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WINTER SURVIVAL AND OVIPOSITION OF ^{14}C-LABELED *CULEX PIPIENS QUINQUEFASCIATUS* SAY IN NORTHERN FLORIDA

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ABSTRACT. Two releases of ^{14}C-labeled females of *Culex pipiens quinquefasciatus* Say were made on an island off the west coast of Florida in December 1971 and February 1973. After being fed ^{14}C-labeled glucose, the mosquitoes oviposited radioactive egg rafts. In 1971, the first radioactive egg raft was collected 5 days after females were released, whereas in 1973 when average maximum and minimum temperatures were more than 8° C lower, it was 18 days before the first radioactive egg raft was detected. The released females were able to survive outside temperatures as low as —0.6° C and to oviposit 38 days after release.

Because *Culex pipiens quinquefasciatus* Say (=*C. fatigans* Wiedemann) is an important disease vector throughout the world, our laboratory has conducted extensive biological studies with this species since 1966 and has evaluated the sterile-male release technique as a control method. In support of this effort, techniques were developed to incorporate radioactive phosphorus (^{32}P) into egg rafts deposited by female *C. p. quinquefasciatus* so reproductive behavior and longevity during the months of peak populations (from May to September) could be determined (Lowe et al. 1973, Patterson et al. 1970, Smittle et al. 1973, Weidhaas et al. 1973). Also, seasonal fluctuations in an isolated population of *Culex* species were investigated by collecting all egg rafts from April 1971 to September 1972 (Lowe et al. 1974).

Likewise studies were made during the winters of 1971–1972 and 1972–1973 of the survival and oviposition of females during the cool winter weather. For this test, a technique was developed whereby ^{14}C-labeled glucose fed to adult females was subsequently incorporated into their eggs.

METHODS AND MATERIALS. The study site was Seahorse Key, an uninhabited island in the Gulf of Mexico, near the town of Cedar Key, Florida. Since mosquitoes from an indigenous population may be better adapted for survival than those from a laboratory colony, egg rafts were collected from this area and used to start a colony to produce the mosquitoes used in this study.

For the 1971 release, we planned to hatch and rear larvae in outdoor cages, but ambient temperature ranged from 4 to 25° C so hatch and survival were poor. Therefore, the additional mosquitoes required were reared in the laboratory where temperatures ranged from 18 to 29° C. Pupae were separated by sex with a plate glass separator (Fay and Morland 1959) and placed in screen cages for eclosion. Adults were manually sexed after eclosion and counted in a cold room (1° C). Females were placed in 37 x 38 x 46-cm screen cages (approximately 2000

per cage) inside a large outdoor screen building where for 4 days they were offered 2.5% sugar water containing 1 μCi of ^{14}C-labeled glucose/ml.

For feeding, 10 ml of radioactive solution was placed in a petri dish (60 x 15 mm), and a circle of molded plastic foam (50 mm in diameter and 3.2 mm thick) containing numerous holes (4.8 mm in diameter) was floated on the surface of the solution. Two such dishes were placed in each cage. This technique allowed females to rest on the plastic foam and ingest the ^{14}C-labeled sugar water without getting excessive external contamination. Sugar water levels in each dish were checked daily and additional ^{14}C-labeled sugar water added when necessary. Temperatures ranged from 16 to 27° C during the feeding period. Separated males were maintained identically except they were offered unlabeled sugar water. Males were released with females so adequate numbers of males would be present in the population for insemination.

All larvae used for the 1973 release were reared in the laboratory, and adults were separated and caged as in 1971. Also, adults were fed as described except the concentration of ^{14}C in the sugar water was increased to 2 μCi/ml. The day after mosquitoes were placed in the outdoor screen building, subfreezing temperatures were forecast so they were returned to the laboratory. Temperatures in the laboratory during the remaining 3 days of feeding ranged from 21 to 27° C.

