TECHNIQUES FOR ^{32}P LABELING AND ASSAY OF EGG RAFTS FROM FIELD-COLLECTED *CULEX PIPIENS QUINQUEFASCIATUS* SAY[1]

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ABSTRACT. Over 154,000 radioactive mosquitoes were produced by holding late third- and early fourth-instar larvae in trays of water containing 2.0 to 2.5 nanocuries of ^{32}P per ml with 1 larva per ml until pupation. When pupae were transported to Seahorse Key, Florida, and the resulting adults released, over 10,000 radioactive egg rafts were collected 4 to 29 days after release. The egg rafts were then assayed in a liquid scintillation counter for radioactivity. In addition to providing a label for the egg rafts produced, radioactivity data were used to estimate the number of first and second egg rafts deposited during the second ovipositional cycle. Deposition of first egg rafts occurred to a varying degree during the second ovipositional cycle. Furthermore, deposition of a very few first egg rafts occurred as late as 25 days after adult emergence.

INTRODUCTION. We have been studying the population dynamics of *Culex pipiens quinquefasciatus* Say and the application of the sterile male release technique for the control of this species. Such research requires survey techniques that permit the capture of both native and released insects and tagging systems that permit their identification. In Florida, we have been unable to trap significant numbers of adult *C. p. quinquefasciatus* by standard survey techniques such as the use of light, carbon dioxide, or animal-baited traps or natural resting sites. However, ovitraps have been extremely useful because we have been able to collect good numbers of egg rafts. Thus, a system of marking the eggs deposited by released females was needed.

Smittle and Patterson (1970) reported that female *C. p. quinquefasciatus* exposed to ^{32}P in the larval stage transferred 40 percent of their radioactivity to their first egg raft. These egg rafts could then be identified by this radioactive tag. Patterson *et al.* (1970) and Weidhaas *et al.* (1973) used this tagging method in the field to identify egg rafts from small numbers of released females. Therefore, we selected this method of tagging large numbers of mosquitoes that were to be released in a large-scale test of the biology and population dynamics of *C. p. quinquefasciatus* (Lowe *et al.*, 1973). The present paper presents the methods used in rearing mosquitoes and tagging them with ^{32}P and analyzes the data obtained with radiolabeling and recovery of radioactive egg rafts. The insects so tagged were used by Lowe *et al.* (1973) for studies on population dynamics of this species.

MATERIALS AND METHODS. Two releases of radiolabeled mosquitoes were made, the first in May 1971, (50,000 females and an equal number of males) and the second in July (27,000 females and an equal number of males). Mosquitoes for this study were from a laboratory colony started from native females a few months before the May release. This colony was programmed to produce 700 egg rafts (ca. 126,000 eggs) on a given day. Thus, when insects were to be used in a release, 13 egg rafts (ca. 2,300 eggs) were placed in each of 50 rearing trays (45 x 56 x 8 cm), each tray containing 4 liters of water. Two g of a mixture of brewer's yeast, liver powder, and hog supplement (1:1:1) was added to the water just before the rafts were introduced. Additional food was added as required.

When the larvae reached the late third and early fourth instar, they were removed from the trays by screening. Then the larvae from two trays were consoli-

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.

dated and placed in a tray containing 3500 ml of clean water to which the ^{32}P had been added for a total of 25 treated trays. After the appropriate exposure time of the larvae in the water with ^{32}P, 500 ml of rearing water (saved from the first trays) was added to each treatment tray. This procedure provided 4000 ml of treatment solution, 1 ml for each larva.

The treatment solution used with the mosquitoes released in May contained 2.0 nanocuries of ^{32}P per ml after the rearing water and food was added after the larvae had been exposed for 6 hours. Before the July release, an attempt was made to increase the radioactivity of the larvae by increasing the amount of ^{32}P to 2.5 nanocuries per ml and exposing the larvae for 18 hours before the rearing water and food were added. This procedure increased the radioactivity of the larvae slightly but the delay in feeding prolonged the development time and spread the time for pupation.

The larvae were maintained in the infused ^{32}P solution until pupation. Additional larval food was added as required. Pupation began about 48 hours after the beginning of the exposure to the ^{32}P solution, so each day thereafter larvae and pupae were screened from the treatment trays, and the pupae were separated from the larvae by the ice water technique of Wethersby (1963) as modified by Hazard (1967). Then the larvae were returned to the ^{32}P solution, and the pupae were separated sexually by size. Since most larvae pupated within a 3-day period and since pupae of the same ages were used for the releases, the releases had to be made on 3 consecutive days.

