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TRANSFER OF RADIOACTIVITY TO EGGS AND LARVAE BY FEMALE *Culex pipiens quinquefasciatus* SAY TREATED AS LARVAE WITH ^{32}P[1]

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Patterson *et al.* (1968) reported that male *Culex pipiens quinquefasciatus* Say exposed to ^{32}P as larvae transferred detectable quantities of radioactivity to the females they inseminated. Similar results have been obtained in tests with other species of mosquitoes (Dame and Schmidt, 1964; Quraishi *et al.*, 1966; Smittle *et al.*, 1969). Also Quraishi *et al.* (1966) reported that female *Aedes vexans* (Meigen) exposed to ^{32}P in the larval stage transferred radioactivity to their eggs. This paper presents the results of a study of the relationship between the radioactivity contained in female *C. p. quinquefasciatus* and that found in their eggs and larvae.

MATERIALS AND METHODS. Third and fourth instar larvae of *C. p. quinquefasciatus* from the laboratory colony were placed in distilled water for 4–6 hours and then transferred to distilled water containing 0.25 microcurie of ^{32}P per milliliter of water. The volume of treatment solution ranged from 409 to 512 milliliters and one larva per milliliter was introduced. After 48 hours, the larvae were removed from the solution, placed in distilled water for 10 minutes to remove any external contamination, and transferred to distilled water for pupation. Throughout the exposure and after it, finely ground laboratory chow was fed routinely until all larvae had pupated.

Adults from the treated larvae were allowed to mate and take a blood meal. Then, 3 days later, the females were placed in individual cups covered with nylon net to which hay infusion water had been added as an oviposition medium. Control females were set up in a similar manner. The control and treated females and their egg rafts were assayed for radioactivity. Also, females that had not oviposited were assayed.

The measurements of radioactivity (counts per minute less background) were made with both the G-M and liquid scintillation counters. The G-M counts were obtained with a Nuclear Measurements Corporation DS-1A scaler equipped with a 1.4 milligrams per square centimeter end window G-M tube. The liquid scintillation counts were obtained with a Packard Tri-Carb® liquid scintillation spectrometer (Model 3365D). Twenty milliliter low-potassium glass vials with foil caps were used. The scintillation fluid was composed of 4.0 grams of PPO (2,5-diphenyloxazole) and 0.1 gram of dimethyl POPOP (2,2'-*p*-phenylene-bis(4-methyl-5-phenyloxazole)) per liter of toluene. Also thixotropic gel powder (Cab-O-Sil®) was added at the rate of 30 grams per liter of solution to keep the mosquitoes and egg rafts suspended in the scintillation fluid.

Females and egg rafts were weighed for comparison with radioassays. Some egg rafts were not counted in the liquid scintillation counter, but were allowed to hatch so we could evaluate fertility and radioactivity in the larvae.

RESULTS. Fifteen gravid females that had not oviposited, 25 females that had oviposited, and 25 egg rafts were analyzed for radioactivity with the G-M counter. These same samples (except 15 of the egg rafts) were also analyzed with a liquid scintillation counter. The results were as follows:

	Average Counts Per Minute	
	G-M	Liquid Scintillation
Females not ovipositing	4,451	49,106
Females that oviposited	2,648	30,582
Egg rafts	1,802	21,518

Ovipositing females therefore retained about 60 percent of their radioactivity. The other 40 percent was found in their egg rafts.

Seventeen gravid females weighed an average of 2.62 milligrams per female before oviposition and 1.56 milligrams after oviposition—a difference of 1.06 milligrams. Their egg rafts averaged 1.24 milligrams per raft. Thus, the females lost about 40 percent of their weight during oviposition, and the loss of weight and radioactivity correlated exceptionally well.

Quraishi *et al.* (1966) reported that female *A. vexans* lost only 13 percent of their radioactivity by oviposition. However, this discrepancy between their results and ours may be attributed to the difference in the number of eggs oviposited, as *A. vexans* averaged only 41 eggs per female while *C. p. quinquefasciatus* averaged 155 eggs per female.

