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MATING COMPETITIVENESS OF CHEMOSTERILIZED HYBRID MALES OF *AEDES AEGYPTI* (L.) IN FIELD TESTS

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ABSTRACT. Field tests were conducted to study the effects of heterosis on mating competitiveness (c) of chemosterilized males of *Aedes aegypti* (L.). When males of the normal strain were sterilized by exposing pupae to *P,P*-bis(l-aziridinyl)-*N*-methylphosphinothioic amide and released along with untreated males and virgin females of the same strain, c averaged 97%. When hybrid males replaced either the sterile or untreated normal males in releases designed to measure the effect of heterosis, no discernible effect was noted, whether the hybrids were sterile (c = 97%) or fertile (c = 109%). The results indicate that chemosterilized males may be suitable for genetic control of this species, but that hybrid vigor does not necessarily increase the competitiveness of the released males.

Research on the sterile male technique for mosquito control was stimulated by the report (White 1966) that thiotepa (tris(l-aziridinyl)phosphine sulfide) could be used to chemosterilize pupae and subsequently by the report of Lofgren et al. (1973) that some analogues of thiotepa were even better pupal sterilants. The use of these chemicals for sterilizing mosquito pupae is simpler than treatment of larvae or adults and also produces a fairly competitive insect apparently because of the low level of somatic damage. Thiotepa was used as the sterilant in an experiment that eliminated *Culex quinquefasciatus* Say from an island off the Gulf Coast of Florida (Patterson et al. 1970). One of the thiotepa analogues, *P,P* - bis(l - aziridinyl) - *N* methylphosphinothioic amide, was the sterilant used in the successful control of a population of *Anopheles albimanus* Wiedemann in the Lake Apastepeque area in El Salvador (Lofgren et al. 1974). Likewise, thiotepa was used to sterilize the mosquito species, *Culex fatigans*, in release experiments in India (Rao 1974). The results of the Indian experiments were not conclusive in terms of population reduction, but the best estimate of the performance of the sterilized males indicated good competitiveness. The same laboratory in India also worked extensively with thiotepa as a sterilant for *Aedes aegypti* (L.) (Grover and Sharma 1974), generally via a somewhat traditional approach including studies of sterilizing treatments, permanency of sterility in males, and mating competitiveness.

We have also been involved in studies on using chemosterilants for the control of *Ae. aegypti*, but our approach has been different. In our studies we have used one of the analogues of thiotepa and have made an attempt to increase the competitiveness of chemosterilized males by taking advantage of heterosis. In an earlier paper, we

reported results of outdoor cage tests that indicated a significant increase in competitiveness in hybrid sterile males compared with the parent strains (Seawright et al. 1975). After obtaining these favorable results, we proceeded to test the mating competitiveness of chemosterilized normal strain and hybrid males at simulated breeding sites in a field situation during the summer of 1975. The results of these latter tests are summarized in the present paper.

MATERIALS AND METHODS

Larval rearing of *Ae. aegypti* used in these tests was conducted in an insectary at 30°C. Groups of ca. 1400 first-stage larvae were set in trays containing 5 liters of water infused initially with liver powder (0.95 g) and brewer's yeast (0.47 g). After 52–54 hr, 1.5 g of ground hog supplement were added to each tray followed by an additional 1.5 g the next day. Pupae were picked the 5th and 6th days after setting, and sexual separation was accomplished with a device similar to that described by Fay and Morlan (1959).

Two wild-type strains were used in the tests. The strains were collected in Gainesville (GV) and Orlando (ORL), Florida, during June 1975 and were cultured for three generations prior to their use in our experiments.

Three simulated breeding sites were built 6 miles north of Gainesville, Florida, in a mixed pine-cypress forest managed by the University of Florida. Prerequisites for site selections included moderately dense vegetation, absence of alternate breeding sites, and isolation. Prerelease oviposition and adult surveys indicated that no indigenous *Ae. aegypti* were breeding in the area. The release sites were located about 0.5 mile apart. Each release site contained a small hut (2.4 x 1.2 x 1.2 m), two oviposition sites (3-gal buckets containing water and lined with filter paper), and a caged rabbit. The rabbit and oviposition buckets were placed at the release sites 1 wk preceding each release to sample oviposition by native mosquitoes. The following competitive mating tests were run and each replicate within a test was conducted at a different release site.

1. Sterile GV♂: normal GV♂ : normal GV ♀.
2. Sterile F_1 ♂ (ORL x GV):normal GV ♂: normal GV ♀.
3. Normal F_1 ♂ (ORL x GV): sterile GV ♂ : normal GV ♀.

By testing these combinations we hoped to measure the effect of chemosterilization (test 1), the effect of heterosis (test 3), and the joint effects of chemosterilization and heterosis (test 2) on competitiveness. Each population was tested twice and consisted of 1000 of each type (sterile male, fertile male, fertile female) released each day for 4 days. The tests were conducted so that replications of the same type of population were not conducted simultaneously to avoid using mosquitoes reared together in replicates. Male mosquitoes were sterilized by treatment of pupae in 1% aqueous *P,P*-bis(1 - aziridinyl) - *N* - methylphosphinothioic amide for 30 minutes. This treatment has consistently induced 99.5% (or greater) sterility in males of *Ae. aegypti*.

