

## SUSCEPTIBILITY STATUS OF *Aedes albopictus* TO THREE TOPICALLY APPLIED ADULTICIDES

BIN KHONG KHOO,<sup>1</sup> DONALD J. SUTHERLAND,<sup>1</sup> DANIEL SPRENGER,<sup>2</sup> DAVID DICKERSON<sup>2</sup>  
AND HUNG NGUYEN<sup>2</sup>

**ABSTRACT.** The baseline susceptibility of *Aedes albopictus* (Sabah strain) to malathion was determined. Both laboratory-colonized and field-collected *Ae. albopictus* at Harris County, TX are resistant to malathion but susceptible to Scourge®. The acute toxicity of bendiocarb to *Ae. albopictus* is confounded by its rapid knockdown and delayed recovery syndrome of poisoning.

### INTRODUCTION

The presence of established populations of *Aedes albopictus* (Skuse) was first discovered in Harris County, Texas in August, 1985 (Sprenger and Wuithiranyagool 1986). Subsequent surveillance by others revealed the presence of other populations in 12 states (Centers for Disease Control 1986). Black et al. (1988) suggested that infestations in other areas of the country may have stemmed from three relatively large independent introductions into the U.S.A. All North American strains of *Ae. albopictus* appear to be genetically similar. Allozyme analyses of worldwide strains suggest that the introductions originated from Japan (W. C. Black and K. S. Rai, personal communication).

Since there is a lack of information in Japan on the susceptibility of *Ae. albopictus* to insecticides (Y. Wada, personal communication), a preliminary study was conducted on the susceptibility of *Ae. albopictus* to the three adulticides currently employed by the Harris County Mosquito Control District to control *Culex quinquefasciatus* Say. This information will be essential for any future control efforts. In the two years since its discovery, *Ae. albopictus* has already become an important nuisance species in Harris County.

### MATERIALS AND METHODS

Both laboratory-colonized and F<sub>1</sub> females (3–4 day old) of field collected *Ae. albopictus* were employed in the study. The laboratory colony of *Ae. albopictus* was established at the Harris County Mosquito Control District (HCMCD) on April 1, 1986. To ensure genetic diversity, larvae from 10 widespread collection sites were used to establish the colony as described by Sprenger et al. (1987). The F<sub>1</sub> females originated from progenies of adults reared from larvae col-

lected from six separate locales (Fig. 1). For determination of the baseline toxicity of this species of mosquito to malathion, the Sabah strain provided by G. B. Craig (University of Notre Dame) was employed. This strain was derived from larvae collected from bamboo stumps in August, 1986 in Sabah, Malaysia by L. Munstermann (University of Notre Dame).

The three adulticides tested were analytical grade malathion (American Cyanamid), Scourge®, a formulation of resmethrins and piperonyl butoxide (Penick-Bio UCLAF), and technical grade bendiocarb (Nor-am). The method of topical application as described by Khoo and Sutherland (1983) was employed in the treatment of adults. For the Harris County Mosquito Control District (HCMCD) laboratory-colonized *Ae. albopictus*, at least 50 females were treated for each dosage level. Treatments of adults were conducted at 21°C. Additional treatments with Scourge were conducted at 27°C. The baseline susceptibility of *Ae. albopictus* (Sabah strain) to malathion was obtained by probit analysis (Finney 1971) using SAS procedures (SAS Institute 1982).

### RESULTS AND DISCUSSION

As depicted in Fig. 2, the log dosage-probit mortality (ld-pm) regression for the Sabah strain of *Ae. albopictus* is linear and steep (slope = 8.72) and it may represent the baseline toxicity of malathion to this species of mosquito based on 24 h posttreatment mortality. The LD<sub>50</sub> is 5.1 ng/female (4.88–5.31 fiducial limits) and the LD<sub>95</sub> is 7.9 ng/female (7.30–8.68 fiducial limits). The ld-pm response curve of the F<sub>12</sub> generation of the HCMCD laboratory-colonized *Ae. albopictus*, however, shows a distinct plateau which represents the range of dosage where there is virtually no increase in response to malathion. This response curve is typical of a heterogeneous population consisting of a mixture of susceptible and resistant individuals (Tsukamoto 1963). Similar results were obtained when treatments with malathion were repeated with the F<sub>17</sub> generation of the HCMCD laboratory-colonized *Ae. albopictus*. Although

<sup>1</sup> Mosquito Research and Control Unit, Dept. of Entomology and Economic Zoology, Cook College, P. O. Box 231, New Brunswick, NJ 08903.

