ARTICLES

RADIOACTIVE TAGGING OF *CULEX QUINQUEFASCIATUS* (SAY) WITH P^{32}

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Previous workers (Hassett and Jenkins 1949; Bugher and Taylor 1949; Thurman and Husbands 1951) have demonstrated the value of laboratory determinations of many factors in the rearing, handling, and radioactive tagging of mosquitoes before field dispersion studies are undertaken. Practical consideration must be given (a) to obtaining a suitable initial supply of specimens, (b) to estimating the final adult yield in terms of sex, survival, vigor and radioactivity levels and (c) to determining any species idiosyncrasies in assimilating and retaining the radioactive material. The present paper describes these evaluations as applied to *Culex quinquefasciatus* (Say) adults exposed as larvae to solutions of $Na_2HP^{32}O_4$.

LIFE HISTORY DATA. *Culex quinquefasciatus* (Say), a tropical and subtropical species, is found in foul waters often contained in rain barrels, catch basins, cesspools, ditches, and other similar habitats. The presence of all immature stages, egg rafts, larvae and pupae, in the same breeding sites offers practical difficulties since larvae of uniform age are best suited for tagging with $Na_2HP^{32}O_4$ solutions. Thus, the use of the egg rafts to secure the desired larvae appeared as the best solution. Therefore laboratory studies were made to determine (a) the rate of egg production of adult females, and (b) the possibilities of delaying egg hatch so that eggs from several days could be combined.

Colonies, each containing 100 male and 100 female adults, were offered a chicken as a source of blood every other night. The 5- to 9-day-old females laid an average of 37 egg rafts per colony. In the next 96 hours, an average of 34 egg rafts per colony was obtained. Counts on 38 rafts showed from 110 to 249 eggs per raft with mean and standard deviation values of 158 and 27 eggs per raft, respectively.

At 60°–80° F., essentially 98 percent of the eggs hatched within 24 hours. However, rafts maintained at 50° F. for 24, 48, 72, and 96 hours, showed hatching rates of 89, 97, 72, and 11 percent, respectively, within 24 hours after removal from refrigeration. Thus eggs could be held at 50° F. for 1 to 3 days and combined with freshly laid eggs to secure a uniform starting source of insects.

Eighty-seven of the larvae from approximately 500 eggs per liter of water pupated in 11 to 21 days when reared at 80° F. and 70 percent relative humidity. All successful adult emergence (92.4%) occurred within a 2-day period after pupation. The final distribution of the sexes was essentially equal. The percentages of each sex which emerged from pupae of days 11 through 21 were as follows: for males 8.8, 18.5, 26.9, 14.4, 15.1, 9.4, 2.8, 1.7, 1.0, 1.1, and 0.3, respectively; for females 0.0, 1.9, 6.7, 8.7, 12.2, 29.7, 7.7, 14.5, 9.8, 7.8, and 1.0, respectively.

Based on these production values (namely: 35 egg rafts per 100 females in a 4-day period, a mean number of 158 eggs per raft, 98 percent egg hatch, 87 percent pupation, and 92.4 percent adult emergence with equal numbers of each sex), one million adult females for dispersion studies

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would require (a) the collection of 100,000 pupae in a 2-day period and the rearing of the first four days of eggs produced by the resultant adults, or (b) the collection of about 16,000 egg rafts within a 4-day period. The latter starting point appears the more feasible although a combination of methods (a) and (b) could be considered.

To reduce the amount of radioactive solution and the attendant radioactivity hazards, larvae should be concentrated as much as possible during the tagging period. Since crowding might influence the growth and survival values for the species, 3rd instar larvae, reared by the standard insectary procedure, were concentrated at densities of 5,000, 10,000, 15,000, and 20,000 larvae per square foot of water surface. The concentrations were prepared in a series of 8-inch cubical paraffin-lined wooden boxes filled to a depth of 4 inches with tap water (0.5, 1.0, 1.6 and 2.1 larvae per ml. of water, respectively). On the day of concentration, 0.55 to 0.6 mg. of coarsely ground dog biscuit per larvae was provided. During the next 3 days, 0.55, 0.2, and 0.1 mg. of food per larva were added. The pupae were not collected, but daily counts were made on adults caught in cages designed to enclose the wooden boxes.