We placed treated and nontreated pupae in the hut at each release site, although the release of pupae may be less desirable in an actual control effort because of the chemosterilant residues present in pupae. For our experimental purposes we felt this to be a better procedure, primarily because transporting pupae is easier, requires less space, and is not as deleterious to the mosquitoes as transportation in the adult stage.

Competitiveness was based on percentage egg hatch of field-collected eggs and by scoring, as sterile or fertile, females collected at the release site. We attempted to capture at least 50 gravid females during the conduct of each test. These females were taken to our laboratory and allowed to oviposit individually in vials. The eggs collected by both methods were kept moist and incubated at 27°C for 1 week before hatching in a vacuum chamber. Laboratory control groups, consisting of 100 males and 100 females, were used to assay the efficacy of the sterilant treatment and

also the fertility of the untreated types in each release.

RESULTS AND DISCUSSION

Examination of the data in Table 1 revealed a considerable variation in the percentage competitiveness that was calculated by the 2 methods. The widest disparity was observed for populations 2 and 4. For population 2, the average of the 2 values was 99%, which might be interpreted as an effect of differential sampling. The same could be true for population 4, though the average (83%) suggests a reduction in competitiveness of the sterile males. The χ^2 analyses show for the 2 methods are not comparable, because of the increased sensitivity in obtaining significant differences with the larger sample sizes of the field-collected eggs. In a separate report (Seawright et al. 1977) we recorded an average fecundity of 95 eggs/female/oviposition by *Ae. aegypti*. When the egg totals in Table 1 are divided by this value to translate the totals into sterile and fertile females, the χ^2 analyses showed no significant differences, which means that the sterile males were competitive.

Competitiveness averaged 88.5% for the 6 experiments shown in Table 1. This level of competitiveness is acceptable from the standpoint of genetic control.

There was no discernible effect of heterosis on the hybrid males (c for sterile was 97, and c for fertile was 109) and although this was disappointing and seemingly contrary to the results we obtained in outdoor cage studies (Seawright et al. 1975), there is perhaps a plausible explanation. The cage studies were conducted with strains of *Ae. aegypti* originally collected in Gainesville and Ocala, Florida, and both strains were cultured in the laboratory for a much longer duration than the 2 strains used in these field tests. Culturing strains under laboratory conditions will alter the gene pool and most likely fix different alleles for different strains. The strains used in the field tests were in the F^3 generation of laboratory colonization suggesting that both parent populations were still fairly heterogeneous. If this were true, then the 2 strains had little effect on heterogeneity.

The results obtained in this present study, coupled with other information, are encouraging evidence that the sterile male technique can be used for control of *Ae. aegypti*. First, the overall competitiveness of the chemosterilized males was adequate and comparable to results reported by Grover and Sharma (1974) for the competitiveness of thiotepa-sterilized males of *Ae. aegypti* in laboratory and outdoor cage tests.

Second, there are several chemosterilants that can be used to sterilize mosquitoes in the pupal stage. Most of these chemical compounds are similar in structure to thiotepa, and the only real differences between these chemicals lies in their water solubility and rate of uptake by the pupae (Seawright et al. 1973b).

Third, residue analysis techniques are available for the majority of the effective sterilants for mosquitoes (Seawright et al. 1973b). By monitoring the mass rearing and sterilizing techniques, it has been possible to minimize the mutagenic residues to <1 ng per mosquito by simply holding the sterilized adults until they are 2–3 days old (Seawright et al. 1971; Seawright et al. 1973a; LaBrecque et al. 1972; and Seawright et al. 1976). It seems unlikely that residues of <1 ng/mosquito would do any substantial ecological damage.

The last item for consideration involves the ability to mass rear a sufficient number of males for sterilization and release. This should pose no problem. In our work with *Ae. aegypti*, we have encountered little trouble in rearing large numbers for experimental purposes. Also, an efficient and relatively inexpensive rearing system was devised by Singh et al. (1974) for the production and sexing of *Ae. aegypti*.

Although we did not demonstrate any heterosis in the results of our tests herein, we feel that the idea is a valid one and that an effort should be expended to avoid using an inbred, homogeneous population as a source of sterile males.

Table 1. Percentage competitiveness (c) of chemosterilized males of *Aedes aegypti* calculated according to the method of Fried (1971).

Test population[a]				Competitiveness based on						
				Viability of field-collected eggs			Mating of collected females			
				% Hatch			Number collected			
♂	♂	♀	Population	Observed	Expected[c]	C	Fertile	Sterile	C	Mean c
S GV	GV	GV	1	49.5	46.0	85[d]	22	23	104	
			2	56.3	48.2	71[d]	26	33	127	97
S Hyb	GV	GV	3	44.2	49.1	122[d]	28	28	100	
			4	62.2	49.2	58[d]	38	31	108	97
Hyb	S GV	GV	5	42.4	41.8	97	32	29	89	
			6	55.9	55.3	98	29	23	79	91[e]

[a] S denotes sterile male; Hyb denotes F_1 ♂ (ORL ♀ X GV ♂).
[b] 2750–4000 eggs examined per test.
[c] Calculated from laboratory controls.
[d] Significantly different from expected (χ^2; $P < .05$).
[e] C for hybrid = 109.

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