Although, as noted, the majority of the insects tagged with ^{32}P was used by Lowe *et al.* (1973) in their studies on Seahorse Key, we kept some at the laboratory so we could measure the radioactivity in the emerging adults and in the egg rafts deposited by the females. Thus, samples of 5 to 20 adult males and females from each day's release were analyzed the day after emergence. Other males and females were placed in screen cages (25 x 25 x 15 cm) and provided with sugar water on cotton, a one-day-old chicken for a blood meal, and oviposition media. The resulting egg rafts were analyzed for comparison with those returned from Seahorse Key. Also, in July, after the females had mated and taken a blood meal, 200 were placed in individual vials with infusion water as oviposition medium; females that oviposited were given another blood meal and placed in vials to collect the second egg raft.

The measurements of radioactivity in adults or egg rafts were made with a Packard Tri-Carb® liquid scintillation spectrometer. The scintillation fluid was composed of 5 g of PPO (2, 5-diphenyloxazole) and 10 g of thixotrophic gel powder (Cab-O-Sil®) per liter of toluene. For assay, either adults or egg rafts were placed individually in glass vials containing 10 ml of the scintillation fluid. Vials containing scintillation fluid only were used for determination of background radioactivity. The counts/min for each adult or egg raft were recorded automatically on data sheets. The results are reported as counts/min minus background.

RESULTS AND DISCUSSION. The females used for the May release averaged 1855 counts/min (range 1349–2674); the males averaged 893 counts/min (range 559–1373). Females used for the July release averaged 1945 counts/min (range 803–2765); the males averaged 987 counts/min (range 112–1455). Thus, individual mosquitoes varied considerably in the amount of radioactivity assimilated. The counts for the treated adults from both groups indicate that females contained an average of about 0.9 and males an average of 0.4 nanocuries. Then since each tray contained either 8 μCi (May release group) or 10 μCi (July release group) and produced about 4000 adults (half males and half females), we estimate that 13 to 16 percent of the radioactivity in each tray was assimilated and retained by the mosquitoes.

Since the assay for radioactivity killed the mosquitoes, other adults emerging from the treated pupae were used to pro-

duce egg rafts in the laboratory for comparison with those collected on Seahorse Key. Twenty-five of the first egg rafts from adults prepared for the May release had an average of 264 counts/min. Females held from the July release produced 163 first egg rafts that averaged 406 counts/min and 42 second egg rafts that averaged 161 counts/min.

Table 1 reports the radioactivity of eggs collected from Seahorse Key. As Lowe *et al.* (1973) report, there was a 4-day oviposition cycle after the May release and a 3-day cycle after the July release. They also discuss the oviposition and longevity of adults as they relate to environmental factors.

In the May release, 39 percent of the egg rafts were obtained on days 7, 8, and 9 in the first oviposition cycle, and 24 percent were obtained on days 11, 12, and 13 during the second oviposition cycle. In the July release, 42 percent of the egg rafts were obtained on days 6, 7, and 8 during the first oviposition cycle, and 36 percent were obtained on days 9, 10, and 11 during the second oviposition cycle.

The average radioactivity of the egg rafts during the 3 days of the oviposition cycle after the May release was 274 counts/min. After the July release, it was 416 counts/min. Thus the radioactivity of the first egg rafts from both releases was comparable to those of the first egg rafts obtained from the two groups of control females retained at the laboratory. However, the radioactivity of the rafts collected during the first 3 days of the second oviposition cycle after the May release was 37 percent lower than the radio-

Table 1.—Radioactivity and egg rafts collected and analyzed after two releases of ^{32}P-labeled females on Seahorse Key.