<sup>2</sup> Harris County Mosquito Control District, 1646 Old Spanish Trail, Houston, TX 77054.

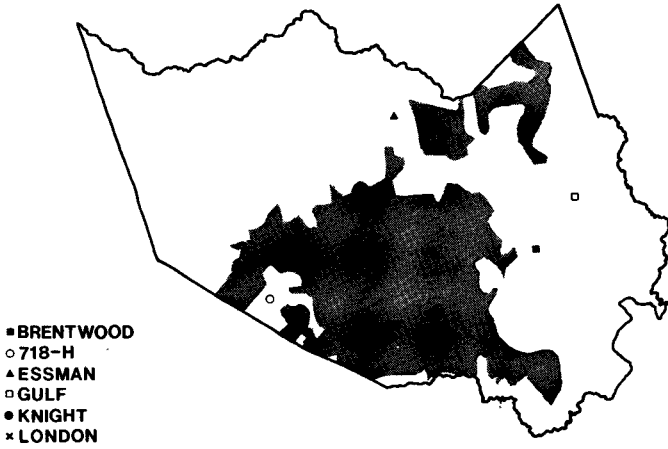


Fig. 1. Locales where F<sub>1</sub> females of field-collected *Aedes albopictus* originated. The shaded area represents the Houston city limits within Harris Co., TX.

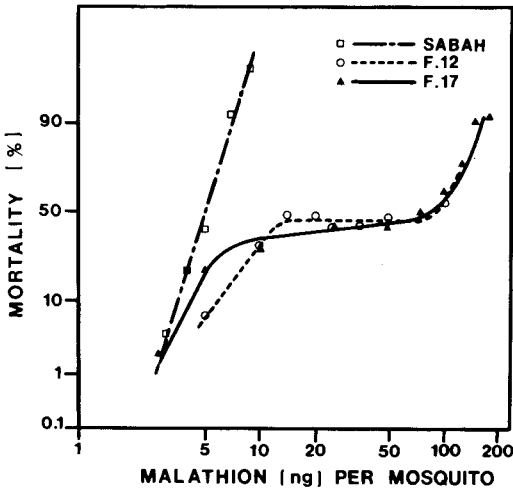


Fig. 2. The Ld<sub>50</sub>-pm lines of a homogeneous Sabah strain as compared to the F<sub>12</sub> and F<sub>17</sub> generations of the Harris County Mosquito Control District (HCMCD) laboratory-colonized *Aedes albopictus*.

the study is preliminary, it can be concluded that at least 50% of the laboratory-colonized populations of *Ae. albopictus* are resistant to malathion.

Table 1 summarizes the percent mortality of field-populations (F<sub>1</sub> generations) of *Ae. albopictus* females from various locales treated with malathion. The small sample size at Brentwood and 718-H preclude any comments regarding their resistance status with respect to malathion. However, it is evident that the populations at Essman, Gulf, Knight and London exhibit resistance to malathion.

