RELEASE STRATEGY EVALUATION OF STERILE MALES OF *ANOPHELES ALBIMANUS* WITH COMPETITIVE MATING[1,2]

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ABSTRACT. Field competitive mating tests were conducted in El Salvador, C.A., to assay the dispersal ability of sterile MACHO males, the genetic sexing strain developed for *Anopheles albimanus* Wiedemann mosquitoes. The tests were designed to study the strategy of releasing sterile males at sites 200 m apart. Chemosterilized MACHO males were released from 2 sites, and males and females from a newly colonized wild-type strain were released from a third site. Sterility of recaptured females was used to compute an average competitiveness factor (C) of 0.74 for the sterile MACHO males, which indicates that the sterile males effectively dispersed throughout the release area and that the 200-m distance between release sites allowed effective contact between sterile males and wild females.

INTRODUCTION

Kaiser et al. (1979) reported successful mating competitiveness in the MACHO strain of *Anopheles albimanus* Wiedemann, which is the genetic sexing strain developed for sterile-male releases on the coast of El Salvador, C.A. They calculated a mean competitiveness factor (C) of 0.785 for the field releases, which compares favorably with that of the sterile males used in eliminating a population of *An. albimanus* at Lake Apastepeque, El Salvador (Weidhaas et al. 1974). During the initial releases at Lake Apastepeque, the ratio of sterile males to native males was judged to be ca. 2:1, and it was concluded that the elimination of *An. albimanus* from the lake area would have been complete had the sterile males been only 25% competitive.

In a more recent evaluation of the use of the sterile male technique for control of *An. albimanus*, approximately 1,000,000 sterile males were released per day on the coastal plain of El Salvador (Bailey et al. 1980). Although large population peaks occur at the beginning of the wet and dry seasons, estimates of the average density of the native population indicated that the ratio of sterile to wild males in the coastal release zone was higher than that observed at the onset of the Apastepeque experiment. However, even though the releases were large enough theoretically to effect suppression, the reduction of the native population was less than expected. The competitiveness of the sterilized male was tested and found satisfactory, i.e., the effects of colonization, mass production, sterilization, etc., were not too detrimental (Kaiser et al. 1979). However, the success of the sterile male technique depends on other important factors, viz., strategic placement of release sites, effective dispersal of sterile males, and immigration of fertile females into the release zone.

The experiment reported herein was designed as a simulation of the release strategy used throughout the coastal release zone of El Salvador. Sterile male release sites were systematically placed 200 m apart along known or suspected breeding sites so that emerging native females would be no farther than 100 m from a release site. Researchers have usually assumed that *An. albimanus* mating

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takes place near breeding sites, although this is not well documented in the literature. If mating activity is very localized, a uniform dispersal of 100 m by sterile males would saturate the breeding sites. The results reported here evaluate the effectiveness of our release strategy and the dispersal of the sterilized MACHO males.

MATERIALS AND METHODS

STRAINS. Three strains of *An. albimanus* were used in the dispersal studies: (1) SANTA TECLA—a wild-type strain from females collected in District 13 of the coastal plain of El Salvador in 1975; (2) MACHO—a strain heterozygous for a Y-autosome translocation and an inversion. The MACHO strain has been maintained by inbreeding with SANTA TECLA females since February 1977. MACHO males used in the dispersal tests originated from eggs incubated in a 0.01% aqueous solution of propoxur, *o*-isopropoxyphenyl methylcarbamate, for 24 hr, which eliminated the propoxur-susceptible (pr^s/pr^s) females; (3) CAMPO—a new wild-type strain started from females collected on the coastal plain of El Salvador in January 1979. This colony was inbred; however, in an effort to maintain a vigorous, competitive organism for testing, field-collected stock was added to the colony daily until the conclusion of testing. Unfortunately, there was no way to insure that added collections were incorporated into the existing CAMPO strain.

COLONY MAINTENANCE. The procedures followed in maintaining the 3 strains were described by Bailey et al. (1980). One-ml samples of pupae from each strain were taken daily for determination of the number of pupae per ml and the sex ratio.

STERILIZATION. We sterilized pupae by placing them in an aqueous solution of 1% bisazir (*P,P*-bis (1-aziridinyl)-*N*-methylphosphinothioic amide) for 1 hr. They were then transferred to a rinse bath for ½ hr., measured volumetrically, and packaged for transport to the field (Bailey et al. 1979).

