SEPARATION OF SEXES OF ADULT *ANOPHELES ALBIMANUS* BY FEEDING OF INSECTICIDE-LADEN BLOOD[1,2]

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ABSTRACT. A technique was devised for removing the adult female *Anopheles albimanus,* which were potential vectors of malaria, from populations reared for release of sterile males in a coastal area of El Salvador. The adults, chemosterilized as pupae, were held in cages for 48–72 hr after emergence and then offered a blood meal of warm (42–44°C) citrated bovine blood containing 0.05% malathion (*0,0*-dimethyl phosphorodithioate of diethyl mercaptosuccinate; AI3-17034) from natural membrane condoms for 20 min. Comparisons showed that it was possible to remove more than 95% of the females before the field releases; the remaining females would not survive more than a few days, and therefore could not be implicated in disease transmission. With this level of female removal, there was a corresponding loss of approximately 25% of the males available for release.

One of the major problems in sterile male release (SMR) programs for mosquito control has been the difficulty of removing the females, which are potential disease vectors. During a SMR study with *Anopheles albimanus* Wiedemann in El Salvador in 1972, pupae were mechanically separated by sex to an average of 86% males (Fay and Morlan 1959, Dame et al. 1974), chemosterilized, and transported to the field for adult emergence. The 14% females that remained had been treated with the chemosterilant, so their non-viable eggs were not a contention in control of the population. However, the females could have been capable of disease transmission (Omar et al. 1974). In addition, as population control was achieved, these released females represented a large percentage of the field-collected adults. Since they could not be identified as being previously released, they were included in assays of the induced level of sterility (Lofgren et al. 1974).

In 1975 a much larger SMR program was undertaken in an effort to control *An. albimanus* in a coastal area of El Salvador. Problems encountered with pupal releases included vandalism and damage to the release containers and predation of the emerging adults. These obstacles were overcome by making daily releases of adults each evening. Bailey et al. (1979) discussed the methods used for sterilization and packaging of the adults, and Lowe et al. (1980) described the techniques for transportation and release. Initially, however, the releases were made with adults that had been mechanically sexed as pupae so they continued to include some females.

Earlier a method was devised for maintaining an adult colony of *An.*

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[1] This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. Also, mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.

[2] Due to the extensive amount of data collected from the many and varied combinations of comparisons, tabular results have been deleted from this report. The data are available to interested individuals by personal communication with the authors.

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albimanus by feeding preserved blood through natural membranes (Bailey et al. 1978), and this method was modified for routine use in the mass rearing facility in El Salvador (Savage et al. 1980). Once it was determined that the adult colony females were attracted to the warm bovine blood and that they fed readily through the membranes, we began studies to develop the use of this technique to separate the adults by sex. The objective was to contaminate the preserved blood with an insecticide that would kill the feeding females but not affect the nonfeeding males.

MATERIALS AND METHODS

Initial studies were conducted with citrated bovine blood contaminated with trichlorfon (diemethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate; AI3-19763), malathion (*0,0*-dimethyl phosphorodithioate of diethyl mercaptosuccinate; AI3-17034), synergized pyrethrins, or nicotine sulfate.

The blood was heated to about 40°C and poured onto stretched Parafilm® membranes supported on the top of adult cages. Subsequent additional trials were made with only malathion and synergized pyrethrins, but in this case a natural animal membrane condom was filled with the contaminated blood and hung inside the adult cage.

After it was determined that malathion was the insecticide of choice, numerous tests were made with bovine blood contaminated with this chemical. Originally, the adult cages had cuphooks inserted into the tops to support the clips that sealed the condom membranes (Savage et al. 1980), but later the membranes were placed into net Tubegauze® bags suspended from openings cut into the top of the cages (Bailey et al. 1980).

It was also necessary to determine the maximum number of adults that could emerge in a cage of limited size, survive until the separation, and still provide the best response to the feeding stimulus.

During the 14 months that we separated the sexes with contaminated blood, large numbers of pupae were produced in the laboratory, such that a daily average of 532,500 males was available for field preparation. With these numbers it was possible to complete numerous replicated experiments to determine the efficiency of the adult separation. In these tests we compared the effect of: (1) density in the cages, (2) area available for resting space; (3) conditioning by dehumidification; (4) the availability of sugar water pads during blood feeding; (5) citrated versus defibrinated bovine blood; (6) the number of membranes used per cage; (7) location of the membranes within the cage; (8) various types of net bags used to support the membranes; (9) the type or brand of membrane; (10) the effect of blood temperature on feeding response; (11) light intensity during feeding; and, (12) loss of males during the procedure. All tests were conducted a minimum of 4 times, each on a different day, and the more promising methods were evaluated in 7–14 replicates.

