae washed into the trap by the advancing tide. On the 18th day, trap A was removed since its presence was thought to inhibit migration of larvae, and from that time on, all traps caught labeled adults.

To determine whether any radioactive material had been carried from one trap to another by the tides, six smears of mud from beneath each trap were made on cards and exposed to X-ray film. From under trap A four out of the six smears were active, B, C and D were all inactive, while one smear from trap E was radioactive. Hence there was a positive indication that the radioactive material was being distributed by tidal action.

The foregoing examples show that the techniques of labeling larvae with radioactive substances, and then recapturing the

TABLE 3.—Numbers of radioactive *C. barbosai* (numerators) recovered from each of five emergence traps in a mangrove swamp after the mud beneath the centre trap A had been treated with P-32. Total numbers of *C. barbosai* are indicated by the denominator.

Days after treatment	Traps				
	A	B	C	D	E
8			Traps set		
11	8/27	0/8	0/9	1/14	0/7
14	0/0	0/0	0/0	0/4	0/0
18	18/24	0/6	0/7	3/13	0/11
21	10/13	0/3	1/5	5/23	0/9
25	Discontinued	2/12	1/11	0/18	4/10
29		2/16	1/7	2/6	2/7
TOTAL	36/64	4/45	3/39	11/78	6/44

adults is definitely a feasible proposition. By applying a very dilute concentration of P-32 to the habitat, the progressive accumulation of radioactive material by the larvae counteracts to a great extent the decay of the material so that even with a substance with a half-life as short as 14.2 days, labeled adults were still detectable 67 days after the initial treatment. Although the numbers used in these examples are small, emergence rates of 500 per sq. ft. per week are not uncommon in some localities, and as such would be ideal for dispersal studies.

All standard precautions for use with radioactive materials should be taken. However, once the dilute solutions have been prepared (0.25–5.0 μc/ml.) they can be kept in thick glass containers, since, due to the self-absorption of the solution, very little radiation passes the glass, and only normal care is required in handling. In the field, freshly treated mud may have an activity approaching the maximum safe contamination level stipulated for laboratory benches and equipment, and should be treated with respect. Experimental areas should be well away from footpaths to avoid disturbance by inquisitive persons. They should be well signposted with warnings and the actual treated area covered with wire mesh to prevent the unwary from walking on it. Expensive equipment is not essential, but some type of counting equipment (scaler and G-M tube) should be available for monitoring glassware, benches etc., and for estimating radiation levels.

SUMMARY. Three examples of means by which *Culicoides* larvae can be labeled with radiophosphorus are given. In all of these the radioactivity is carried over to the adult and may therefore be used for adult dispersal studies. Two of the methods involve applying weak active solutions either to mud containing larvae in a dish in the laboratory or directly to the habitat. Radioactivity is detected by means of radiograms on X-ray film.

ACKNOWLEDGMENTS. I am most grateful to Dr. C. T. Lewis for suggesting this technique, and for his later advice and encouragement; and to the Ministry of Health, Jamaica, for permission to publish this paper.

References Cited

FREDEEN, F. J. H., SPINKS, J. W. T., ANDERSON, J. R., ARNASON, A. P., and REMPEL, J. G. 1953. Mass tagging of black flies (Diptera: Simuliidae) with radiophosphorus. Can. Jour. Zool. 31, pp. 1–15.

LEWIS, C T., and WARLOFF, N. 1964. The use of radioactive tracers in the study of dispersion of *Orthotylus rivescens* (Douglas & Scott) (Miridae: Heteroptera). Ent. exp. & appl. 7, pp. 15–24.

PROVOST, M. W. 1952. The dispersal of *Aedes taeniorhynchus* 1. Preliminary studies. Mosq. News 12, No. 3, pp. 174–190.