FIELD TESTS. Field releases were conducted near the Amayo River in the same area previously described by Kaiser et al. (1979). The releases were made during the dry season, however, the rocky river bottom still contained numerous large pools of water; the dense vegetation and rocky terrain near the river provided an excellent habitat for *An. albimanus* similar to that described by Breeland (1972). In fact, the majority of the pools contained *An. albimanus* larvae. Ten days prior to the first release, methoprene, isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, as Altosid-SR-10® briquets, were placed in all pools within 1 km of the release site. This aided in the elimination of wild females from our captures, and helped prevent the establishment of a natural population of *An. albimanus* which may have resulted from our fertile adult releases. Test releases were followed by routine sterile male releases at 5 release sites along the Amayo River to insure suppression of local *An. albimanus* populations.

The test releases consisted of 2 replicates, both at a ratio of ca. 20 sterile MACHO ♂:1 CAMPO ♂:1 CAMPO ♀. Our goal was to release 10,000 ♀/replicate since previous releases at that rate resulted in captures adequate for reliable interpretation by statistical methods. A 1-ml volumetric sample of CAMPO pupae was scored for sex by examination of the pupal terminalia, and the desired number of females was calculated and measured for release. The number of CAMPO males (estimated from the sex ratio) included with the females was then used to determine the number of MACHO males needed for the releases to approximate the 20:1 male ratio. The final ratio was determined by adjusting the release numbers for pupal mortality.

Sterile MACHO males were released for 2 days before the CAMPO males and females to maximize dispersal of sterile males throughout the test site. The first

replicate consisted of 5 releases of MACHO and 3 of CAMPO, and the second of 4 releases of MACHO and 2 of CAMPO.

The test site included: (1) Metal racks placed on trees near the edge of the river to provide shelter for cups containing pupae (Lowe et al. 1980). In the previous competitive mating release (Kaiser et al. 1979), sterile males were placed at the same release site as the wild-type males and females. However, since we intended to study the dispersal capabilities of the MACHO males the sterile MACHO males were placed at 2 release sites that were 100 m up- and downstream from the central CAMPO release site. (2) Tack-Trap® was placed on the trees around the racks to exclude predators. (3) Calf-baited traps (Lowe and Bailey, unpublished) were placed approximately 125 m up- and downstream from the CAMPO release site, and a calf was placed in each at 1800 hr and removed the following morning by the collection team.

Adult mosquitoes were collected daily from each calf trap, and the females were returned to the laboratory. Efforts were made to collect only females from the traps, but to insure the removal of all males the daily collections were screened in a cold room (3.3°C). Females were held in cages in the laboratory for 2 days and then immobilized in a cold room and placed individually in 32-ml plastic vials; 5 ml of H_20 were added later. Oviposition was noted on the following day. Females that failed to oviposit were transferred to a cage, blood-fed, then handled as newly captured females. The incubation time for *An. albimanus* eggs is ca. 48 hr.; however, we held the eggs for 5 days before they were checked for sterility to ensure sufficient time for all eggs to hatch. An egg hatch with 0–5% hatch was recorded as sterile, and hatches containing <15 eggs were discarded.

CONTROLS. The following quality control measures were established: (1) Spermathecae were removed from a sample of ovipositing females captured in the calf traps. They were assayed for sperm since uninseminated females often deposit normal complements of eggs that do not hatch and cannot be distinguished from those resulting from sterile matings. This precluded the necessity of maintaining a CAMPO ♀ × CAMPO ♂ control cage. (2) Daily collections from the 2 calf traps were begun 11 and 8 days prior to the 1st and 2nd releases, respectively. Spermathecae of the collected females were observed for sperm, and the average number of mated females collected per day for the period preceding each release was used as a correction factor in determining sterility for that release. (3) Cups used in the releases were returned to the laboratory after eclosion of adults, and mortality was calculated. This observed mortality was used to determine the actual numbers of mosquitoes released. (4) One hundred CAMPO females and 100 sterile MACHO males were placed in a colony cage for mating. Since the same sample of sterilant was used for all MACHO males, the 100 control males were taken from those treated on the final treatment day of each of the 2 replicates. The females were assayed for sterility as previously described and all egg batches from the 2 tests were determined to be sterile. (5) Males were found in the calf traps and at times were unavoidably included in the female captures. Since males of both strains readily mate in small cages in the laboratory, there was sufficient reason to assume they would also mate in the calf traps and in the small cages used for transporting the daily captures. (We have previously mentioned that males were removed from captures once they were returned to the laboratory.) In an effort to measure this possible source of bias, females collected in the evening outside the calf traps were assayed for sterility. The status of these females was compared to that of females exposed to males inside the calf traps and transport cages, and no significant difference in sterility was observed between these groups.

