COMPETITIVENESS OF STERILE MALE *CULEX PIPIENS QUINQUEFASCIATUS* SAY RELEASED INTO A NATURAL POPULATION [1]

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ABSTRACT. When known numbers of chemosterilized males, normal males, and ^{32}P-labeled females of *Culex pipiens quinquefasciatus* Say were released at a ratio of 1:1:2 into a natural isolated environment, and all egg rafts collected were assayed, rafts from released and indigenous females could be distinguished and sterility ascertained. Four such tests made over 18 months provided information about female longevity, dispersal, and reproductive activity. In addition, the design of each test was altered to release the different groups at various times and locations to alleviate biases of dispersal and longevity. The first 3 tests involved single releases of large numbers of adults and produced average sterilities of only 27 percent. The fourth test involved daily releases for 10 days and produced a sterility of 42 percent (expected sterility: 50%). Thus the sterile males were competitive when released over a sufficient period to allow all ages of adults to become balanced within a population.

INTRODUCTION

Before it will be possible to utilize recent advances in knowledge of controlling insects using methods other than traditional applications of pesticides, we need a broad knowledge of the population dynamics of the species. However, for mosquitoes, especially for *Culex* species that have world-wide importance as disease vectors, considerable biological data are available. Lindquist *et al.* (1967) released *Culex pipiens fatigans* Wiedemann (=*quinquefasciatus* Say) labeled with radioactive phosphorus (^{32}P) to show that dispersal was adequate for sterile male release control programs. Patterson *et al.* (1970b) showed that when dispersal was required over only short distances, releases of sterile males eliminated an isolated population of *C. pipiens quinquefasciatus* Say, and Weidhaas *et al.* (1971) reported information concerning the biotic increase of this species. Hagstrom (1971) studied the survival and dynamics of *C. tarsalis* Coquillett also using an isolated population.

More recently, Weidhaas *et al.* (1973) made daily releases of *C. p. quinquefasciatus* tagged with ^{32}P, or sterilized with thiotepa, to determine migration distances of the males and survival of the females. Smittle *et al.* (1973) described techniques for ^{32}P labeling of large numbers of *C. p. quinquefasciatus* and methods of assaying field-collected egg rafts. Lowe *et al.* (1973) utilized these techniques to determine the effect of seasonal changes on ovipositional patterns and adult longevity. In these previous tests, treated pupae were transported to the field, and releases occurred by eclosion at the release site. In more recent studies, known numbers of adults were released. For example, Lowe *et al.* (1974) conducted a 17-month survey of *Culex* species present at an isolated location to determine the relative abundance and ovipositional patterns of these species at various times of the year. Also, we recently released adults of *C. p. quinquefasciatus* labeled with ^{14}C to observe the overwintering capabilities of this species (Smittle *et al.* 1974). We found that in 2 successive years the released adults survived long enough to oviposit during warm periods; moreover, they apparently continued breeding throughout the winter in northern Florida.

In the present paper, we report the results of a series of tests in which adult *C. p. quinquefasciatus* were released to

[1] Mention of a pesticide in this paper does not constitute recommendation of this product by the U.S. Department of Agriculture.

determine the competitiveness of sterile males in a natural environment. Tagged mosquitoes were used in both single and multiple releases to establish which method provided more meaningful data.

EXPERIMENTAL LOCATION AND METHODS

Seahorse Key, an island in the Gulf of Mexico near the Florida coastal town of Cedar Key, was chosen as the location for the study. Several factors make this an excellent site for a biological investigation: (1) it is isolated from other islands and from the Florida mainland by 2 miles of salt water, and the prevailing winds are from the Gulf of Mexico; (2) an indigenous population of *C. p. quinquefasciatus* occurs throughout the year; (3) the biology of this species on the island has been well-defined by previous releases; (4) the island is a national wildlife refuge, and there are large rookeries of white ibis, brown pelicans, cormorants, and frigate birds to provide the blood meals required by the mosquitoes; and (5) the island is fairly large, a mile long and up to ¼ mile wide. Permission to use Seahorse Key for the studies was granted by the U.S. Department of Interior, Fish and Wildlife Service, and the University of Florida, which maintains a marine laboratory on the Island.