Days after release	May release (50,000 ♀)		July release (27,000 ♀)	
	No. radioactive egg rafts	Average counts/min	No. radioactive egg rafts	Average counts/min
4	..	..	7	248
5	6	229	55	423
6	255	321	191	437
7	1039	292	400	459
8	1367	285	269	416
9	1087	242	141	344
10	555	229	460	364
11	588	189	137	322
12	997	172	82	244
13	533	139	116	180
14	455	130	60	139
15	194	94	43	147
16	291	92	8	94
17	235	94	26	113
18	234	66	17	96
19	246	62	16	66
20	205	55	3	49
21	149	48	2	25
22	81	33	..	..
23	99	35	..	..
24	109	39	..	..
25	56	30	..	..
26	27	25	..	..
27	13	17	..	..
28	29	21	..	..
29	19	19	..	..
Total	8868	..	2033	..

activity of those collected during the same period of the first cycle. The 4 days that lapsed between the peaks would account for a reduction of only 18 percent based on the 14.3 day half life of ^{32}P. Therefore, the remaining reduction was probably the result of ^{32}P lost by incorporation into the first egg rafts. Smittle and Patterson (1970) showed that female mosquitoes incorporated approximately 40 percent of their radioactivity into an egg raft. Then since the females used for the July release that were held in the laboratory produced first egg rafts averaging 406 counts/min (vs. 416 counts/min for those released in the field), we estimate that these females had an average of 1015 counts/min before oviposition and 609 counts/min after oviposition. Furthermore, since the laboratory mosquitoes were egged 7 days apart, a decay correction factor of 0.712 must be introduced. In other words, at the time of the second oviposition, the females should have had an average of 434 counts/min, and the egg rafts should have had an average of 174 counts/min. This theoretical value compares very closely to the 161 counts/min actually recorded from the second egg rafts from females retained in the laboratory.

The close correlation between the actual radioactivity of the second egg rafts and the expected radioactivity made it possible to estimate the number of females producing a second egg raft in the second oviposition cycle in the field. In the May release, the average radioactivity of the 3493 rafts collected on 3 days during the first oviposition cycle was 274 counts/min, and the females that produced these eggs would be expected to produce egg rafts 4 days later that had about 135 counts/min. Egg rafts from females that waited until the second oviposition cycle to deposit the first egg raft would be expected to have about 227 counts/min. The average radioactivity of the 2118 egg rafts collected on 3 days in the second cycle was actually 168 counts/min. Then from our calculations about 777 (37 percent) were first egg rafts and 1341 (63 percent) were second egg rafts.

Similarly, after the July release, the 738 egg rafts collected during 3 days of the second oviposition cycle had an average of 352 counts/min. Then from the same calculations the first egg rafts from females that oviposited during the second cycle would be expected to have 381 counts/min, and the second egg rafts would have 229 counts/min. On this basis about 607 rafts (82 percent) were first egg rafts and about 131 (18 percent) were second egg rafts.

We subsequently extended our calculations concerning the second oviposition to determine the number of second egg rafts produced on days 10 to 14 after the May release and days 8 to 12 after the July release. In making these calculations, we assumed that all egg rafts through day 9 after the May release would be first egg rafts and that second egg rafts would be produced 4 days after the first egg raft. Also, we assumed that all egg rafts through day 7 after the July release were first egg rafts and that second egg rafts would be produced 3 days after the first egg raft.

The results of these calculations are shown graphically in Figure 1. After the May release, the number of second egg rafts reached a peak on day 12, that is, 4 days after the peak of first egg rafts. Also, on days 10, 11, 12, and 13, the number of second egg rafts ranged from 29 to 44 percent of the number of first egg rafts on days 6, 7, 8 and 9. After the July release, there was no definite peak for second egg rafts; the number ranged from 39 to 61 on days 9 through 12. The large number of females that waited until day 10 to deposit their first egg raft may have resulted in part from the environmental conditions (Lowe *et al.* 1973).

The number of second egg rafts was not calculated beyond day 14 after the May release and day 12 after the July release. After these days, the apparent production of third egg rafts caused the average observed radioactivity to drop below

