THE SUSCEPTIBILITY STATUS OF *AEDES SOLLICITANS* ADULTS TO TOPICALLY APPLIED MALATHION

B. K. KHOO AND D. J. SUTHERLAND

Mosquito Research and Control, Cook College, NJAES, Rutgers University, New Brunswick, NJ 08903

ABSTRACT. The status of susceptibility to malathion of wild-caught *Aedes sollicitans* and their F_1 progenies from 5 areas in New Jersey was determined by topical application. Laboratory strains of this species from Louisiana and Texas, established in 1981, exhibit baseline susceptibility (LD_{50}s: 2.1 ng/male, 3.4 ng/female Louisiana; 2.3 ng/male, 3.6 ng/female Texas). However, in all New Jersey populations tested, a plateau in their respective log dosage-probit lines is distinguishable suggesting incipient resistance to malathion. The relationship between topical application data and malathion aerial ULV space sprays is discussed.

INTRODUCTION

Since 1970, malathion has been the major adulticide used to control *Aedes sollicitans* (Walker) populations in New Jersey as a supplement to water management and larviciding. While this adulticide as aerial ULV has been effective, the long interval of its usage warrants the monitoring of the malathion susceptibility of this important New Jersey species. Information gained from such monitoring would provide justification for possible adjustments in the malathion formulation or for selection of alternate adulticides, in order to insure continued effective control of this species by the State Airspray Program.

The standard technique for determining the susceptibility or resistance of mosquitoes to an adulticide involves their exposure to WHO (World Health Organization) insecticide impregnated papers. However, this technique has a drawback with regard to stability of biodegradable pesticides (Gilotra et al. 1972, Rathor and Toqir 1980). Although Rathor and Toqir (1980) overcame this problem by preparing their own malathion papers, we elected to employ the method of topical application which allows for the delivery of a known amount of insecticide to each mosquito.

This paper describes the laboratory colonization of *Ae. sollicitans*, and the detailed protocol for topical application, and presents the susceptibility status to malathion of wild-caught *Ae. sollicitans* and their F_1 progenies from 5 areas in New Jersey as compared to that of laboratory strains derived from Louisiana and Texas.

MATERIALS AND METHODS

FIELD COLLECTIONS AND STRAINS. Female *Ae. sollicitans* were collected from Atlantic County (Mott's Creek), Cape May County (Eldora and Goshen), Cumberland County (Port Norris) and Ocean County (West Creek). Two methods of collection were employed. When the adult landing rate was adequate, the mosquitoes were aspirated manually into pint-size cartons whose ends were replaced with fine gauze. When landing rates were low, CO_2-baited CDC traps

were employed for overnight collection followed by introduction into cartons. To avoid overcrowding, only 50 to 60 mosquitoes were introduced into each carton and provided with a 4 cm cotton dental roll wetted with 10% sucrose solution. Each carton was enclosed in a clear polyethylene bag and held overnight in darkness at room temperature to reduce possible stress and also to insure that only undamaged healthy survivors were available for topical treatment the next day. Strains from Louisiana (Cameron Parish) and Texas (Beaumont) were established in 1981 and maintained according to the procedures described below.

REARING AND PROPAGATION. For studies on the F_1 progenies and the maintenance of the Louisiana and Texas strains, mated females were fed on a guinea pig and confined in groups of five to a glass vial (8 dram) containing an oviposition surface, a cotton plug moistened with distilled water. After a minimum of 14 days, distilled water was added to the vials, and the eggs were hatched *in vacuo* (55 cm Hg). One hundred to 150 newly hatched larvae were introduced into a white enameled pan (300 × 900 × 50 mm), containing 1.2 liters of saline water (5.0 ppt Instant Ocean®, Aquarium Systems, Mentor, OH, in distilled water). Larval food was BYLP (1:1 mixture of brewer's yeast and liver powder, ICN Pharmaceuticals, Inc., Cleveland, OH) and HS (finely ground, 250 μm, Hog Supplement, Agway, Inc., Hightstown, NJ). On the first day, these larvae were provided with 10 mg BYLP and 15 mg HS sprinkled onto the water surface. On the second day, the water surface was skimmed with paper toweling, 50 ml saline water was added, and the larvae were provided with 20 mg BYLP and 30 mg HS. On the third day, the larvae were recovered by straining the pan contents, rinsed with tap water, and transferred to clean pans of saline water. Larval density was reduced to 50–55 larvae of similar size per pan, and 100 ml of 'old' water (the previous rearing water) and 30 mg BYLP and 120 mg HS added. After the third day, the water was changed daily, followed by the addition of 'old' water and food as provided on the third day. On the sixth day, the majority of larvae were in the late 4th instar, recognizable by their greenish tinge. Larval density was adjusted to 100–150 per pan, and the larvae provided with 20 mg BYLP and 60 mg HS. The rearing chamber was maintained at 27°C with a 16L:8D photoperiod. These strict rearing procedures insure that larval size is optimized and the larval mortality is below 5%. The reintroduction of 'old' water appears to synchronize larval development so that most adult eclosion occurs within a 24 hr period.