The island rises sharply from the shore to a high central ridge that runs lengthwise north and south. Dense woods border narrow sand beaches on the west side and an area of mangrove swamp and sawgrass on the east side. A narrow clearing around the marine laboratory near the dock extends to an old lighthouse at the top of the ridge. No fresh water is available for breeding except a septic tank and a fresh water cistern, both of which were sealed during the present studies. In earlier studies, Patterson *et al.* (1970a) established that breeding of the indigenous population of *Culex* sp. occurs near the cleared areas in natural objects that can hold rainwater.

The present study involved 4 releases of large numbers of adult mosquitoes between September 1971 and May 1973. The methods of rearing, treatment, transport, release, and egg raft collection and assay were the same for each release. Also, all releases were composed of 3 groups: normal males, sterile males, and normal females. In 3 releases, various combinations of each group were labeled with ^{32}P. However, each release differed either in the time or location of release of each group and in the length of time that egg rafts were collected and assayed. All oviposition sites were monitored for various periods before each release to obtain data concerning the populations of *Culex* sp. present and their relative abundance.

Rearing of the mosquitoes for the releases was accomplished in the laboratory by using large plastic trays each containing about 2000 larvae hatched from 13 to 15 egg rafts. These trays were maintained on a normal rearing schedule until the larvae reached the third instar. To obtain ^{32}P-labeled mosquitoes, we removed the third instar larvae from the rearing water and treated them with radioisotopes by the techniques described by Smittle *et al.* (1973); then they were returned to the tray, and allowed to pupate. For treatment, each tray received approximately 0.0025 μCi of ^{32}P/ml per larva. When radioactive labels were not required, the larvae were maintained to pupation in the original rearing trays.

After pupation, all mosquitoes were sexed with a pupal separator. Then since both normal and sterile males were needed for each release, half the male fraction of pupae was treated with the chemosterilant thiotepa by placing them in a 0.7 percent solution for 4 hours and then holding them in clear rinse water for 1 hour. Groups of pupae were put into separate cages according to treatment. Within 24 hours after emergence, the adults were immobilized in a cold room, the unwanted sex in each sample was manually removed, and the adults were counted and placed in aluminum

cages (37 x 37 x 46 cm) for release. The cages were sealed in cardboard boxes containing wet sponges (for humidification), the boxes were transported to the island, and the mosquitoes were released upon arrival. The number of dead mosquitoes was tabulated, and the release records were adjusted accordingly. All released adults were less than 48 hours old except in 1 test when the release of females was delayed 2 days because of weather.

Eggs from the native population and from the released females were obtained from artificial oviposition sites: 38-liter plastic wash tubs containing 9.5 liters of water infused with a 1:1:1 mixture of liver powder, brewer's yeast, and hog supplement. Fourteen such tubs were used as ovitraps, 7 at the bottom of the hill, 3 midway up the hill near a generator shed and salt water cistern, and 4 on the ridge. Some tubs at each elevation were near the interface of the woods; some were in open areas near the buildings; and some were sheltered. The tubs were emptied and reset every 3rd or 4th day to reduce predator breeding and surface scum and to provide an active infusion for optimum attractancy.

All rafts were collected daily after each release and returned to the laboratory in individual vials. After the first release, the egg rafts were assayed for radioactivity in a proportional counter, then they were separated according to ovitrap location and presence of radioactivity and placed in vials of infusion water to check hatch. A later modification of this system (unpublished data) was to observe hatch first, and then assay rafts and larvae in a liquid scintillation counter.

The experimental design of each release was adjusted to attain a measurement of the competitiveness of the sterile males.

