A CAGE TEST FOR EVALUATING MOSQUITO ADULTICIDES UNDER FIELD CONDITIONS

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During 1956, the Entomological Research Center of the Florida State Board of Health, working in cooperation with the Indian River Mosquito Control District, initiated a comprehensive research program to gain information on the most efficient use of equipment and insecticides in the mosquito adulticiding program in Florida.

Some of the equipment and techniques are similar to those used by many research agencies. Certain other procedures are believed to be different from previously published methods.

In selecting a procedure for adulticide studies, it was felt that each major facet of the overall problem could best be handled as a separate experiment. This procedure should reduce confounding of results by eliminating the necessity of trying to evaluate many different problems from a single set of data.

Information on effective dosage of various insecticides, some old and some new, was selected as the most critical need in the Florida adulticiding program. This paper presents the methods that have been developed for these dosage tests to date.

Although Brescia (1946), Clark (1947), Horsfall (1950) and others have demonstrated that mosquitoes in screen cages can be killed with aerosols, the landing rate method of evaluating dosage has been more widely used under field conditions. It was felt that caged mosquitoes would allow a more accurate evaluation of dosage levels under the conditions of these tests.

METHODS. *Rearing and Handling Test Insects.* When available, the larvae and pupae of the salt-marsh *Aedes* are collected in natural breeding areas and reared to the adult stage in the laboratory. When larvae or pupae are not available in the field, sods are brought to the laboratory and flooded to hatch the eggs. Fifty pupae are placed in a pint Mason jar filled with tap water. On the top of each jar is mounted with masking tape a 14 by 18 mesh screen cage, six inches high and the same diameter as the inside of the standard Mason jar cap, which is used as a top; see Fig. 1(1). An inverted paper funnel is fitted into the open end of the cage with two small pieces

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of tape. The funnel, which is made by cutting the tip from cone-shaped drinking cups prevents the mosquitoes from returning to the water, where many specimens would become caught in the surface film.

The adults are allowed to emerge into the cage during 48 hours, then the cages are taken from the jars, the paper funnel is removed, and the open end of the cage is closed with a friction-fitting screen cap, Fig. 1(2). At the time the cage is removed from the emergence jar, a pad of cotton saturated with sugar water is placed on the screened top; the pad is moistened daily with tap water. Test insects are all between the ages of two and eight days, and, when possible, all mosquitoes used in a single night's testing are of the same age.

No attempt is made to separate the two species of salt-marsh mosquitoes in the cages, but test mortality statistics are recorded separately for each species. Male mosquitoes are also left in the cages, but because of the higher natural mortality rate of the males, only female mosquitoes are used in mortality counts.

Test Procedure. The test area is an undeveloped subdivision where there are good dirt roads and a minimum number of trees and tall shrubs. Stations are permanently marked along streets at 165, 330, and 660-foot intervals in the four compass directions. This system allows fast preparation for testing regardless of wind direction. The station distances correspond to one-half, one, and two average city blocks respectively.

Two cages of mosquitoes are suspended horizontally by means of small-gauge copper wires from heavy-gauge wire "arms" which are attached to one inch by two inch by six foot wooden stakes (Fig. 2). One cage is two feet and the other cage six feet above the ground. Stakes are provided with a pointed, strap iron spike, which facilitates their mounting in the ground without driving.

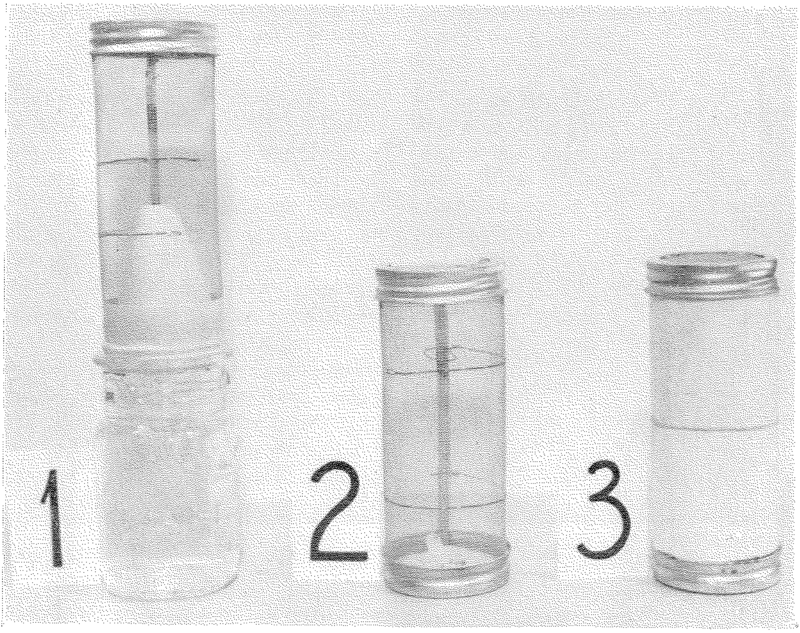

Fig. 1.—1. Emergence jar with cage mounted on top; 2. Caged mosquitoes with cotton pad on top; 3. Clean cage.

