naled-malathion mixture, although the total volume was only half as large and the total weight of insecticide was one-sixth as large.

SUMMARY. Field tests with low volume sprays of undiluted technical insecticides for the control of salt-marsh mosquitoes were made in Florida citrus groves. Application dosages were figured on the basis of the volume of liquid (fl. oz.) and the weight of active ingredient (lbs.) per acre. Control of 95 percent or higher was obtained 6 hours after treatment with 0.5 fl. oz. (0.05 lb.) naled per acre, 0.5 fl. oz. (0.05 lb.) naled plus 0.5 fl. oz. (0.04 lb.) malathion per acre, 0.5 fl. oz. (0.05 lb.) naled plus 0.5 fl. oz. (0.04 lb.) glycol per acre, and 1 fl. oz. (0.11 lb.) naled plus 4 fl. oz. (0.32 lb.) of malathion per acre. After 24 hours a mixture of 1 fl. oz. (0.11 lb.) naled and 4 fl. oz. (0.32 lb.) malathion gave 97 percent control, and a mixture of 0.8 fl. oz. (0.05 lb.) fenthion plus 1.6 fl. oz. (0.02 lb.) Baygon gave 93 percent control.

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STANDARDIZED FEEDING OF *AEDES AEGYPTI* (L.) MOSQUITOES ON *PLASMODIUM GALLINACEUM* BRUMPT-INFECTED CHICKS FOR MASS SCREENING OF ANTIMALARIAL DRUGS [1]

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In the course of a pilot study on the practicability of mass screening of antimalarial drugs against the parasitic stage (sporogonic cycle) in the mosquito vector, a number of techniques have been developed or improved. Techniques that are successful in a small number of tests in a research laboratory are not necessarily adaptable or efficient when applied to a line production type of drug screening.

Observations on the effect of anti-malarial drugs on the sporogonic cycle in the mosquito vector have been made by a number of investigators. Lumsden and Bertram (1940a) stated that the most delicate criterion for the estimation of the action of a drug was the development of the parasite in the mosquito. They selected the oocyst count as most suitable for their purpose. In another paper, Lumsden and Bertram (1940b) discussed the effects of plasmoquine and praequine on the subsequent development of the gametocytes

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of *Plasmodium gallinaceum* in *Aedes aegypti*. Though oocyst counts are quite indicative, the absence of viable infective sporozoites in the salivary glands of the mosquito is the ultimate criterion of the effectiveness of the test drug. In the experiments described by the above investigators, the drug was fed to the vertebrate host and the results observed in the invertebrate vector.

Terzian (1947) was evidently the first to feed the drug directly to the invertebrate vector. He presented evidence to show a definite relationship between drug activity and parasite development in the infected *Aedes aegypti*. Thus it should be possible to evaluate drugs for their prophylactic activity using the *Aedes aegypti* mosquito as the vector. Terzian fed the drugs to mosquitoes by offering cotton balls soaked in a solution of drug and 4 percent sucrose as nutrient. He judged his results by microscopic examinations of mosquito suspensions or by the development of parasitemia in chicks following the injection of ground mosquito suspension. Johnson and Akins (1948), using techniques similar to those of Terzian, observed that paludrine hydrochloride and quinine sulphate were inhibitory to the development of sporozoites. The salivary glands of the mosquito were dissected out and examined for presence of sporozoites.

Geigy and Rahm (1949) placed emphasis on the dissection and observation of oocysts as a criterion for efficacy of drugs fed to *Aedes aegypti*. They conclude as others have, that this type of experiment is of importance in the search for prophylactic anti-malarial drugs.

Additional research along these lines was reported by Terzian, Stahler and Weathersby (1949), Terzian and Weathersby (1949), and Terzian, Ward and Stahler (1951). Singh, Narayandas and Ray (1953) tested proguanil, pyrimethamine and M3349, a "proguanil precursor," against the sporogonic cycle in *Aedes aegypti*. The mosquitoes imbibed various concentrations of the drug in 4 percent glucose before and after an infective blood meal. Proguanil was found to be effective in that none of the mosquitoes showed salivary gland infection at drug concentrations of 0.01 to 1.0 percent; however, oocysts were found in the gut. Similar results were noted with pyrimethamine. Concentrations of 0.001 and 0.0001 were found to be inadequate to inhibit the development of oocysts or sporozoites. M3349 was ineffective at all concentrations. Narayandas and Ray (1954) reported on the effect of pentaquine, pamaquine and primaquine on the sporogonic cycle in *Aedes aegypti*.