EXPERIMENTAL DESIGN AND RESULTS

TEST 1. The first test began September 17, 1971, with a release of 8000 sterile males at the base of the hill near the marine laboratory. We assumed that the natural mortality rate of sterile adults was about 25 percent per day (from previous releases), and we wanted to establish that sterile males were still competitive 4 days after release. Then by September 21, there should have been approximately 2500 of the released sterile males present, so we released 2500 fertile males and 5000 ^{32}P-labeled virgin females, both 24 hours old, at the same site to obtain a 1:1:2 ratio of sterile males, fertile males, and fertile females, respectively.

Egg rafts were collected for 20 days after September 21. Of the total 272 rafts collected during the major oviposition period (from September 25 to October 7), 11 were nonradioactive, that is, from indigenous females; therefore, the recapture rate from the released females was 5.2 percent. Peaks of oviposition by *C. p. quinquefasciatus* occurred every 3rd day after the first radioactive rafts were collected. Of the 261 radioactive egg rafts, 26 percent (68 rafts) were sterile (Table 1). The expected sterility of 50 percent would have confirmed a 1:1 ratio of sterile to fertile males and a daily mortality rate of 25 percent, but the observed sterility of 26 percent indicated a ratio of 1 sterile mating for every 3 fertile matings and suggested an effective population of sterile males of only 830 instead of 2500 on the 4th day after their release. The reason for this variation from the expected is not clear; however, it was apparently associated with (1) a daily mortality of sterile males in excess of 25 percent, (2) a lack of competitiveness in the sterile males, or (3) experimental bias favoring the fertile males. The latter seemed a logical explanation because the fertile males were released at the same site as the females, and the sterile males had 4 days to disperse from that site.

TEST 2. In the second test, to overcome the apparent problem of dispersal from the release site, we released adults from each group at each of 3 locations: 37 percent at the bottom of the hill near the

TABLE 1. Fertility of egg rafts collected on Seahorse Key after release of sterile and normal males and fertile females of *C. p. quinquefasciatus*.

Test	^{32}P Labeled			Collected rafts		Percent sterility
	S ♂	N ♂	N ♀	Sterile	Fertile	
1 (9/71)	—	+	+	68	193	26
2 (6/72)	—	+	+	365	993	27
3 (9/72)	+	+	+	63	169	27
4 (4/73)	—	—	—	1239	1687	42

marine laboratory, 37 percent at the middle of the hill near the generator sheds, and 26 percent at the top of the hill near the lighthouse. (Evening observations of mosquito activity during all previous releases had shown that mating swarms occurred in these areas.) All releases were near the interface of wooded and cleared areas, but the 4 ovitraps at the top of the hill were all in more open areas. On June 15, 1972, 19,935 sterile males were counted into 8 cages (approximately 2500 per cage) for transport and release. The observed average cage mortality due to handling and transport was 50 adults (2%) so that approximately 19,500 males were released. By June 18, the estimated natural mortality (25% per day) should have decreased this total to 8226, so this number of fertile males was released on that date in the respective proportions at each location. This release occurred on an exceptionally stormy day that preceded an early-season hurricane. Plans called for the release of 12,276 females on the next day, June 19 (ratio of 1:1:2, accounting for an extra day of mortality for all males), but this release was delayed 2 days because of gale winds and heavy rains accompanying hurricane Agnes; it was finally accomplished June 21 when the females were 3 to 4 days old.

Egg rafts were collected for 22 days after June 21. Of the total 1599 rafts collected during the major oviposition period (from June 22 to July 7) 241 were nonradioactive, that is, from native females. Therefore, the recapture rate was 12.3 percent for released females. Peaks of oviposition occurred in 3- to 4-day cycles, as in the past, despite the disturbed weather due to the hurricane. Of the 1358 radioactive egg rafts, 27 percent (365 rafts) were sterile (Table 1). This sterility was similar to that for the previous test, and again indicated a ratio of 1 sterile to 3 fertile matings, and suggested an effective population of only 2740 sterile males instead of 8225 on the 3rd day after release. Thus, though the possible bias had been alleviated, the lower sterility continued. It could still have been caused by a daily mortality rate of sterile males greater than 25 percent, a lack of competitiveness, or greater dispersal out of the area during the additional 3 days in the environment.

