Insecticide Susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand

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ABSTRACT *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), two important vectors of dengue fever and dengue hemorrhagic fever, were collected from Mae Sot, Nakhon Sawan, Nakhon Ratchasima, Surat Thani, and Phatthalung, Thailand, from July 2003 to April 2004. The patterns of insecticide susceptibility to temephos, malathion, and permethrin of both *Ae. aegypti* and *Ae. albopictus* larvae were determined. *Ae. aegypti* from all study sites were resistant to permethrin, they but were susceptible to malathion. Resistance to temephos was detected in all strains of *Ae. aegypti*, except those from Nakhon Ratchasima. *Ae. albopictus* larvae had low levels of resistance to all three insecticides, except Mae Sot and Phatthalung strains, which were resistant to permethrin.

KEY WORDS insecticide resistance, *Aedes*, temephos, malathion, permethrin

DENGUE FEVER (DF) AND dengue hemorrhagic fever (DHF) are vector-borne diseases of public health importance in tropical, subtropical, and temperate regions of the world (Gubler 1998, Pancharoen et al. 2002). Millions of people are infected by DF and DHF annually (Jacobs 2000). Thailand has suffered from one of the highest rates of DF and DHF in the world since DHF first occurred in the country in 1958 (Nimmannitya 1987). Currently, a dengue vaccine is not available, and the only effective vector intervention involves well-organized larval control measures (Swaddiwudhipong et al. 1992, Gratz 1993, Pancharoen et al. 2002, Kantachuvessiri 2002).

In Thailand, organochlorines (DDT), organophosphates (temephos, fenitrothion, malathion, and chlorpyrifos), carbamates (propoxur, pirimiphosmethyl, and bendiocarb), pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin, and etofenprox), and biologicals (*Bacillus thuringiensis israelensis* and *Bacillus sphaericus*) have been used to control mosquito vectors (Bang et al. 1969, reviewed in Chareonviriyaphap et al. 1999). After the first DHF outbreak in Thailand in 1958, DDT was widely used to control *Aedes* mosquitoes. Control of *Aedes albopictus* (Skuse) is more difficult than *Aedes aegypti* (L.) because the habitat of the former encompasses a wider range. *Ae. albopictus* is most commonly found in suburban and rural areas where there are open spaces with considerable vegetation (Estrada-Franco and Craig 1995).

The first report of DDT resistance in *Ae. aegypti* in Thailand (from Bangkok and Nakhon Ratchasima) was published by Neely (1964). In 1966, larval *Ae. aegypti* collected from 14 areas in Bangkok showed resistance to DDT, but they were susceptible to organophosphate compounds of which temephos (Abate) and chlorpyrifos (Dursban) were the most effective larvicides (Bang et al. 1969). From 1986 to 1993, resistance of *Ae. aegypti* to temephos, malathion, and fenitrothion was reported from many regions of Thailand (Chareonviriyaphap et al. 1999). Currently, temephos is the most widely used product for control of *Ae. aegypti* larvae. During the peak period of adult *Aedes* populations, especially during the rainy seasons, fogging applications of cypermethrin [10% (wt:vol)] and deltacide [bioallethrin, 0.09% (wt:wt); deltamethrin, 0.06% (wt:wt); and piperonyl butoxide, 11.9%] are used. Use of household sprays, especially those containing organophosphates and pyrethroids, has increased recently in homes in Thailand.

The insecticide susceptibility of *Ae. albopictus* in Thailand is poorly documented. In 1967, temephos applications and malathion fogging on Samui Island in the Gulf of Thailand did not reduce the percentage of containers with *Ae. albopictus* larvae (Gould et al. 1970). In contrast, their study reported that the number of *Ae. aegypti* adults and the percentage of infested containers declined after insecticide applications. In 1997, a small number of adult *Ae. albopictus* reared from larvae collected from three locations in northern Thailand were assayed following the standard World Health Organization contact test against DDT, permethrin, and fenitrothion (Somboon et al. 2003). These results indicated that *Ae. albopictus* populations from these sites were resistant to DDT but susceptible to permethrin and fenitrothion.

