*Journal of the American Mosquito Control Association*, 20(1):45–54, 2004


# EXCITO-REPELLENCY OF DELTAMETHRIN ON THE MALARIA VECTORS, *ANOPHELES MINIMUS, ANOPHELES DIRUS, ANOPHELES SWADIWONGPORNI,* AND *ANOPHELES MACULATUS,* IN THAILAND

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ABSTRACT. This study compared the behavioral avoidance responses of 4 mosquito malaria vectors, *Anopheles minimus, Anopheles dirus, Anopheles maculatus* form B, and *Anopheles swadiwongporni,* to deltamethrin, the primary insecticide used for indoor residual spraying for malaria vector control in Thailand. Six test populations, representing 4 laboratory colonies and 2 wild-caught populations, were observed during and after exposure to deltamethrin at the operational dose (0.02 g active ingredient/m$^2$) in excito-repellency escape chambers. The laboratory colonies included a deltamethrin-susceptible colony and a deltamethrin-resistant colony of *An. minimus* species A, 1 colony of *An. dirus* species B, and 1 colony of *An. maculatus* form B. The 2 wild-caught populations included *An. swadiwongporni* and members of the *An. dirus* complex. Times to escape by female mosquitoes during 30 min of exposure to deltamethrin-treated papers were observed in all populations and compared to nontreated paired controls in contact and noncontact test configurations. Strong behavioral avoidance was observed in the deltamethrin-resistant colony of *An. minimus,* followed by *An. swadiwongporni* and *An. maculatus.* The slowest escape response was observed in the colony of *An. dirus* species B. All 6 populations of *Anopheles* showed marked contact irritancy to deltamethrin compared to paired controls and noncontact repellency trials, in both controlled laboratory colonies and field-caught populations. The degree of repellency was less profound than irritancy but, in most cases, produced a significant escape response compared to paired controls. Avoidance behavior appears to be an innate behavior of mosquitoes, as indicated by the general avoidance response detected in all 4 species, regardless of deltamethrin susceptibility status, age, or nutritional and physiological status. Excito-repellency assays of the type described in this study should become an integral part of the overall assessment of an insecticide's ability to control disease transmission in any given area.

**KEY WORDS** Behavioral avoidance, irritancy, repellency, deltamethrin, *Anopheles minimus, Anopheles maculatus, Anopheles dirus, Anopheles swadiwongporni,* Thailand

## INTRODUCTION

In Thailand, malaria remains a major and reemerging health problem (Chareonviriyaphap et al. 2001). The primary vectors in Thailand include *Anopheles dirus* Peyton and Harrison, *Anopheles minimus* Theobald, *Anopheles maculatus* Theobald, and *Anopheles swadiwongporni* Rattanarithikul and Green, all members of the subgenus *Cellia.* Each species represents a member in broader species complexes, including *An. dirus, An. minimus,* and *An. maculatus* (which contains *An. swadiwongporni*), respectively (Rattanarithikul and Green 1986, Subbarao 1998). Many members within these species complexes exhibit both endophagous and exophagous behavioral patterns conducive for efficient malaria transmission (Pinichpongse and Bullner 1967, Suwonkerd et al. 1990, Chareonviriyaphap et al. 2000). *Anopheles dirus* and *An. minimus* are members representing individual species complexes, of which the respective sibling species often are not distinguishable morphologically from one another (Baimai 1989, Rattanarithikul and Panthusiri 1994). *Anopheles maculatus* and *An. swadiwongporni* are morphologically distinct members in the *An. maculatus* group (Rattanarithikul and Green 1986). One of the principal methods of malaria abatement in Thailand has been use of various methods of vector control to reduce transmission risk. For many years, DDT was the chemical of choice and was used extensively in malaria-endemic areas. Because of reported adverse impact on the environment and general negative public attitudes, DDT use was gradually phased out between 1995 and 2000 for the control of malaria vectors in Thailand (Chareonviriyaphap et al. 1999).

Deltamethrin, a common synthetic pyrethroid, is frequently and widely used for indoor residual spraying of house surfaces to control anopheline mosquitoes (Patipong 2000). This compound generally is applied in 1 or 2 spray rounds per year in malaria-endemic areas of Thailand (Ministry of Public Health 2000). The true mode of action of deltamethrin on the control of vectors and malaria is still open to investigation in terms of the relative importance of the lethal properties and behavioral responses of vector populations (Roberts et al. 2000). Because most pyrethroids demonstrate a significant and immediate excito-repellency action on exposed mosquitoes, the proposed wide-scale use of deltamethrin for malaria control in Thailand has stimulated the need for well-designed studies on the significance of pyrethroid avoidance behavior and its overall efficacy in reducing human–vector contact. Moreover, the respective roles of irritability and repellency of deltamethrin against the impor-

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tant malaria vectors in Thailand merit careful investigation before launching programs that use this compound exclusively.