CAGE TYPE. Three types of cages were tested as small holding cages for the adult mosquitoes. These consisted of a 3 x 2½" glass jar with a bakelite screw top lid, a cardboard half pint ice cream carton and a circular plastic container with a screw top lid. The plastic container 3" in diameter and 4" high proved most successful for drug feeding purposes and for later blood feeding on chicks. A circle 3" in diameter was cut out of the plastic lid and an 18 mesh plastic screen was fastened to the lid by heat sealing. When it was found necessary to use an aspirator to remove or handle any of the mosquitoes in the cage, a small hole ¾" in diameter was made in the bottom of the cage and a slit heavy rubber dam was glued over the opening. Waterproof tape can be used to cover this slit if leakage occurs due to enlargement of the slit. Each cage conveniently holds 30 adult female mosquitoes. To avoid transfer and loss of mosquitoes, 30 female pupae are introduced into the container in ½ inch of water. The pupae emerge in 24–48 hours and the excess water is drained off by inverting the cage. The pupae and adults are held in a room maintained at 80° F. and 80 percent relative humidity.

MOSQUITO FEEDING. For drug feeding purposes, the feeding pipette was considered more efficient than the use of cotton balls or paper pads soaked in the feeding solution.

A suspended pipette outside the cage, with the mouth resting on the screening and filled with the feeding solution, was

found to be readily accessible to the mosquitoes due to the greatly increased resting surface on the screening. The amount of solution that could be withdrawn (±1 c.c.) was sufficient for 3 days' consumption (±0.6 c.c.). The tapered tip should be removed and the end ground to provide a larger resting surface on the screen and allow direct access to the drug solution. The escape problem was eliminated as the cage did not have to be opened to refill or remove the pipette.

A rack (fig. 1) constructed of ¼" x 1" x 36" aluminum bars bored at 4" intervals with ¼ inch holes was used to hold and suspend the feeding pipettes over the cages. Four such bars are bolted together to form a rack.

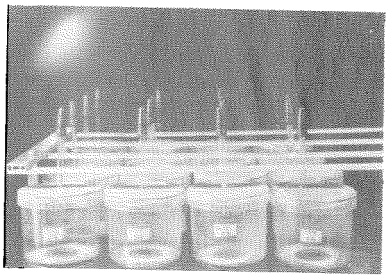

FIG. 1.—Mosquito feeding on drugs from suspended pipettes in aluminum rack.

CONCENTRATION OF SUCROSE. In order to determine the concentration of sucrose required for adequate longevity and subsequent blood feeding, tests were conducted comparing 1 percent, 4 percent, 8 percent, 10 percent, 16 percent and 32 percent concentrations. Various dilutions of pure honey at 20 percent, 40 percent, 60 percent, 80 percent and 100 percent were also tested. The presence of a sucrose meal in the mosquito diverticulum was detected by the addition of 10–20 crystals of methylene blue to 100 c.c. of the solution. Dissections were performed daily. Ten percent sucrose appeared to give the best results.

Tests were conducted to determine the time required for 100 percent of the mosquitoes in a cage to take a minimum of one sucrose meal; 92–100 percent of the mosquitoes fed at least once during 3 days. It was observed that sucrose-fed mosquitoes, regardless of the concentration of sucrose, did not readily feed on the chicks, unless submitted to a starvation period prior to the blood meal. A starvation period of 48 hours following 72 hours of feeding on 10 percent sucrose gave the highest percentage of blood feeding (Table 1). The only sources for food and water during each test is either the drug-sucrose mixture or the infective blood meal which was from an 8A strain of *Plasmodium gallinaceum* infected chick.

TABLE 1.—Comparison of blood feeding of mosquitoes after 48 hours starvation and 72 hours prior feeding on various concentrations of sucrose.