Given the limited information on susceptibility of dengue vectors to insecticides in Thailand, our objective was to determine the current susceptibility of *Ae. aegypti* and *Ae. albopictus* larvae collected from geographically distant areas to three commonly used in-

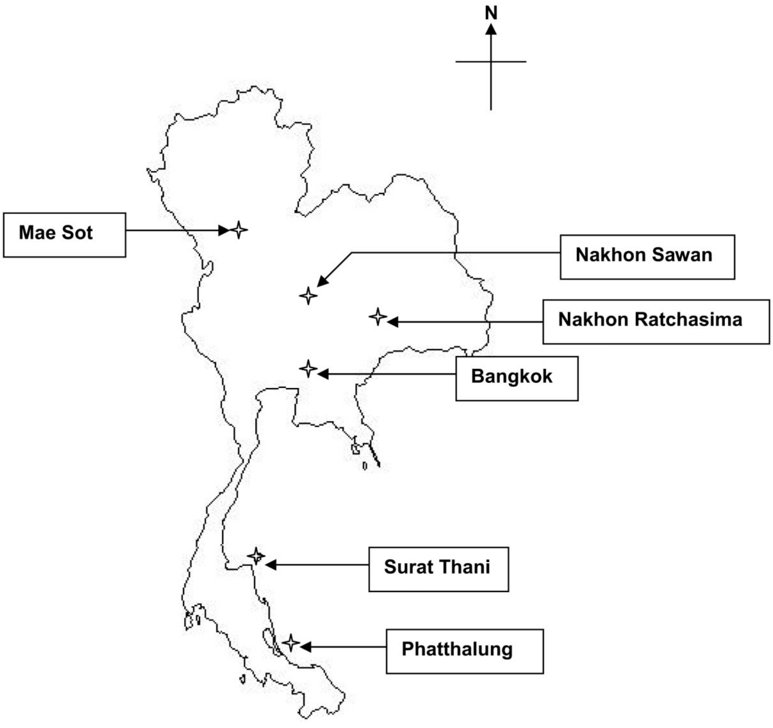

Fig. 1. Collecting sites in Thailand used in this study from June 2003 to April 2004 are indicated by an asterisk (*).

secticides. Documentation of insecticide resistance will identify insecticides that are no longer effective and is a critical first step toward developing a resistance management program (National Research Council 1986).

Materials and Methods

Field Sites. From July 2003 to April 2004, larvae were collected from the majority (≥80%) of positive containers at the following five field sites ranging from Mae Sot in northern Thailand (16° 45′ N, 98° 34′ E) to Phatthalung in southern Thailand (7° 30′ N, 100° 3′ E) (Fig. 1).

1. Ban Pai Lom and Ban Lao Bao in Mae Pa subdistrict, Mae Sot district (MS), Tak Province, ≈500 km northwest of Bangkok.

2. Village 3 of Sa Thale subdistrict (Tambon) is located in Phayuhakhiri District (Amphoe), Nakhon Sawan (NS) Province, which is in the central part of Thailand. NS is ≈240 km from Bangkok and is the gateway to northern regions.

3. Village 5 of Ban Nong Suang Phattana, Nong Suang subdistrict, located in Kham Thale So District, Nakhon Ratchasima (NR) Province. NR is located 260 km northeast of Bangkok.

4. Village 5 of Ban Thung Plub is in Samor Thong subdistrict, Tha Chana District, Surat Thani (ST) Province, 640 km south of Bangkok.

5. Village one of Ban Na Node is in Ban Na Node subdistrict, Muang District, Phatthalung (PT) Province. PT is located in southern part of Thailand ≈840 km from Bangkok.

Global positioning system (GPS) coordinates of these sites are presented in Table 1. The sites were chosen because each had a high rate of recent dengue cases reported by the Department of Epidemiology, Ministry of Public Health, Thailand 2003 (Mekong Basin Disease Surveillance Collaboration 2003).