FIG. 2.—Stake with cages attached as in field test.

Each test or replicate consists of the cages from two sets of stakes, or a total of twelve cages. In almost all tests to date, four cages of mosquitoes picked at random from the test group and placed in a similar manner in another subdivision about two miles away have served as checks for the tests.

Cages are exposed to the test insecticide in the usual manner of driving the fogging vehicle along the upwind side of the plots. Starting ten minutes after the test begins, the treated mosquitoes are transferred to clean cages at the stake and cages of untreated mosquitoes are hung on the stake for the next test.

The technique for transferring treated mosquitoes to clean cages is similar to a procedure used by the Orlando, Florida laboratory of the U. S. Department of Agriculture. The treated cage is tapped several times on a horizontal surface to force the mosquitoes to the bottom of the cage. The loosened top is then quickly pushed off by an upswing of the clean cage, which is held bottom up in the opposite hand; this motion is completed by bringing the open end of the clean cage into position over the open end of the treated cage. The positions of the two cages are then reversed and the mosquitoes are forced to the bottom of the clean cage by tapping it several times on the horizontal surface. Before the mosquitoes can recover, the treated cage is flipped off and the top is placed on the clean cage. The entire process can be completed in 5 to 10 seconds. The clean cages are wrapped with a paper towel held in place by a rubber band, Fig. 1(3). This is to reduce chances of contamination from the operators' hands. Also, a number 6–6 Lily paper cup lid is placed in the bottom of the cage to facilitate counting the mosquitoes.

Three experienced workers can transfer the twelve cages of treated mosquitoes to clean cages and prepare the next group for testing in a period of about 10 minutes.

Wind velocities are recorded with a cup anemometer at six feet above the ground during each of the three minutes required to treat a replication. The temperature at six inches and at six feet above the ground, and the relative humidity are also recorded for each replication as a routine procedure. Soap bubbles have proved helpful in ascertaining wind direction and behavior in connection with the tests.

Treated insects and checks are held for 12 hours, out-of-doors, protected from rain, and then examined for mortality. A mosquito that is able to fly is counted as being alive. All cages, including the checks, are cleaned with an acetone-water-acetone wash after each test.

EVALUATION OF TEST. Although laboratory precision cannot be expected in a field test, especially with problems presenting as many variables as do wind-borne aerosols, the data obtained in these cage tests have been very satisfactory. The reproducibility of results, especially with the more effective dosage levels, has been excellent. Data from 18 replications with the same dosage of malathion show a coefficient of variation of only 12.5 percent and a standard deviation of 2.59.

The accuracy of the test is also supported by a straight-line dosage mortality curve with three dosage levels of malathion, Fig. 3. The average percent kills with their corresponding dosages are 60.0 percent at 0.13 pound per acre, 88.1 percent with 0.27 pound per acre and 98.8 percent at 0.54 pound per acre. The 0.27 pound per acre dosage represents 18 replications, the other two dosages three replications each.

The effectiveness of the technique used in rearing and handling the test insects is supported by data from check mortalities. A total of 34 replications averaging 58 female mosquitoes per replication, show an average mortality of only 1.1 percent with a range of 0 to 5.7 percent. Although corrected data based upon check mortalities are used, they are so similar to the uncorrected data that little is gained by the corrections.

EXPERIMENTAL DESIGN. The test is easily adaptable to the randomized blocks design, using time as blocks and randomizing treatments within blocks. This design has the advantage of allowing any treatment to be dropped at any time without affecting other treatments or the statistical analysis of the data. However, due to weather variations, etc. between the blocks, larger differences between treatment results may be necessary to show significance

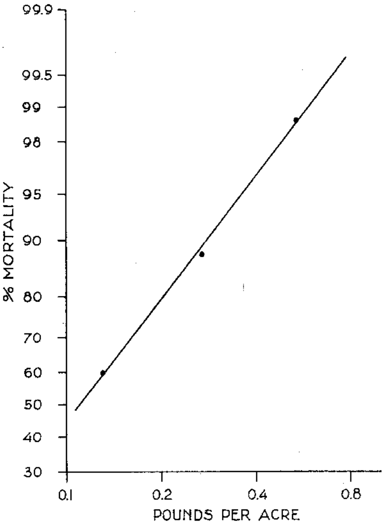

FIG. 3.—Dosage-mortality curve based upon three dosage levels of malathion.

if the treatments are too numerous to be run on a single night.

DISCUSSION. Using a screen bottom instead of a solid bottom on test cages might improve the technique by allowing maximum chance for coverage inside the cage.

Although the test has been used only for dosage experiments with mosquito adulticides, it probably can be utilized in swath determination and similar problems.

Also, it appears to have potential utility in field testing insecticides against other insects, such as black flies and house flies.

The test has been evaluated only with thermal aerosols, therefore its usefulness in testing other methods of application is not known at this time.

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