The procedure with pupae destined for field release was as follows: they were chemically sterilized by immersion for 1 hr in bisazir (*P,P*-(1-aziridinyl)-*N*-methylphosphinothioic amide), rinsed in water baths, and measured volumetrically to determine numbers. Then about 300 ml of water was poured into a square plastic dish (10 × 14 × 6.5 cm deep), 2,500 pupae were added and the dish was placed in an emergence cage. Cotton pads saturated with 10% sugar water as a food source were put into cups inside the cage or suspended in the net bags through the ports in the cage top. Adults were allowed to emerge for 24 hr, and then the dishes were transferred to new cages, thus insuring that all adults in a cage were of a fairly uniform age. The adult cages were held an additional 48 hr in rooms maintained at 27.8±2.2°C and 70–85% relative humidity (RH) to maximize the number of females that would be ready to accept a blood meal.

The blood for the treatment of females was prepared in a special blood processing area. First, 0.05 ml of technical grade malathion was added per 100 ml of preserved bovine blood. Then approximately 200 ml of this blood were poured into the membrane, the open end was secured by a metal clamp, and the membranes were put into a water bath and heated to 42–44°C. The blood was then put immediately into the cages for ca. 20 min by which time it had cooled and lost most of its attraction. These membranes were removed and reheated for use in other cages until all feeding was accomplished as early in the day as possible. One half hour after feeding was finished, the dead females were removed from the bottom of the cage with an aspirator, and the live adults were transferred to a walk-in cold room to be prepared for release (Bailey et al. 1979, Lowe et al. 1980). After each day, the blood was discarded but the membranes were rinsed and used for 7 days or until they were unserviceable. The blood processing and feeding areas and all equipment were decontaminated with acetone each afternoon.

Accurate data on the efficiency of each technique was obtained by handling all cages used for experiments individually. Also, known numbers of pupae were inserted, and all dead adults were removed immediately before the insertion of the membranes. The number of each sex was recorded during the 72 hr holding period; similar counts were made with the dead adults removed 30 min after feeding was completed; and a final count was made during packaging for field release. These data were then evaluated for percent mortality and survival for each sex at each stage of the process.

RESULTS AND DISCUSSION

After preliminary tests had shown that adult females were unable to obtain a blood meal offered in latex-based condoms, studies were conducted to examine results with other materials. The females were able to feed through stretched Parafilm and natural membrane condoms, and these materials were used for the toxicant feedings. The results of the initial studies showed that trichlorfon, malathion, and synergized pyrethrins killed females that were 48 hr old or older. Nicotine sulfate proved to be repellent or distasteful, and the poor feeding gave a low percentage of mortality. Additional trials with trichlorfon and synergized pyrethrins in membranes hung inside the cages revealed an increase in mortality of both sexes which was attributed to fumigant action rather than direct ingestion. Results showed malathion gave high levels of female mortality without adverse effects on the males. Hanging the membrane inside the cage increased the feeding activity of the females, and since this technique was logistically easier to use in the large scale necessary for the release program, it and the faster-acting malathion were adopted as a routine method for removal of females.

After a review of all data obtained from the numerous comparisons and experiments, it was shown that the only variable that significantly affected sexing was the time of conditioning by dehumidification at ca. 40% RH. Table 1 shows that this criterion affected both the female removal and the loss of males at a statistically significant level. Results of the other comparisons were then reevaluated for their economic or logistical benefits, and the useful techniques were used in the routine field releases. The method of choice was: (1) cages were stocked with 15,000 pupae that were either from the first pupal collection (mostly males) or manually-sexed pupae from the second separation (to obtain mostly males); the adults were allowed to emerge for 24 hr; and the cages were held for 72 hr; (2) the sugar pads were removed from the top ports and the adults were preconditioned at ca. 40% RH for 30 min; (3) two thin Patrician® membranes (Patrician Products, Inc., 580 Bryant St., San Francisco, CA 94107) filled with 0.05% malathion-laden, citrated blood (42–44°C) were

Table 1. Effects of conditioning *Anopheles albimanus* adults in a dehumidified room on the percentage of females in releases and the total male survival when offered malathion-laden blood.

	Females in releases (%)			Total male survival (%)		
Conditioning time (hr)	Mean	Standard error	t-test	Mean	Standard error	t-test
0	27.8	4.5	t = 3.01	65.8	1.8	t = 3.35
3.5	11.6	2.5	p = 0.0395	51.6	5.1	p = 0.0285

placed in support bags at the front of the cage for 20 min; and (4) the sugar pads were returned immediately thereafter and the cages were moved to humidified areas (70–85% RH) until the adults were removed for field release.