Since the HCMCD laboratory-colonized *Ae. albopictus* stemmed from wild-caught mosqui-

Table 1. Susceptibility of *Aedes albopictus* F<sub>1</sub> females to malathion.<sup>a</sup>

Locale	Dose (ng/♀)	No. treated	% mortality
Brentwood	25	3	100
	100	39	72
718-H	100	8	100
Essman	25	99	47
	35	58	69
	50	60	82
Gulf	100	40	75
	25	55	62
Knight	100	60	73
	10	10	70
	25	60	50
London	35	60	55
	100	40	62
	25	60	33
	35	59	46
	50	90	42
	100	56	48

<sup>a</sup> Mortality is based on 24 h posttreatment.

toes and after 12 to 17 generations of colonization free of any insecticidal pressure, yet at least 50% of the populations are still resistant to malathion (not unlike those from Knight and London), it suggests no reversion to malathion susceptibility. This implies the establishment of a Hardy-Weinberg equilibrium (Suzuki and Griffiths 1976) in the laboratory colony with virtually no difference in fitness between the susceptible and resistant genotypes. One would expect a reversion to susceptibility had the resistant genotypes been less fit than the susceptibles.

Table 2 summarizes the percent mortality (based on 48 h posttreatment) of the HCMCD lab-colonized *Ae. albopictus* with respect to dosage of Scourge applied. One hundred percent

Table 2. Susceptibility of the F<sub>12</sub> laboratory-colonized *Aedes albopictus* to Scourge®.

Dose (ng/♀) <sup>a</sup>	No. treated <sup>b</sup>	% mortality
0.7	50 (50)	42 (4)
1.0	50 (50)	64 (32)
1.5	50 (50)	96 (72)
3.0	40	100
5.0	60	100
10.0	90	100
14.0	40	100

<sup>a</sup> Expressed as actual resmethrins; piperonyl butoxide included at 3 × the dose.

<sup>b</sup> Treated mosquitoes were held at 21°C. Those held at 27°C are in parentheses. All mortalities were 48 h posttreatment.

mortality was obtained with a dose as low as 3.0 ng Scourge/female. This, however, does not necessarily suggest absence of cross-resistance of malathion to Scourge because the formulation of Scourge included the synergist, piperonyl butoxide (PB), a compound noted for overcoming resistance that involves the mixed-function oxidase (MFO) system (Hodgson and Tate 1976).

The results (Table 2) also establish that Scourge is more toxic to *Ae. albopictus* at low (21°C) than at high (27°C) temperature. This phenomenon of the inverse relationship of toxicity and temperature is well-documented for pyrethroid insecticides (Khoo and Sutherland 1981).

Although laboratory determinations showed high susceptibility of *Ae. albopictus* to Scourge, it does not follow that operational application of ULV Scourge would prove to be efficacious. Label recommended rates of Scourge may be inadequate (Khoo et al. 1987). The operational performance of Scourge requires evaluation independent of the laboratory results.

Table 3 summarizes the percent mortality (based on 48 h posttreatment at 21°C) of the F<sub>1</sub> generations of wild-caught *Ae. albopictus* with respect to dose of Scourge applied. Not unlike the HCMCD laboratory-colonized *Ae. albopictus* (Table 2), a high mortality was obtained with a dose of 1.5 ng Scourge/female.

Table 4 summarizes the percent mortality of HCMCD laboratory-colonized and wild-caught populations (F<sub>1</sub> generations) of *Ae. albopictus* females with respect to dose of bendiocarb applied. Mortality was observed at 24, 48 and 72 h respectively, because bendiocarb appears to be slow-acting although knockdown occurs within minutes. Females treated at the lower doses recovered from knockdown by 24 h posttreatment but those treated at the higher doses remained knocked-down for 48 h or longer. Those that recovered at the higher doses appeared weak and it was necessary to probe some of the

Table 3. Susceptibility of field populations of *Aedes albopictus* to Scourge®<sup>a</sup>.

Locale	Dose (ng/♀)	No. treated	% mortality
Essman	1.5	40	77
	14.0	40	100
Gulf Knight	1.5	40	82
	1.5	40	97
London	14.0	10	100
	1.5	40	90
	14.0	80	100

<sup>a</sup> Treated mosquitoes were held at 21°C. Percent mortality is based on 48 h posttreatment.

Table 4. Susceptibility of *Aedes albopictus* to bendiocarb<sup>a</sup>.