Larval Collection. *Ae. aegypti* larvae (from MS, NS, NR, and ST) and larval *Ae. albopictus* (from MS, NS, ST, and PT) were collected from containers in each village by using turkey basters and placed in 50-ml Falcon tubes (Corning Glassworks, Corning, NY) and covered with a cotton plug. As adult mosquitoes emerged, they were aspirated from tubes, knocked down

Table 1. Description and GPS coordinates of field sites where collections were conducted from July 2003 to April 2004

Site	Village	Subdistrict	District	GPS coordinates	
Mae Sot	Pai Lom	Mae Pa	Mae Sot	16°45′N	98°33′E
	Lao Bao	Mae Pa	Mae Sot	16°45′N	98°34′E
Nakhon Sawan	1	Sa Thale	Phayuhakhiri	15°29′N	100° 8′E
Nakhon Ratchasima	5	Nong Suang	Kham Thale So	15°05′N	101°54′E
Surat Thani	5	Samor Thong	Tha Chana	9°34′N	99°07′E
Phatthalung	1	Ban Na Node	Muang	7°30′N	100°03′E

Table 2. Susceptibility of *Ae. aegypti* larvae to temephos

Strain	n	LC_{50}[a] (95% CI)	LC_{95}[a] (95% CI)	RR_{50}	RR_{95}	Slope (SE)
MS	930	24 (15–36)	100 (60–780)	4.4	10	2.0 (0.2)
NS	1,100	13 (8–18)	70 (40–240)	2.4	7.0	2.2 (0.16)
NR	790	1.4 (1.3–1.5)	3.3 (3.0–4.0)	0.3	0.3	4.3 (0.3)
ST	990	440 (300–540)	1,300 (990–2,000)	82	130	3.6 (0.4)
ROCK	900	5.4 (4.8–6.4)	10 (8–20)			5.7 (0.5)

Explanation for strain abbreviations is in text.
[a] Values are in parts per billion.

Table 4. Susceptibility of *Ae. aegypti* larvae to permethrin

Strain	n	LC_{50}[a] (95% CI)	LC_{95}[a] (95% CI)	RR_{50}	RR_{95}	Slope (SE)
MS	1,420	7.0 (5.8–8.3)	24 (18–38)	10	13	3.1 (0.2)
NS	1,350	19 (15–25)	57 (40–120)	27	32	3.5 (0.2)
NR	790	10 (8–16)	40 (20–160)	14	22	3.2 (0.3)
ST	930	30 (20–40)	130 (70–620)	43	72	2.4 (0.2)
ROCK	920	0.7 (0.5–0.9)	1.8 (1.2–9.1)			3.7 (0.3)

Explanation for strain abbreviations is in text.
[a] Values are parts per billion.

on wet ice, and identified to species by morphological characteristics according to Rattanarithikul and Panthusiri (1994). *Ae. aegypti* and *Ae. albopictus* collected at each site were placed in separate 30-cm^3 plastic cages (Megaview Science Education Service Co., Ltd., Taichung, Taiwan) by species and collection sites. Mosquitoes were provided cotton soaked with 20% sucrose. Both sexes were held together to ensure mating (≈20% males per cage). Adults and larvae were returned to the laboratory at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, where they were held at 27 ± 1°C and 75% RH.

Mosquito Colonies. Emerged *Aedes* mosquitoes were allowed to feed on a human forearm (A.P. Cornell University Human Subjects Protocol # 1-10-036) or on a chicken hung in a stocking inside the cage for 30 min two times per week (Cornell University Animal Use Protocol # 01-56). Oviposition containers filled halfway with clean drinking water and lined with paper towels were placed inside cages. Eggs on papers were removed and dried for 1 d and then stored in a Ziploc bag with wet paper towels to maintain high humidity. Eggs were hatched under a vacuum for 30 min. A pinch of fish food (Hi Pro, Specialists Fish food, Famedy Co., Ltd., Bangkok, Thailand) was added to the flask and held overnight until larvae were large enough to sort into trays of 200 per tray. All mosquitoes were reared to fourth instar F_1 for bioassays.

Bioassay. Ten fourth instars were put into a plastic cup (≈7 cm in diameter and 5 cm in depth) containing 99 ml of distilled water. Temephos (98%), malathion (99.2%), and permethrin (98%), were obtained from Chem Service (West Chester, PA). Insecticides were diluted in acetone and stored at 4°C. One milliliter of appropriate insecticide solution was dispensed with a pipette above the water surface in each cup. Each bioassay consisted of a minimum of six concentrations, at least two replicates per concentration, and two control replicates (acetone alone). Each bioassay was repeated 3–7 d for a minimum of six to 14 replicates per concentration. All containers were held in an incubator at 26°C. After 24 h, mortality was recorded. Larvae were considered dead if they could not be induced to move when probed with a needle.