The island was prepared for the releases by placing ovitraps similar to those used in previous studies (Lowe et al. 1973, Patterson et al. 1970, Weidhaas et al. 1973) at various locations after all known natural breeding sites had been destroyed. Normally, all *Culex* sp. egg rafts deposited in the ovitraps were collected every 2 to 6 days; however in 1971, collections were suspended once for 20 days due to the unseasonably warm temperatures during the Christmas and New Year holiday period. Each collected egg raft was placed in an individual vial with water and taken to the laboratory for hatching. A sample of larvae from each raft was removed and reared to the fourth-instar for identification. The remaining larvae, egg shells, and unhatched eggs were poured through a small strainer (2 cm in diameter) consisting of fine mesh nylon cloth glued to a wire loop. After the strainer was placed momentarily on blotted paper to remove excess water, a fine stream of air was directed so as to blow the larvae and eggs into a liquid scintillation vial. These vials contained a liquid scintillation fluid prepared by adding 5 g of PPO (2, 5-diphenyloxazole) and 10 g of thixotrophic gel powder (Cab-O-Sil®) per liter of a mixture of toluene and ethyl alcohol (3:1). Radioactivity was measured as counts per minute with a Packard Tri-Carb® liquid scintillation spectrometer, and adjusted for background radioactivity. Nonradioactive egg rafts were assumed to have been laid by indigenous females and were identified to provide a record of the species present on the island.

RESULTS AND DISCUSSION. In 1971, 4 releases totaling 24,000 females and 14,000 males were made on the island over a 7-day period. Samples of radioactive females had an average of 2,042 counts/minute (range 205–6603) at the time of release. Radioactive egg rafts collected from ovitraps averaged 238 counts/minute (range 41–852). The first radioactive rafts were collected 5 days after the beginning of the release, and the last ones were collected 26 days after the last release (Table 1). No radioactive rafts were found during the following 18 weeks.

In 1973, 2 releases totaling 5000 adults of each sex were made over a 3-day period. Samples of radioactive females had an average of 10,908 counts/minute (range 4047–20,100) at the time of release. Radioactive egg rafts collected from the ovitraps averaged 831 counts/minute (range 20–2661). The first radioactive rafts were collected 18 days after the beginning of the release, and the last ones were collected 38 days after the last release (Table 1). No radioactive rafts were

Table 1. Oviposition of ^{14}C-labeled *C. p. quinquefasciatus* females after release in December 1971 and February 1973.

Days after first release	Number of radioactive egg rafts collected	
	1971	1973
5	6	0
7	35	0
10	13	0
12	56	0
18	NC[a]	2
20	NC	1
25	NC	44
27	NC	73
31	NC	25
34	2	19
38	0	7
41	0	2

[a] NC = No collections made.

found during the following 3 weeks. In our previous studies using ^{32}P-labeled females, 26 days after females were released was the maximum time that radioactive egg rafts were collected.

The difference in 1971 and 1973 in the interval after release before first oviposition was apparently caused by differences in temperature during the 20 days after the releases. The average minimum and maximum temperatures in 1971 were more than 8° C higher than in 1973. In 1973, most oviposition occurred during the second 20-day period when the average minimum and maximum temperatures were 2° C higher than in 1971. During 1973, the minimum temperature recorded was —0.6° C, 9 days after release; the maximum temperature that same day was 2.8° C. There were no inhabited buildings on the island to provide heated shelter and since oviposition began 9 days after this cold day, *C. p. quinquefasciatus* adults apparently can survive temperatures below 2.8° C for more than 24 hours. However, females may find shelter inside buildings or cisterns, which may provide a warmer environment.

These studies show that an active field population of *C. p. quinquefasciatus* females can survive at least 38 days during the winter in northern Florida despite fluctuating temperatures as low as —0.6° C. In addition, the data indicate that females delay oviposition during periods of cool weather but oviposit when warm temperatures return. This indicates that the females did not go into a true diapause but became inactive during unfavorable conditions. Observation of activity during the winter has been reported for other *Culex* species. Hays (1973) observed that *C. p. pipiens* L. will leave hibernation sites in the Ohio-Mississippi River Basin during periods of warm weather during the winter. Lowe et al. (1974) collected egg rafts from *C. restuans* Theobald and *C. nigripalpus* Theobald as well as *C. p. quinquefasciatus* during the winter in Florida.

The techniques developed using ^{14}C-labeled females provide a useful tool for long-term biological studies of mosquitoes in the field.

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