	15 minutes feeding time			
% Sucrose concentration	4	10	16	32
No. mosquitoes engorged	171	133	93	80
No. mosquitoes exposed	281	141	116	116
Percent engorgement	60.8	94.3	80.9	68.9

BLOOD FEEDING. Various methods of blood feeding were tested. The method of Eldridge and Gould (1960) was tested on 3-4-week-old chicks. This technique provides for one cage of mosquitoes to be fed on each of the chick legs. A high percentage of engorgement was obtained. This method however, was somewhat time consuming and presented some disadvantages when working with infected materials. In addition, condensation of moisture in the mason jar type of cage resulted in high mortalities. Moreover, when large numbers of cages had to be used, mosquitoes were apt to escape. The technique in which a chick was trussed and placed on top of a plastic cage was tried and found to be excellent and eliminated the escape of mosquitoes. However, only one cage could be fed at a time and it required transferring mos-

quitoes to the cage prior to drug feeding which could lead to possible losses of mosquitoes during the change of the drug container.

A cage holder[3] (Fig. 2) was devised

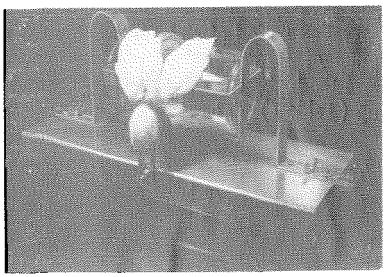

FIG. 2.—Cage holder for blood feeding of mosquitoes.

that could hold two of the aforementioned plastic cages at a time, with the chick sandwhiched between them. The cages were held in position by two movable holders and the screened lids of the plastic cage were pressed against each side of the chick. The chick legs and head were held in place by straps of Velcro,[4] a self-locking cloth. The cage and chick holder are made of aluminum and can be easily cleaned.

The operation is very rapid, one minute is required to have chicks and cage in position, and there is no loss of mosquitoes as there is no need to open the cage. The feeding percentages are increased when the cages to be fed are taken to the feeding room about one hour before the operation starts for conditioning of the mosquitoes. When young chicks are used no plucking is necessary; however, with older chicks it is recommended to remove some of the down to increase the area of bare skin available. Maximum engorgement is usually obtained within 10–15 minutes. As many as 36 cages of mosquitoes have been fed in succession on a single chick, with no observable adverse effects on the chick, and the average feeding percentage remained at 91.6 percent.

It has also been observed that the parasitemia rate does not vary significantly in the infected chick during a long feeding period. In one experiment in which a series of caged mosquitoes were fed consecutively on a chick, the total feeding period was 4½ hours and the parasitemia rate fluctuated between 9.5 and 11.5 percent.

OOCYST AND SPOROZOITE DEVELOPMENT. After the mosquitoes have fed on an infected chick the cages are held in a holding room in which an 80° F. temperature and 80 percent relative humidity are maintained. The pipette feeders containing sucrose and drug are again placed on the cages and the mosquitoes are kept for 7 days before dissection to determine oocyst production. Even with the blood meal only partially digested, small oocysts may be seen on the mid-gut 48 hours after blood feeding. On the fourth day after the infective blood meal, large numbers of oocysts can be readily seen, and may cover the entire surface of the gut. For practical purposes, it has been deemed more convenient to let the sporozoites reach the salivary glands before any dissection is performed. Dissections for oocysts and sporozoites may then be deferred until day 9 after blood has been offered. With this technique, no slit is needed in the rubber dam at the bottom of the cage. The cage can be completely sealed and therefore eliminates all chances of escape. Cages with infected *Aedes aegypti* are chilled for 5 to 10 minutes before the mosquitoes are transferred to a glass jar and chloroformed, thus keeping the chemical from dissolving the plastic.

SUMMARY. Mass screening of antimalarial drugs can be conducted by a standardized method of feeding *Aedes aegypti* mosquitoes on *Plasmodium gallinaceum* infected chicks. Drug consumption takes place before and after an infective blood meal. Those mosquitoes starved to death that do not imbibe the candidate drug and sucrose are eliminated

[3] Now manufactured by Cornell Chemical & Equipment Co., 1115 N. Rolling Road, Baltimore, Maryland 21228.

[4] E. I. duPont deNemours, Inc. registered trade mark.

from the test system. Any mosquito surviving until dissection (9th day after infective blood meal) must have fed on the drug-sucrose mixture.

Full blood engorgement of mosquitoes occurred after 48 hours of withdrawal from drug-sucrose mixture. Ten to 15 minutes were required for blood feeding. No significant variation in the parasitemia rate in the chicken occurred in 4½ hours of consecutive mosquito feeding.

Newly designed laboratory apparatus, including cages, blood-feeding holders and drug feeders, are described, and have been found efficient in line production.

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