Table 3. Susceptibility of *Ae. aegypti* larvae to malathion

Strain	n	LC_{50}[a] (95% CI)	LC_{95}[a] (95% CI)	RR_{50}	RR_{95}	Slope (SE)
MS	1,320	78 (62–95)	260 (190–430)	1.6	2.9	3.2 (0.2)
NS	610	150 (140–170)	590 (460–840)	3.0	6.6	2.8 (0.3)
NR	1,610	99 (90–110)	200 (160–290)	2.0	2.2	5.5 (0.4)
ST	1,030	170 (150–200)	370 (290–590)	3.4	4.0	4.8 (0.3)
ROCK	920	50 (43–58)	92 (74–140)			6.2 (0.4)

Explanation for strain abbreviations is in text.
[a] Values are in parts per billion.

Baseline Susceptibility. The baseline toxicity of *Ae. aegypti* and *Ae. albopictus* to temephos, malathion, and permethrin was determined using the standard susceptible Rockefeller (ROCK) strain of *Ae. aegypti* housed at Cornell University.

Analysis of Data. Data were analyzed using probit analysis (SPSS for Windows version 11.5, SPSS Inc., Chicago, IL) to determine the 50% lethal concentration values (LC_{50}) and 95% lethal concentration values (LC_{95}). Control mortality was corrected using Abbott's formula. Resistance ratios (RR_{50}) were calculated as the ratio of LC_{50} for field strains divided by the LC_{50} of the susceptible (ROCK) strain. Similarly, RR_{95} were calculated and reported.

Results

Insecticide Susceptibility in *Ae. aegypti*. Considerable variation in temephos resistance was noted at the four sites. High levels of resistance (>100-fold greater than ROCK strain) were detected from the Surat Thani populations (RR_{95} = 130), although the Nakhon Ratchasima strain was three-fold more susceptible to temephos than the ROCK strain. Low (<10-fold) to moderate (10–100-fold) levels of resistance were detected in Nakhon Sawan (RR_{95} = 7.0) and Mae Sot (RR_{95} = 10) (Table 2). *Ae. aegypti* from all four sites had low levels of resistance to malathion, with a RR_{95} values ranging from 2.2 to 6.6 in Nakhon Ratchasima and Nakhon Sawan, respectively (Table 3). Conversely, at all four sites, larvae showed moderate levels of permethrin resistance (Table 4). The highest level of permethrin resistance was at Surat Thani (RR_{95} = 72), followed by Nakhon Sawan (RR_{95} = 32), Nakhon Ratchasima (RR_{95} = 22), and Mae Sot (RR_{95} = 13), respectively.

Insecticide Susceptibility in *Ae. albopictus*. Low levels of temephos resistance were detected in *Ae. albopictus* larvae from Mae Sot (RR_{95} = 6.4), and Phatthalung (RR_{95} = 3.0) (Table 5). Larvae from Surat Thani were almost as susceptible as the ROCK strain (RR_{95} = 1.7). Two populations of *Ae. albopictus* in southern Thailand had very low levels of resistance to malathion compared with the standard susceptible

Table 5. Susceptibility of *Ae. albopictus* larvae to temephos

Strain	n	LC_{50}[a] (95% CI)	LC_{95}[a] (95% CI)	RR_{50}	RR_{95}	Slope (SE)
MS	560	26 (23–28)	64 (56–77)	4.8	6.4	4.1 (0.3)
ST	840	9.8 (7.7–14)	17 (13–70)	1.8	1.7	6.7 (0.5)
PT	550	15 (10–20)	30 (20–70)	2.8	3.0	6.4 (0.6)
ROCK	900	5.4 (4.8–6.4)	10 (8–20)			5.7 (0.5)

Explanation for strain abbreviations is in text.
[a] Values are in parts per billion.

strain, with RR_{95} values of 1.1–2.5 from Phatthalung and Surat Thani, respectively (Table 6). Permethrin resistance in *Ae. albopictus* larvae varied from low (Surat Thani, RR_{95} = 2.6; Nakhon Sawan, RR_{95} = 6.7) to moderate (Phatthalung, RR_{95} = 16.7; Mae Sot, RR_{95} = 42) across the four collection sites (Table 7).