RESULTS AND DISCUSSION

The number of each type of adult mosquito released per day for the 2 replicates appears in Table 1. MACHO and CAMPO emergence was 81.4 and 81.0%, respectively, for the 1st replicate, and 74.3 and 80.3%, respectively, for the 2nd replicate.

Results of the test releases with sterile MACHO males, CAMPO males, and CAMPO females appear in Table 2. Numbers of sterile or fertile females were adjusted according to the results of the applicable controls. Percentages of ovipositing field-collected females checked for insemination for the 1st and 2nd replicates were 23 and 30, respectively, and the percentages of unmated ovipositing females for the 2 replicates were 13 and 15%, respectively. Also, the number of fertile females was adjusted downward in accordance with field collections made prior to the releases from the 2 calf traps. Mean numbers of mated females per trap-night prior to the 1st and 2nd replicates were 1.3±1.4 and 0.625±0.74, respectively.

The ratios shown in Table 2 are a result of calculating the total number of males released (corrected for mortality) and include the sterile MACHO males released for the 2 days prior to the CAMPO release. Adult mortality and emigration of the MACHO males released preceding the CAMPO releases probably reduced the number of sterile males that eventually competed with wild males for wild females. However, no corrections could be made for this reduction.

Very little is known about the behavior of wild *An. albimanus* males, although Breeland (1972) discussed resting sites where males are commonly located, and Hobbs et al. (1974) studied flight patterns of released males. This lack of knowledge concerning male behavior, coupled with our inability to pinpoint the actual loca-

Table 1. Numbers of *An. albimanus* sterile MACHO males (S♂), CAMPO males (♂), and CAMPO females (♀) released and recaptured during competitive mating tests in El Salvador, C.A. in 1979 (All recaptured females were assayed for fertility.)

	Replicate 1		Replicate 2	
Day	No. released[a]	No. ♀ collected	No. released[a]	No. ♀ collected
1	27060 S♂		21115 S♂	
2	32360 S♂		19860 S♂	
3	33950 S♂		63725 S♂	
	1515 ♂		5650 ♂	
	4510 ♀	0	3925 ♀	0
4	28150 S♂		80330 S♂	
	3240 ♂		4330 ♂	
	3140 ♀	5	5080 ♀	0
5	63660 S♂			
	3185 ♂			--
	3040 ♀	51		21
6		181		110
7		445		586
8		95		146
9		19		19
10		9		16
11				4
Total	185 180 S♂		185 030 S♂	
	7 940 ♂		9 980 ♂	
	10 690 ♀	805	9 005 ♀	902

[a] Corrected for mortality.

Table 2. Results of field competitive mating tests with *An. albimanus* sterile MACHO males, CAMPO males, and CAMPO females in El Salvador, C.A. in 1979 (The two strains were released at different sites.)

Replicate	Test population[a]				Mating of[b] field-collected ♀		Percentage sterility of field-collected ♀		Competitiveness[c] (C)
	Sterile MACHO ♂	CAMPO ♂	CAMPO ♀	Male ratio	Sterile	Fertile	Observed	Expected	
1	185180	7940	10690	23.3:1	345	27	92.74	95.88	0.548
2	185030	9980	9005	18.5:1	405	22	94.84	94.87	0.99

[a] Corrected for mortality.
[b] Corrected for fertile indigenous population and ovipositing females without sperm.
[c] Fried, 1971 (in references).

tion of mating with respect to breeding sites, made it very difficult to select a desirable release ratio for sterile MACHO and CAMPO males. An *effective* ratio of 9:1 should yield an expected sterility of 90%. An *effective* ratio refers to the numbers of sterile males actually competing on a successful basis with wild males, not the number of sterile males released, and may be best measured by Fried's (1971) formula for competitiveness. Since our normal release ratio in the large coastal area of El Salvador at the time of these tests was estimated to be more than 9:1 (sterile:wild), and the observed sterility was less than expected, we decided to use higher ratios in our tests to determine the *effective* competing ratio of MACHO males when released at these rates. The ratios of sterile MACHO males to CAMPO males released for the two replicates and corrected for mortality were 23.3:1 and 18.5:1 respectively.

The competitiveness factor (C) was determined with the following formula:

$$C = \frac{S/N \text{ (calculated)}}{S/N \text{ (actual)}}$$

where,

S/N (calculated) = ratio of irradiated males to normal males that will give an expected % sterility if mating N♀ × N♂ gives hatch >5%, and if mating N♀ × S♂ gives 0–5% hatch.

S/N (actual) = the actual ratio used experimentally.