Test 2 provided additional observations concerning the biology of *C. p. quinquefasciatus*. In the first place, even though 26 percent of all mosquitoes were released at the top of the hill, only 9.3 percent of the rafts were obtained from the 4 ovitraps there (Table 2). Similarly, ovitraps in open areas at the bottom of the hill and on the hillside did not attract a proportional amount of oviposition. The tubs in secluded locations or near the interface of wooded areas provided the best oviposition sites, and those in areas near spots where swarming was observed in previous releases definitely produced the highest number of rafts. Secondly, the oviposition rates of released females was even greater than in the first release, so the insemination rate was also high. Thus, the released male mosquitoes apparently secluded themselves and successfully survived the hurricane-force weather.

TEST 3. In the third test, 4900 sterile and 4900 normal males (1:1 ratio) were released near the marine laboratory on

TABLE 2. Effect of location of ovitraps on egg rafts collected during 2 release studies of *C. p. quinquefasciatus* on Seahorse Key.

Ovitrap number	Percentage of total egg rafts collected	
	Test 2 (June 1972)	Test 3 (Sept. 1972)
	Bottom of hill	
1	7.4	6.9
2	4.6	6.6
3	2.9	4.7
4	1.2	6.6
5	3.9	3.7
6	24.0	5.5
7	1.7	0.4
Subtotal	45.7	34.4
	Hillside	
8	19.7	9.1
9	11.2	12.4
10	14.0	6.2
Subtotal	44.9	27.7
	Hilltop	
11	1.8	6.2
12	3.9	16.4
13	3.3	10.6
14	0.3	4.7
Subtotal	9.3	37.9

September 15, 1972; 10,650 ^{32}P females were released at the same location the following day. The weather, both before and during the test, was excellent with warm days and no high winds or rain. Egg rafts were collected only until September 25, since 10 days had been observed to be time enough for most tagged females to oviposit their first eggs. A total of 274 rafts was collected during the major oviposition period (September 18–25), of which 42 were nonradioactive, that is, from indigenous females. Of the 232 radioactive egg rafts, 27 percent (63 rafts) were sterile (Table 1). Again, this percentage sterility indicated a ratio of sterile to fertile matings of 1:3, even though all males were released simultaneously.

Female dispersal during the 3rd test was similar to dispersal during the 2nd test. Even though all adults were released at the bottom of the hill, 34 percent of all rafts collected were in this area, 28 percent were in ovitraps on the middle of the hillside, and 38 percent at the top (Table 2). Thus, the females dispersed uniformly throughout the area, and all ovitraps were attractive in the good weather. Also, the recapture rate of 2.2 percent complemented the data from previous tests that showed that survival and recapture is normally lower in the fall.

A review of the first 3 tests indicated that chemosterilized male *C. p. quinquefasciatus* treated as described were only one-third as competitive as fertile males in a natural environment. This result was in contrast to results of other laboratory and field-cage studies that had shown no apparent differences in competitiveness. Estimates of the density of the natural population based on the number of egg rafts collected from indigenous females demonstrated that the number of native males present on the island was insufficient to bias the results by increasing the number of fertile matings by released females. Furthermore, dispersal and longevity were apparently not the primary reasons for the lower than expected sterility. However, all 3 tests involved single releases of large numbers of males and females; thus, any differences between sterilized and untreated insects could be accentuated because of the limited time that would elapse before the released females would all be mated. To minimize the effect of timing, we therefore arranged a test in which the released population could stabilize numerically so we could observe the true competitiveness of released sterilized males compared with released untreated males.