Discussion

Based on these results, *Ae. aegypti* from all study sites are resistant to permethrin, a compound that is commonly used in Thai households for pest control. All larval populations had low levels of resistance to malathion, suggesting that this compound may still be effective in control programs during DEN outbreaks. All strains of *Ae. aegypti,* except those from Nakhon Ratchasima (RR_{95} = 0.3), were resistant to temephos, the principle larvicide used to control *Aedes* larvae in Thailand. This result was not surprising due to the widespread usage of temephos for larval control (Gratz 1993). Consequently, the use of permethrin and temephos for controlling this vector in Thailand should be used cautiously and rotation programs for resistance management should be implemented immediately.

In 1997, Somboon and colleagues found *Ae. albopictus* populations from Chiang Mai and Nan, Thailand were susceptible to permethrin (Somboon et al. 2003). This study is the first report of pyrethroid-resistant *Ae. albopictus* larvae in Thailand. Low levels of resistance to all insecticides were detected in *Ae. albopictus*, except from Mae Sot and Phatthalung, which were resistant to permethrin alone. Malaria is endemic in Mae Sot and other regions near the Thai-Myanmar border. Past efforts to control malaria vectors, with DDT and pyrethroids (Chareonviriyaphap et al. 1999), may have contributed to the development of resistance in *Ae. albopictus.*

Ae. aegypti and *Ae. albopictus* demonstrated resistance to temephos and permethrin in some regions.

Table 6. Susceptibility of *Ae. albopictus* larvae to malathion

Strain	n	LC_{50}[a] (95% CI)	LC_{95}[a] (95% CI)	RR_{50}	RR_{95}	Slope (SE)
ST	410	150 (130–180)	230 (190–560)	3.0	2.5	8.6 (1.0)
PT	420	60 (40–70)	100 (80–580)	1.2	1.1	6.5 (0.7)
ROCK	920	50 (40–60)	92 (74–140)			6.2 (0.4)

Explanation for strain abbreviations is in text.
[a] Values are in parts per billion.

Table 7. Susceptibility of *Ae. albopictus* larvae to permethrin

Strain	n	LC_{50}[a] (95% CI)	LC_{95}[a] (95% CI)	RR_{50}	RR_{95}	Slope (SE)
MS	890	23 (18–32)	76 (48–200)	33	42	3.3 (0.3)
NS	770	4.1 (3.8–4.5)	12 (9.8–15)	5.9	6.7	3.7 (0.4)
ST	810	2 (1.8–2.2)	4.7 (4.2–5.4)	2.9	2.6	4.5 (0.3)
PT	430	9 (6–14)	30 (17–110)	13	16.7	3.5(0.3)
ROCK	920	0.7 (0.5–0.9)	1.8 (1.2–9.1)			3.7(0.3)

Explanation for strain abbreviations is in text.
[a] Values are in parts per billion.

The habitats of these two species do overlap in many areas of Thailand, and they may be exposed to similar resistance selection pressure. This study found that in most cases *Ae. aegypti* were more resistant than *Ae. albopictus* strains to all three insecticides. One explanation for the higher resistance levels in *Ae. aegypti* is the fact that this species prefers to breed and rest indoors. Therefore, it is likely to be exposed to household insecticides and organized indoor spray treatments by public health workers more often than *Ae. albopictus*, which is more exophilic. In turn, *Ae. albopictus* is likely to be exposed more often to agricultural insecticides.

Although dengue vector control promulgated by the Ministry of Public Health is intended to be the same in all parts of Thailand, insecticide susceptibility patterns of *Ae. aegypti* and *Ae. albopictus* were not homogeneous across geographic regions of Thailand. Variation in health education, control efforts, and the frequency of use of insecticides for both public health and agricultural purposes across sites may be responsible for the variety of the susceptibility patterns of dengue vectors. Our study suggests that continuous resistance monitoring should be conducted on a regional scale in Thailand regularly to identify the efficacy of compounds for dengue control and to facilitate selection of compounds with the greatest promise for halting or minimizing dengue infections. Community awareness, cooperation with public health campaigns to reduce larval *Aedes* breeding sites, and well-managed rotation of the effective insecticides are recommended strategies for controlling dengue vectors.

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