Competitiveness factors for the two replicates were 0.548 and 0.99, respectively, and 0.74 for the combined data. This compares favorably with C = 0.785 for the data summarized by Kaiser et al. (1979) when MACHO and CAMPO were released together. The designs of the 2 tests were similar; however, there were 2 important differences: First, in these trials the MACHO males were not released at the same location as the CAMPO males and females. We assumed that a high proportion of mating would take

place very near the CAMPO emergence site. Therefore, MACHO males randomly dispersing in the direction of the CAMPO emergence site would compete with CAMPO males for CAMPO females. However, since the two C-values are similar and since the C-value for this test is based on the total number of sterile MACHO males released, the C-value of 0.74 indicates several possibilities: (1) the CAMPO females dispersed from their emergence site before mating (the observed level of sterility is indicative of a high ratio of sterile males); (2) the sterile MACHO males were well dispersed throughout the release area; (3) sterile MACHO males released prior to the CAMPO female release dispersed locally (the high level of sterility indicated their presence).

Secondly, a different CAMPO strain was used in this present work than in that of Kaiser et al. (1979). Although the two CAMPO strains were colonized with identical methods and were approximately in the same generation when tested, there was no method by which to assure they were of equal vigor.

To determine the ratio of sterile MACHO males necessary to control the coastal population of *An. albimanus*, we have utilized data collected during the Apastepeque project by Weidhaas et al. (1974), who used population density estimates and sterility data to calculate the rate of increase (RI) of their test population of *An. albimanus*. The mean RI value was 1.37 and the range was 0.5–4.8. By using the highest RI value observed (4.8), and by assigning a level of control to our test population, we were able to calculate the expected sterility necessary to give this control. This relationship can be expressed by the mathematical formula:

$$S = \frac{RI - (F_{1/P})}{RI}$$

where

RI = a ratio of the numbers present during two succeeding generations or units of time, and

$F_{1/P}$ = rate of control for 1 generation or unit of time.

Therefore, with an RI of 4.8, we can use levels of control of 1.0 (equilibrium), 0.5, and 0.1, and calculate the expected sterilities of 0.79, 0.9, and 0.98, respectively. To calculate the ratio of sterile males to native males required to induce these sterility levels, we used the formula:

$$\frac{(C)\,N}{P} = \frac{S}{I\text{-}S}$$

where

C = competitiveness factor
N = sterile males
P = native males

By using our C-value of 0.74, we calculated sterile-male to native-male ratios of 5.1, 12.2, and 66.2, respectively, for sterilities of 0.79, 0.9, and 0.98. This indicates that sterile MACHO males with a C-value of 0.74 would have to be released at a ratio of 12.2:1 to induce, in a single generation, 90% sterility in a population with a 4.8X rate of increase. A summary of release ratios (sterile male to native male) necessary to reduce the wild population by 50% in one generation, using variable rates of increase and competitiveness factors, is given in Table 3.

We think that these tests and the competitive mating tests reported previously by Kaiser et al. (1979) clearly demonstrate the competitiveness of the sterile MACHO male. Also, the MACHO males were competitive within the framework of the design of this test, which indicates: (1) the strategy of placing release sites 200 m apart along known or suspected breeding areas is effective, and (2) the dispersal of the sterile males resulted in a high degree of sterility in the CAMPO population. The rate of increase of the wild population used in these calculations was the highest observed by Weidhaas et al. (1974) at Lake Apastepeque. If population increases are similar throughout the coastal plain, sterile male releases using

Table 3. Release ratios of sterile to wild *An. albimanus* males necessary to reduce the wild population by 50% in one generation.

Rate of increase	Competitiveness factor									Expected sterility
	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	
1X	1	1.1	1.25	1.4	1.7	2	2.5	3.3	5	.5
2X	3	3.3	3.75	4.3	5	6	7.5	10	15	.75
3X	5	5.6	6.2	7.1	8.3	10	12.5	16.7	25	.83
4X	7	7.8	8.75	10	11.7	14	17.5	23.3	35	.875
5X	9	10	11.25	12.9	15	18	22.5	30	45	.9

competitive males at a ratio of 12:1 to native males should control the wild population of *An. albimanus*. However, there are several factors not measured in these tests that could preclude control. One is migration of mated wild females into the release area. We hope that future field studies of *An. albimanus* will show the migration potential of this important disease vector. Another important factor that may account for a discrepancy between success in this type of trial and field releases against native mosquitoes is a possible genetic selection during colonization of the CAMPO strain. In the future we intend to use electrophoretic techniques to discern the salient effects of colonization on various native populations of *An. albimanus*.

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