Each cage consisted of a wooden framework covered on the top and three sides with wire screen and on the remaining side with a cloth sleeve. In the plywood floor of the cage, an opening permitted the cage to slip down snugly over a wooden rearing box. As the cage was lifted off the rearing box, this opening was closed with a sliding panel and simultaneously another panel covered the wooden rearing box. Before removing the cage, all adults were forced from the water surface. Emergence commenced on the 4th day after larval concentration and continued for 5 to 7 days. Adult emergence from the 5,000, 10,000, 15,000 and 20,000 larvae per square foot concentrations were 93, 53, 32 and 8 percent, respectively. At the two lower concentrations, adults were divided equally between the sexes, but at 15,000 larvae per square foot, the females represented only 34 percent of the adults. These tests showed fewer adults from larval concentrations above 10,000 per square foot of water surface. Before these data were fully assembled, however, tests with the actual radioactive material were begun using concentrations of 15,000 larvae per square foot of water surface.

RADIOACTIVE TAGGING DATA. Two series, prepared on successive days using 500 eggs per pan, were reared by routine insectary methods to secure 7-day-old, early 3rd instar and 8-day-old, late 3rd instar larvae, respectively. Larvae were strained from the rearing medium and placed in paraffin-lined wooden boxes at densities of 15,000 larvae per square foot of water surface. Each box, filled to 4 inches, contained 4 liters of water to which 0.1 millicurie $Na_2HP^{32}O_4$ per liter of water was added and thoroughly mixed. The larvae were fed ground dog biscuit according to the schedule described previously. The first adults emerged on the 4th day after concentration of the larvae and emergence continued for 6 to 8 days.

Adults in each cage were collected with a mechanical aspirator and anesthetized lightly with CO_2. Some adults were removed for immediate determination of radioactivity. The remainder were supplied food and held in 1-gallon cardboard cartons for subsequent determinations after 1- and 2-week intervals. For determining radioactivity, each specimen was put into an individual cell, one of a series of holes bored in a wooden paddle and equipped with wire screen tops and bottoms. Specimens of each sex were read in separate paddles.

The mean radioactivity levels for 1-day-old adults from the two age groups of exposed larvae (Table 1) showed highly significant differences between (a) the males and females emerging on the same day from a given set of larvae, and (b) the adults of a given sex emerging the same day from the two sets of larvae. Also, the adults from the exposed 7-day-old larvae

TABLE 1.—Net activity levels (cpm)* of 1-day-old adult *Culex quinquefasciatus* emerging at different intervals after placement of 7- and 8-day-old larvae in solutions containing 0.1 millicurie of $Na_2HP^{32}O_4$ per liter

Days of exposure before emergence	Mean radioactivity (cpm) of adults from larvae exposed at			
	7 days		8 days	
	Male	Female	Male	Female
3	2135	—	290	—
4	1993	2471	419	—
5	1680	2559	539	1050
6	1298	2442	669	1040
7	1105	1924	717	930
8	1020	1801	562	1079
9	847	1485	—	—
10	728	1200	—	—

* Counts per minute.

showed decreasing activity levels as the interval to emergence became longer, whereas the adults from the exposed 8-day-old larvae showed increasing levels (males) or nearly uniform levels (females) as the interval to emergence became longer. Essentially all loss in radioactivity in 8- and 15-day-old adults could be accounted for through decay of the $Na_2HP^{32}O_4$, indicating high retention of absorbed phosphorus by the adults.

Since the adults from larvae exposed at 7 or 8 days of age showed marked differences in radioactivity, further tests were run with 8-, 9-, and 10-day-old larvae in water containing 0.05 millicurie $Na_2HP^{32}O_4$ per liter. Most of the 10-day-old larvae were in the 4th instar. The results (Table 2) re-emphasized the importance of timing the exposure, since the 8-day-old larvae produced adults with activity levels significantly higher than those of adults from larvae exposed at 9 or 10 days. Males from the larvae exposed at 9 days of age showed significantly higher activity levels than males from larvae exposed at 10 days of age. Differences in the adult females were not consistently significant in this latter case. Again with larvae exposed at 8, 9, and 10 days of age,

TABLE 2.—Net activity levels (cpm)* of 1-day-old adult *Culex quinquefasciatus* emerging at different intervals after placement of 8-, 9-, and 10-day-old larvae in solutions containing 0.05 millicurie of $Na_2HP^{32}O_4$ per liter

Days of exposure before emergence	Mean radioactivity (cpm) of adults from larvae exposed at					
	8 days		9 days		10 days	
	Male	Female	Male	Female	Male	Female
3	—	—	491	—	109	—
4	1972	—	736	719	376	500
5	1538	—	527	748	378	563
6	—	—	606	851	508	589
7	—	—	496	800	443	659
8	987	—	466	657	395	584
9	—	—	463	—	287	—
10	638	973	—	477	—	432
11	632	894	—	—	—	—
12	396	798	—	—	—	—

* Counts per minute.