TEST 4. In test 4, the sterile males were released with normal males and females (1:1:2 ratio) daily for 10 days to minimize the possibility that experimental bias would influence dispersal, longevity, and mating aggressiveness by establishing a representative balanced population of released fertile and released sterile males of all ages. The study was begun in April 1973 to minimize any possible effect of

indigenous males, which are normally at a low density at that time of the year.

No radioactive labels were used with any of the released groups so egg rafts from indigenous females could not be distinguished from those of released females. Care was therefore taken to determine the oviposition activity of indigenous females by collecting and identifying all egg rafts from the indigenous population for 10 days before the first release (daily average=15.6 rafts). The actual numbers of egg rafts deposited by the released females could then be calculated by subtracting from the daily collections of rafts of other species and the average number of *C. p. quinquefasciatus* rafts collected during the 10 day pre-release period.

An equal number of sterile and fertile (untreated) males and twice this number of females (1:1:2 ratio) were transported to the island each of the 10 days of release (except that the 6th and 8th days the number of emerged females was low, and the ratio was 1:1:1). The releases were made near the marine laboratory from April 16 through April 25. A total of 10,300 sterile males, 10,300 fertile males, and 17,800 normal females were released. Since egg rafts were collected from the 10 most productive oviposition tubs daily during the releases and for 9 days after the last release, all released females had time to oviposit first egg rafts, and those released early had time to deposit second or third egg rafts. Of the 3138 egg rafts collected during the major oviposition period from April 21 to May 4, 97 percent were *C. p. quinquefasciatus*. Of the 2926 rafts collected from released females, 1239 (42%) were sterile (Table 1). This sterility indicated an effective ratio of 1 sterile to 1.4 fertile males, which approximated the 1:1 release ratio, and suggested fairly equal competitiveness. Thus, sterile males were competitive with fertile males when the two groups were released together and over a period of time sufficient to allow all ages of adults to become balanced within a population.

During this test, samples of adults were withheld, and mating crosses with 25 pairs set up to check sterility. In 3 replicates, 94.4 percent of all egg rafts deposited by females crossed with fertile males hatched. When normal females were crossed with treated (sterile) males, 93.7 percent oviposited, but none of the rafts hatched.

Additionally, biological observations made in test 4 were similar to those for previous tests: dispersal was uniform throughout the test area, and even though rafts were collected for only 9 days after the last release, the recapture rate of 16.4 percent revealed the elevated survival typical of the spring season.

DISCUSSION

Release-recapture studies with marked insects are known to provide an excellent means of studying various aspects of their biology. In the present test, the dual tagging system (sterility and radioactive labeling) used either singly or in combination allowed us to study the competitiveness of sterile male *C. p. quinquefasciatus*. Also, we were able to observe the dispersal, the longevity, and the reproductive behavior of the females. In addition, this study provided information about the differences in reproductive behavior during different seasons of the year in more than one year. These data, in conjunction with those reported from previous tests, show that the recapture rate of released females on Seahorse Key, based on egg raft collections, is always higher in the spring. This phenomenon is directly related to the rate of female survival and is undoubtedly associated with the weather and the availability of hosts for blood meals since the bird rookeries are active. Additionally, dispersal of females was limited only during cold nights, high winds, or in open areas during hot, dry periods. The males apparently dispersed well over the distances expected of them and were able to survive and function normally after sustaining hurricane force winds and rains.

In the first 3 tests, some factor or factors

affected the sterile males and reduced the competitiveness. We were able to alter the design of the releases to minimize experimental bias resulting from dispersal and longevity without eliminating their influence in relation to competitiveness. It is difficult to determine why the sterile males appeared more competitive when they were released daily for an extended period than when released on a single occasion, but they obviously did survive, disperse, and mate almost as well as fertile males in the test when releases were extended. Then, logically, similar releases made over a longer period would increase the sterility even more because the population balance of released males may not stabilize until after several days, and our collections included egg rafts oviposited early in the test. A high rate of sterility can then be induced into an indigenous population of *C. p. quinquefasciatus* by releases of chemosterilized males over an extended period. Releases of sterile males on alternate days might be equally effective.

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