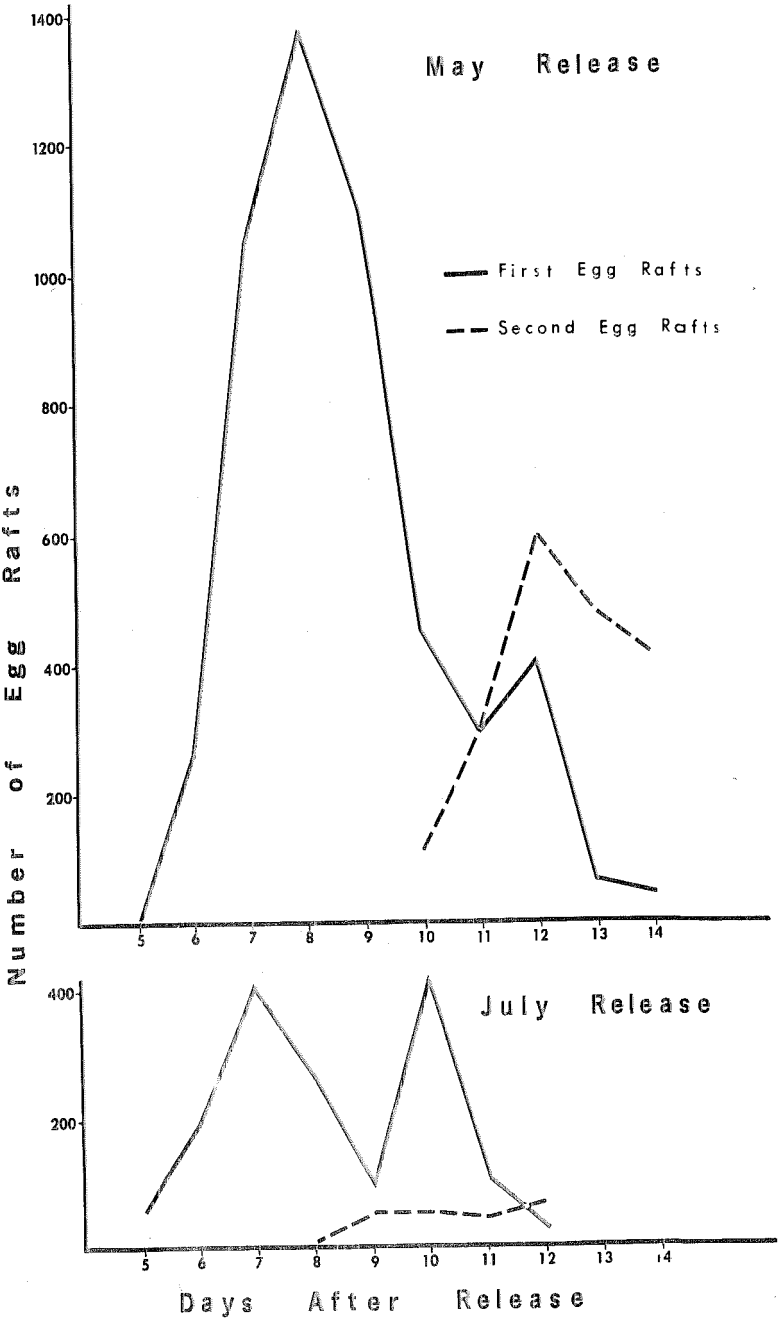

Fig. 1.—Estimated number of first and second egg rafts produced by ^{32}P-labeled females on Seahorse Key.

the theoretical average for the second egg raft average. Also at this time, 78 and 86 percent of the total egg rafts had been collected. Figure 1 also shows two peaks in the deposition of first egg rafts after both releases. In May–June, they were 4 days apart; in July, they were 3 days apart. Therefore, a rhythm was demonstrated for the deposition of first egg rafts in addition to that shown by Lowe *et al.* (1973) for all rafts.

One egg raft with about 200 counts/min was collected on day 25 after the May release, 2 with over 400 counts/min were collected on day 15 after the July release. These were probably first egg rafts since the radioactivity was just slightly lower than the average for the rafts deposited at the time of the first oviposition peak. Therefore, some females will delay first oviposition for as much as 25 days.

The use of radiolabeled female mosquitoes proved to be a valuable tool in studying mosquito biology. It provided a method of identifying the eggs produced, and it can be used quantitatively to obtain information about the time of first oviposition and the number of first and second egg rafts among the rafts collected.

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Cumulative Bibliography of Biological Control of Mosquitoes (Parasites, Predators, Viruses and Related Agents)

It as long been planned to prepare and publish a cumulative bibliography derived from the section "Literature References to Mosquitoes and Mosquito-Borne Diseases." Limitations of time and funding, augmented by the rapidly inceasing number of titles, have prevented the complete realization of this plan, but it was felt that it would be feasible and useful to undertake the project on a special subject basis.

Many readers felt it woud be appropriate to begin with Biological Control. Accordingly, Mrs. Sollers-Riedel has prepared the list of references which begins on page 301 of this issue.