No radioactivity was found in the controls or in egg rafts from untreated females mated with radioactive males.

Only 10 of the 25 egg rafts assayed for radioactivity with the G-M counter were assayed in the liquid scintillation counter. The remaining 15 egg rafts were held in hay infusion until the larvae hatched. These eggs had an average hatch of 92.3 percent compared with 98.1 percent

[1] Mention of a proprietary product in this paper is not an endorsement of this product by the U.S.D.A.

in the controls. The larvae assayed for radioactivity in the liquid scintillation counter averaged 118 counts per minute per larva. Thus, the ^{32}P was carried from the female to the egg and subsequently to the larvae in measurable amounts.

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A QUICK, ACCURATE AND ECONOMICAL METHOD FOR CALIBRATING LOW VOLUME SPRAYING SYSTEMS ON AIRPLANES

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At a point between the control (shut-off) valve and the boom, of a typical airplane spraying system, insert a flow meter with needle valve attached. On the inlet side of the flow meter provide a tee. One leg of the tee will have a closed nipple for attaching a knapsack sprayer.

Remove the spray-wand from the hose of a good quality knapsack hand sprayer. Attach an assembly of pressure gauge, shut-off valve and fitting to connect to the nipple in the tee.

To operate, half fill the knapsack with the spray solution in use. Pump knapsack up to about normal operating pressure. Hang buckets under each nozzle. Turn on knapsack sprayer for one minute. Observe position of ball in flow meter. Measure spray solution collected and adjust rate of flow by means of the needle valve in the flow meter as required.

The spraying system can be accurately calibrated without flying the plane. The pressure within the spraying system will have no bearing on the reading of the flow meter. The flow reading will remain constant for a given solution, unless there is a significant change in the viscosity of the liquid due to temperature.

The flow meter also furnishes a time saving bonus when several nozzles are in use. Normally, it is necessary to land and clean one that becomes plugged. The use of a flow meter allows the plane to continue as long as the pilot can keep the ball on the mark.

A SIMPLE, LIGHTWEIGHT LARVICIDING UNIT

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The use of FLIT® MLO larvicide at low application rates (0.5–2.0 gal./A) has made it possible to employ a compact larviciding apparatus which can be operated from the trunk of an automobile if necessary. The system is pumpless, using CO_2 or compressed air to propel the larvicide. This gives a uniform flow rate even at very low dosages. With no engine noise, larviciding operations can be carried on in crowded residential areas with little annoyance to residents. The unit is a modification of the type developed for aerial ULV spraying (Dearman, *et al*, 1965). It consists of a standard CO_2 bottle with regulator, a 10-gallon pressure tank, and a 100 ft. self-coiling hose with spray gun and nozzle. The self-coiling nylon hose weighs less than a pound and is easily operated by one man without a hose reel.

Components used besides the CO_2 bottle and regulator are:

Tank—Firestone 10-gallon beverage tank with Hanson quick-disconnect couplings. Couplings should be fitted with ¼" NPT hose connections. Available: Southwest Bottlers Supply Co., 405 N. Bowser, Richardson, Tex. This tank is rated at 200 psi and has a pressure relief valve built into the filler cap.

Hose—Synflex® Self-storing Hose, Type S-8 with ¼" NPT swivel fittings. Mfd. by Samuel Moore & Co., Mantua, O. 44255, who can furnish list of local distributors.

Gun & Nozzle—Trigger Teejet® with "D" series orifice disc only. Any of several ¼" models are suitable. Mfg. by Spraying Systems, Inc., Bellwood, Illinois 60104

FLIT® MLO will emulsify and diminish in larvicidal activity if sprayed forcefully into water, so it should be applied as a fine spray or as a low-pressure solid stream. The latter has been found most suitable since there is no drift of atomized particles.

With a D-2 orifice disc under 10 psi pressure a thin solid stream about 10 ft. long is produced which feathers out at the end and will not emulsify the material. The larvicide spreads well enough so that application of this small stream to the center of a 20 ft. wide ditch provides complete coverage. Also, an application around the edge of a house will spread over the entire surface of water standing under the house.