With this method, an average of 92.9% of the females were removed before field release. Also, in view of the results of the controlled tests, almost all the remaining females probably died within a few days and would not be involved as potential malaria vectors.

The major concern relative to the blooding technique for removal of the females was the corresponding loss of males. It was shown that 73.5% of the adult males that died were lost during the 72 hr holding period before the blood feeding. The remaining 26.6% that did not survive died during conditioning, from contact with the membranes, or from contact with the contaminated females. The type of blood or membrane was not a factor, but when cages were stocked with large numbers of unsexed adults and held for longer periods in the dehumified area, male mortality was higher. Also, the increased female activity during the feeding process excited the males into mating activity. This resulted in many males coming into contact with the membranes and the contaminated females, especially when the coupled pairs fell to the floor of the cage during copulation.

The loss of potential sterile males from the field release program was actually greater than the numbers exhibited in test results since the process of mechanically sexing the pupae was based on size differences between the 2 sexes, and there was a large overlap in size. Therefore, many males were separated into the female fraction in the effort to remove as many females as possible. Also, to maintain a proper sex ratio it was sometimes necessary to add additional males to the female fraction used to stock cages for the laboratory colony. Additional mortality occurred during the sterilization and rinsing process, and 8–12% of the treated pupae did not emerge as adults. In fact, during a typical 5-wk period in 1977, the laboratory produced a daily average of 464,300 male pupae, but after tabulating all mortality (including that in packaging and transport of adults), an average of only 171,900 adult males was actually released each day (Savage et al. 1980).

The insecticide-laden blood was subsequently used to remove adult females from the sterile males released for 14 months in El Salvador. The procedure was discontinued only with the advent of a much more efficient method that eliminated females in the immature stages by a genetic sexing technique (Seawright et al. 1978, Kaiser et al. 1978). This new method permitted field release of pupae, which eliminated the handling of adults, and increased the number of males released to more than 1,000,000 per day (Bailey et al. 1980). However, during the period it was in use, the technique of feeding contaminated blood eliminated large numbers of females that could have been disease vectors and allowed the SMR technique to be used for mosquito control. This technique could also be useful for other species in which genetic sexing strains are not available.

References Cited

Bailey, D. L., D. A. Dame, W. L. Munroe and J. A. Thomas. 1978. Colony maintenance of *Anopheles albimanus* Wiedemann by feeding preserved blood through natural membrane. Mosq. News 38:403–408.

Bailey, D. L., R. E. Lowe, J. E. F. Fowler and D. A. Dame. 1979. Sterilizing and packaging males of *Anopheles albimanus* Wiedemann for field release. Am. J. Trop. Med. Hyg. 28:902–908.

Bailey, D. L., R. E. Lowe, D. A. Dame and J. A. Seawright. 1980. Mass rearing the genetically altered MACHO strain of *Anopheles albimanus* Wiedemann. Am. J. Trop. Med. Hyg. 29:141–149.

Dame, D. A., C. S. Lofgren, H. R. Ford, M. D. Boston, K. F. Baldwin and G. M. Jeffery. 1974. Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. II. Methods of rearing, sterilization, and distribution. Am. J. Trop. Med. Hyg. 23:282–287.

Fay, R. W. and H. B. Morlan. 1959. A mechanical device for separating the developmental stages, sexes, and species of mosquitoes. Mosq. News 19:144–147.

Kaiser, P. E., J. A. Seawright and D. A. Dame. 1978. Development of a genetic sexing system in *Anopheles albimanus* Wiedemann. J. Econ. Entomol. 71:766–771.

Lofgren, C. S., D. A. Dame, S. G. Breeland, D. E. Weidhaas, G. Jeffery, R. Kaiser, H. R. Ford, M. D. Boston and K. F. Baldwin. 1974. Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. III. Field methods and population control. Am. J. Trop. Med. Hyg. 23:288–297.

Lowe, R. E., D. L. Bailey, D. A. Dame, K. E. Savage and P. E. Kaiser. 1980. Efficiency of techniques for the mass release of sterile male *Anopheles albimanus* Wiedemann in El Salvador. Am. J. Trop. Med. Hyg. 29:695–703.

Omar, M. F., W. E. Collins and R. W. Gwadz. 1974. Development of *Plasmodium vivax* in chemosterilized anopheline mosquitoes. Am. J. Trop. Med. Hyg. 23:334–338.

Savage, K. E., R. E. Lowe, D. L. Bailey and D. A. Dame. 1980. Mass rearing of *Anopheles albimanus* Wiedemann. Mosq. News 40:185–190.

Seawright, J. A., P. E. Kaiser, D. A. Dame and C. S. Lofgren. 1978. Genetic method for the preferential elimination of females of *Anopheles albimanus*. Science 200:1303–1304.