Pupae were recovered by straining, then rinsed with tap water and added to dishes containing fresh saline water (no more than 100 pupae/dish). Clean water insures successful adult eclosion. In polluted water, partial eclosion and drowning of newly emerged adults occurs, possibly due to a reduction in surface tension of the water. A plastic bag inverted over the pupal dish retained the female and male adults, which were aspirated and separated by sex into cartons (50–60 per carton). Each carton was provided with 10% sucrose solution on cotton rolls, replaced daily thereafter.

Since *Ae. sollicitans* will not mate in captivity, the propagation of the various strains was achieved by induced copulation. Prior to forced mating, newly emerged adults were separated by sex into cartons. The males were supplied with sucrose solution for 2–3 days; the females were offered only water for 1–2 days to insure that most would take a blood meal (guinea pig) just prior to induced copulation. The latter was accomplished as described by McCuiston and White (1976) except that males were aspirated individually into a precooled 4-dram snap-cap vial and immediatey picked up with forceps for pinning before complete hypothermia. Prior to decapitation, all legs of the male were severed to facilitate positioning for mating and to prevent entanglement of the legs of the female.

TOPICAL APPLICATION. In susceptibility determinations, field-caught adults were treated 1 day after collection; for their F_1 progenies and the Louisiana and Texas strains 3–4 day old individuals were employed.

Topical application of acetone solutions of analytical grade malathion (98% purity) was conducted with a microapplicator (Instrument Specialities Co., NE) by means of a 0.25 ml syringe fitted with a 27 gauge hypodermic needle bent at right angles and its tip blunted (Khoo and Sutherland 1981).

To insure randomization, mosquitoes to be treated were pooled into a single carton. From this, 10 males or females were transferred with an aspirator into a 9 cm plastic petri dish through a hole bored in the cover. The base of the petri dish was lined with filter paper. A 2 cm segment of cotton roll moistened with 10% sucrose solution was partially inserted in the hole, in order to prevent wetting of the filter paper and to reduce the chance of intoxicated mosquitoes sticking to the moist roll.

To facilitate handling during topical application, carbon dioxide anesthesia was employed. Just before treatment, each petri dish was placed in the anesthesia chamber and a slow flow of CO_2 was piped into the chamber. The cover of the dish was slightly raised so that CO_2 permeated the petri dish as it displaced air.

After 5–10 sec the immobilized mosquitoes were ready for treatment. Each fragile individual was picked up by the legs with a pair of flexible microdissection forceps (Turtox, NY) and positioned to receive 0.5 μl of the malathion-acetone solution on the pleural area of the thorax. The acetone droplet was evaporated by gently blowing on the mosquito before it was returned to a petri dish base. This prevents accidental absorption of the insecticidal solution by the filter paper. Since the carrier solvent, acetone, is very volatile, only the alternate droplet of the insecticide solution was used for each mosquito. The other alternate droplet was removed by contact with a glass slide. In the control groups, each mosquito received 0.5 μl of acetone. After the treatment the petri dishes were placed in a holding pan, which was covered with a sheet of aluminum foil and placed in a holding chamber at 27°C. Replicated treatments were carried out for at least 4 broods of *Ae. sollicitans*. Mortality was recorded 24 hr posttreatment. The observed mortality was corrected for natural mortality (Abbott 1925) before the data was subjected to probit analyses (Finney 1952).

RESULTS AND DISCUSSION

The susceptibility of the Louisiana and Texas *Ae. sollicitans* to topically applied malathion is summarized in Table 1. Based on the LD_{50}s of both strains, the males are significantly more susceptible than the females. There is no significant difference in malathion susceptibility between the 2 strains. We can, therefore, say with some degree of confidence, that these LD_{50}s represent the base response of *Ae. sollicitans* to malathion.

The status of susceptibility of the salt marsh mosquitoes from 5 areas in New Jersey is summarized in Table 2. The log dosage-probit (ld-p) lines for the Eldora F_0 females and the Goshen F_1 females are graphically presented in Fig. 1 together with the linear regressions obtained for both sexes of the Louisiana strain of *Ae. sollicitans*. For the Eldora F_0 females, the ld-p line shows an inflection beginning at point *a* and ending at point *b*. This interval represents the range of dosage where there is virtually no increase in response because the susceptible subpopulation was eliminated, resulting in a shift in response coinciding with the more tolerant subpopulation. It should be noted that a subpopulation of the Eldora *Ae. sollicitans* was as susceptible to malathion as the Louisiana strain, supporting our assertion that the LD_{50} of the latter represents the base response of *Ae. sollicitans* to malathion. The Goshen F_1 females were essentially similar in their response to malathion as the Eldora F_0 females (Fig. 1).