Locale	Dose (ng/♀)	No. treated	% mortality at:		
			24 h	48 h	72 h
Laboratory (F <sub>12</sub> )	3.0	40	20	32	35
	5.0	40	20	25	37
	7.0	40	10	15	30
	10.0	40	10	30	52
Essman	10.0	40	10	15	27
Gulf	10.0	40	3	15	32
Knight	10.0	40	15	32	42
London	10.0	40	10	32	45

<sup>a</sup> Treated mosquitoes were held at 21°C.

females with a pair of forceps to determine whether they were alive.

Subsequent studies on the syndrome of poisoning by bendiocarb were extended to *Aedes aegypti* (Linn.) and *Aedes sollicitans* (Walker) by Khoo and Sutherland (unpublished data). Observations were increased to 5 days posttreatment and essentially the same syndrome and mortality results were obtained with dosage range spanning 5–35 ng bendiocarb/female mosquito. This ability of the mosquitoes to recover from knockdown may be related to the spontaneous recovery phenomenon reported by many who studied inhibition of acetylcholinesterase by carbamate insecticides (O'Brien 1976).

With respect to wild-caught populations (F<sub>1</sub>) of *Ae. albopictus*, less than 50% mortality was obtained with a dose of 10 ng bendiocarb/female (Table 4).

The study reported here clearly established malathion resistance in populations of the Harris County *Ae. albopictus*. Since the works of Black et al. (1988) suggest that this county could be a source of founding populations found elsewhere in the U.S.A., it would not be surprising if such populations of *Ae. albopictus* are tolerant to malathion as well.

#### ACKNOWLEDGMENTS

The senior author wishes to thank the Harris County Mosquito Control District for providing

technical support and laboratory facilities and American Cyanamid for a grant supporting the studies. This New Jersey Agricultural Experiment Station Publication No. D-40400-01-88 is supported by State Funds and funding from the New Jersey State Mosquito Control Commission.

#### REFERENCES CITED

- Black, W. C., IV, J. A. Ferrari, K. S. Rai and D. Sprenger. 1988. Breeding structure of a colonizing species: *Aedes albopictus* (Skuse) in the United States. *Heredity*. In press.
- Centers for Disease Control. 1986. *Aedes albopictus* infestation—United States, Brazil. *MMWR* 35:493-495.
- Finney, D. J. 1971. *Probit analysis*, 3rd ed. Cambridge Univ. Press, Cambridge.
- Hodgson, E. and L. G. Tate. 1976. Cytochrome P450 interactions, pp. 115-148. *In*: C. F. Wilkinson (ed.), *Insecticide biochemistry and physiology*, Plenum Press, New York.
- Khoo, B. K. and D. J. Sutherland. 1981. Leg fracture in adult mosquitoes induced by bioresmethrin. *Mosq. News* 41:802-804.
- Khoo, B. K. and D. J. Sutherland. 1983. The susceptibility status of *Aedes sollicitans* adults to topically applied malathion. *Mosq. News* 43:441-444.
- Khoo, B. K., D. J. Sutherland and R. Kent. 1987. Actual operational efficacy versus the relative toxicity of Scourge® and malathion to *Aedes sollicitans* (Walker). *Proc. N.J. Mosq. Control Assoc.* In press.
- O'Brien, R. D. 1976. Acetylcholinesterase and its inhibition, pp. 271-296. *In*: C. F. Wilkinson (ed.), *Insecticide biochemistry and physiology*, Plenum Press, New York.
- SAS Institute, Inc. 1982. *SAS user's guide: Statistics*. Cary, NC, 584 pp.
- Sprenger, D., D. Dickerson and H. Nguyen. 1987. Colonization of *Aedes albopictus* (Skuse). *Proc. N.J. Mosq. Control Assoc.* In press.
- Sprenger, D. and T. Wuthiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J. Am. Mosq. Control Assoc.* 2:217-219.
- Suzuki, D. T. and A. J. Griffiths. 1976. *An introduction to genetic analysis*. W. H. Freeman and Co., San Francisco.
- Tsukamoto, M. 1963. The log dosage-probit mortality curve in genetic researches of insect resistance to insecticides. *Botyu-Kagaku* 28:91-98.