TABLE 3.—Net activity levels (cpm)[a] of adult *Culex quinquefasciatus* from 6- and 7-day-old larvae exposed 24 and 48 hours in water containing 0.05 millicurie of $Na_2HP^{32}O_4$ per Liter

Day of pupation[b]	Day of adult emergence	Mean radioactivity (cpm) of adults from larvae exposed at: 6 days, For 24-hr., Males	6 days, For 24-hr., Females	6 days, For 48-hr., Males	6 days, For 48-hr., Females	7 days, For 24-hr., Males	7 days, For 24-hr., Females	7 days, For 48-hr., Males	7 days, For 48-hr., Females
2	4	—	—	—	—	2811	4763	4619	—
	5	—	—	—	—	2469	—	—	—
	6	—	—	—	—	2316	—	—	—
3	4	2959	—	5900	—	2784	5174	5453	8340
	5	2589	—	4774	8242	2446	3903	4867	8933
	6	—	4123	—	—	—	—	4515	—
4	5	2049	3507	4014	8446	1397	3269	—	8318
	6	1088	3065	—	6382	—	3281	—	7416
5	7	—	1609	—	5406	—	—	—	—

[a] Counts per minute.

[b] Day 0 is day of larval exposure to $Na_2HP^{32}O_4$.

the females had significantly higher activity levels than the males; the 8- and 15-day-old adults showed losses in radioactivity almost entirely assignable to normal decay of P^{32}; and the adults from the younger exposed larvae showed progressively lower activity levels as the time to emergence increased, whereas adults from older larvae showed increasing levels with peaks for adults emerging 4 to 6 days after exposure. Although the activity levels for the adult females from 8-day-old larvae exposed to 0.05 and 0.1 millicurie $Na_2HP^{32}O_4$ solutions were quite comparable, parallel observations on the males showed marked differences in the two series (Tables 1 and 2).

To determine the effect of limited 24- or 48-hour exposures on the radioactivity levels of adults, groups of 200 6- and 7-day-old larvae were placed in separate boxes containing 0.05 millicurie $Na_2HP^{32}O_4$ per liter. Half of the larvae from each box were removed at 24 hours, the remainder at 48 hours. Larvae were washed and placed in tap water to complete development. Pupae were collected daily and the early emerging adults for each day's pupae showed the highest activity levels (Table 3). These activity levels were generally higher than those previously obtained, possibly as a result of the differences in numbers of exposed larvae (200 *vs.* 15,000 per square foot). The ratios of activity levels from larvae exposed 48 hours to those from larvae exposed 24 hours were: males—1.89 and females—2.51. This indicates the daily absorption of P^{32} on the 6th, 7th and 8th days was about equal for male larvae but the female larvae absorbed increasing amounts on successive days.

From these studies it appears that the larvae should not be crowded at rates higher than 10,000 per square foot during the exposure period to $Na_2HP^{32}O_4$ and that the length of the exposure may be limited to 48 hours if 6-, 7-, or 8-day-old 3rd instar larvae are utilized. Concentrations of 0.05 and 0.1 millicurie of $Na_2HP^{32}O_4$ per liter did not appear to affect the activity or longevity of the adult mosquitoes.

In view of the somewhat extended period of 7 to 9 days for adult emergence from a given set of exposed larvae, the adults would have to be held if the speed of dispersion was being measured. In this case, adults emerging the first 4 days (principally males) could be destroyed, and adults emerging days 5 to 8 would include about two-thirds of the available females.

SUMMARY. One million radioactively tagged adult female *Culex quinquefasciatus* (Say) for dispersion studies can be obtained (a) by the collection of 100,000 pupae in a 2-day period and the rearing of the first 4 days of eggs produced by the resultant adults, (b) by the collection of about 16,000 egg rafts within a 4-day period and the rearing of the resultant larvae, or (c) by a combination of the two methods (a) and (b). Based on insectary studies, production values are as follows: 35 egg rafts per 100 females in a 4-day period, a mean of 158 eggs per raft, 98 percent egg hatch, 87 percent pupation and 92.4 percent adult emergence, with equal sex division. Exposure of 6- to 8-day-old larvae at concentrations of 10,000 larvae per square foot of water surface for periods of 24 hours or longer in 0.05 to 0.1 millicurie $Na_2HP^{32}O_4$ solutions will yield adults with activity levels detectable for a period of at least two weeks.

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