In all the New Jersey populations tested, a plateau in their respective ld-p line is distinguishable (Table 2). Since the ld-p lines are not linear, the data are unsuitable for probit analyses (Tsukamoto 1963). The LD_{50}s and

Table 1. Susceptibility of Louisiana and Texas *Aedes sollicitans* to topically applied malathion.

Strain	ng/individual LD_{50}	ng/individual LD_{95}	LD_{50} fiducial limits (P = 0.05)	Slope
Louisiana (♂)	2.1	3.6	2.0–2.2	6.9
(♀)	3.4	5.7	3.3–3.5	7.5
Texas (♂)	2.3	3.7	2.0–2.7	8.4
(♀)	3.6	5.1	3.4–3.7	10.5

Table 2. Susceptibility status of various New Jersey *Aedes sollicitans* populations to malathion.

Area sampled	Generation	ng/individual LD_{50}	ng/individual LD_{95}	Range of plateau (ng)
Mott's Creek[a]	F_0 (♀)	4.8	26.0	4.5–11.0
Eldora[a]	F_0 (♀)	4.0	23.0	3.6–12.0
Goshen	F_0 (♀)	5.5	26.0	5.0–15.0
	F_1 (♂)	3.0	25.0	3.5–15.0
	F_1 (♀)	5.0	25.0	4.0–11.0
Port Norris	F_0 (♀)	4.5	23.0	3.0–11.0
	F_1 (♂)	2.3	13.0	3.0– 7.0
	F_1 (♀)	3.5	20.0	3.0–11.0
West Creek	F_0 (♀)	10.0	55.0	5.5–11.0
	F_1 (♂)	6.5	35.0	7.0–15.0
	F_1 (♀)	10.0	60.0	9.0–20.0

[a] There were insufficient F_1 progenies for susceptibility determinations.

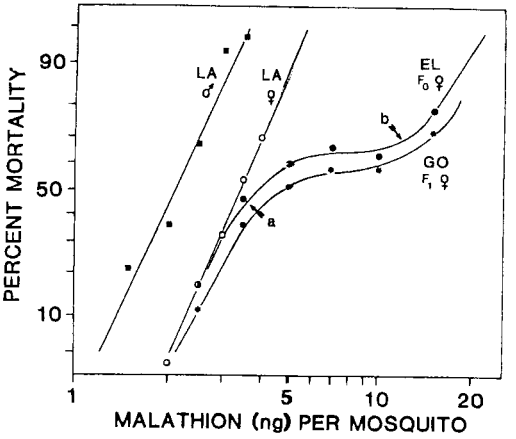

Fig. 1. The shape of the ld-p lines of a homogeneous Louisiana (LA) strain as compared to the heterogeneous Eldora (EL) and Goshen (GO) *Aedes sollicitans* from New Jersey.

LD_{95}s presented were obtained graphically. The results showed that these populations of *Ae. sollicitans* have become tolerant to malathion. The results of a preliminary adult selection study with the highly heterogeneous West Creek population of *Ae. sollicitans* (Khoo and Sutherland, unpublished) have established a genetic basis for the increase in tolerance to malathion.

Although the standard WHO method for susceptibility determination with adult mosquitoes involves their exposure to insecticide impregnated paper, we have opted for the method of topical application not only because it is more precise but also because the results obtained have a direct relationship to aerial application of ULV malathion. For example, based on the LD_{95}s for the Louisiana and West Creek *Ae. sollicitans*, Table 3 was constructed. It indicates that a single 100 μm droplet is more than sufficient to kill one Louisiana or one West Creek female adult. On the other hand, for 10 μm droplets, 10 are required for the Louisiana and 94 for the West Creek female. Clearly, an increase in tolerance poses a problem in terms of the number of droplets that must impact on the mosquito. In turn, the probability that a mosquito will collect a lethal dose, acquired by combinations of malathion droplets, is dependent on the number distribution of each class of droplets and the time the mosquito spends in flight. Since delivery of ULV space spray to the target mosquito is in the form of airborne droplets, conceivably, data from topical application could serve as a basis in the tailoring of field application rate and volume of a given adulticide such that the most effective dosage is contained in the optimum droplet size of the aerosol.

Table 3. Number of ULV malathion droplets required to kill a ♀ *Aedes sollicitans*.

Droplet diameter (μm)[a]	No. of droplets[b]	
	Louisiana	West Creek
100	1	1
50	1	1
25	1	6
20	2	12
15	3	28
10	10	94

[a] Based on 91% malathion (sp. gr.:1.2315); 1 cc = 1.120665×10^9 ng. Volume = $0.5236\ (D \times 10^{-4})^3$ cc where D = droplet diameter (μm).

[b] Based on the LD_{95} dose (LA: 5.7 ng; West Creek: 55.0 ng).

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