Two different types of behavioral avoidance responses by mosquitoes are recognized: irritancy and repellency (Rutledge et al. 1999, Roberts et al. 2000). Irritability occurs when insects actually make physical contact with chemical residues before eliciting a stimulus-mediated response, whereas repellency is defined as a stimulus acting from a distinct distance from the insecticide-treated surface that deters insects from entering treated areas or otherwise disrupts normal patterns of behavior. Excito-repellency bioassays for describing and quantifying the irritant effects of insecticides on mosquitoes were developed beginning in 1963 and have been modified over the years (Rachou et al. 1963, Shalaby 1966, WHO 1970). Initial laboratory investigations on behavioral response of *Anopheles* to various insecticides were conducted by using the World Health Organization (WHO) excito-repellency test box design (Coluzzi 1963, Bondareva et al. 1986, Pell et al. 1989, Quinones and Suarez 1989, Ree and Loong 1989). Presently, no method for the assessment of mosquito behavioral responses has been universally endorsed as a standard for conducting excito-repellency testing, data analysis, and interpretation (Brown 1964, Roberts et al. 1984, Evans 1993, Rutledge et al. 1999, Roberts et al. 2000, Chareonviriyaphap et al. 2001). Recently, a controlled-design excito-repellency box was developed for testing both contact irritancy and noncontact repellency (Roberts et al. 1997, Chareonviriyaphap et al. 2001). This initial system has been modified further into a collapsible chamber designed for greater ease of use (Chareonviriyaphap et al. 2002). Described herein are the behavioral responses when using contact and noncontact assays and colonized *An. minimus* species A, *An. maculatus* form B, and *An. dirus* species B, and 2 field populations, 1 of *An. swadiwongporni* and the other of members of the *An. dirus* complex, against the standard field dosage of deltamethrin (0.02 g/m$^2$).

## MATERIALS AND METHODS

The irritability and repellency of deltamethrin were determined by observing the number of mosquitoes escaping from matched test and control chambers when using 4 species of *Anopheles* mosquitoes considered vectors of malaria in Thailand. Of the 6 different populations tested (Chareonviriyaphap, unpublished data), only 1 was considered to be resistant to residual deltamethrin based on the standard WHO contact bioassay (WHO 1975). All behavioral tests were conducted under near-identical laboratory-controlled conditions (temperature and humidity), between 0800 and 1630 h, at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

*Test populations:* *Anopheles dirus* species B (DISB) originally was collected from wild-animal footprints in Ban Paung District, Chantaburee Province, eastern Thailand, in 1987, and was maintained in insectary-controlled conditions at the Armed Forces Research Institute of Medical Science (AFRIMS), Bangkok, Thailand. The colony was obtained by the Malaria Division, Department of Communicable Disease Control (CDC), Ministry of Public Health, Nontaburi, Thailand, in 1995 and obtained in 1998 by the Department of Entomology, Kasetsart University, for the purposes of this study. This colony was found to be completely susceptible to deltamethrin at the field operational dosage of 0.02 g/m$^2$ when using the standard WHO contact bioassay and impregnated papers supplied by WHO. Susceptible *An. minimus* species A (MISA) originally was collected from animal quarters in Rong Klang District, Prae Province, northern Thailand, in 1993 and maintained in insectary-controlled conditions at the CDC, Nontaburi, beginning in 1995. The colony was received from the CDC in 1998 and raised in the Department of Entomology, Kasetsart University. This colony was determined to be completely susceptible to the field dosage of deltamethrin. The origins and colonization of resistant *An. minimus* species A (MIRA) have been described in a previous study (Sungvornyothrin et al. 2001). This colony exhibited between 50 and 60% resistance to deltamethrin at the operational dosage based on standard contact bioassay. *Anopheles maculatus* form B (MASB) was obtained from resting collections in animal quarters at Ban Khun Hauy, Mae Sot District, in 1999. The colony was initially maintained at the Department of Entomology, AFRIMS, and was provided to the Department of Entomology, Kasetsart University, in February 2002. The colony was found to be completely susceptible to deltamethrin. *Anopheles swadiwongporni* (SASA) was collected by evening resting collections from animal quarters in Ban Pu Teuy, Tri Yok Noi, Kanchanaburi Province, during January and February 2001. The wild-caught females were determined to be completely susceptible to deltamethrin. *Anopheles dirus* complex (DISC) was obtained from human-landing collections in the foothill area of Ban Pu Teuy during January and February 2001. A determination of the ratio of *An. dirus* species A, B, C, and D in the collection was not made. The field-caught females were determined to be susceptible to deltamethrin.

*Mosquito rearing:* Mosquito colonies were reared by following the method of Chareonviriyaphap et al. (1997), with only minor modifications. Each colony was maintained in separated rooms within a common insectary under controlled conditions (25 ± 5°C; 80 ± 10% relative humidity) at the Department of Entomology, Kasetsart University. Adult insects were provided cotton pads soaked with 10% sugar solution from the day of emergence and were maintained in 12 × 12 × 12-in. screened cages. Female mosquitoes were permitted to imbibe a blood meal from restrained laboratory hamsters on the 4th day after emergence. Depending on the mating requirements, some strains required forced copulation before oviposition. Approximately 2–3 days after bloodfeeding, oviposition dishes (moist filter paper in petri dishes) were placed in the cages with the gravid females. Larval stages were reared in enameled pans under identical physical and nutritional conditions throughout the study period.

*Insecticide-impregnated papers:* Only a single standard field dose of deltamethrin was used in this investigation, based on current malaria control policy in Thailand. The amount of active ingredient varied only slightly from the dosage (0.025 g/m$^2$) generally recommended by WHO (WHO 1992). Test papers (27.5 × 35.5 cm$^2$), impregnated with 0.02 g/m$^2$, were purchased from WHO, Vector Control Unit, Penang, Malaysia. All papers were treated at the rate of 2.75 ml of the insecticide solution per 180 cm$^2$ and used before their specified time of expiration.

*Behavioral tests:* Tests were conducted to compare the behavioral responses (irritancy and repellency) of *An. minimus* species A, *An. dirus* s.l. and species B, *An. maculatus* form B, and *An. swadiwongporni* to an operational dosage of deltamethrin applied to a paper surface. For all bioassays, slightly modified test chambers from those previously described were used in paired control and treatment trials (Chareonviriyaphap et al. 2002). Details of the chamber design and test methodology follow closely those of Sungvornyothrin et al. (2001) and Roberts et al. (1997). For colonized populations, only unfed, nulliparous female specimens were used in excito-repellency tests, whereas field-collected mosquitoes represented a mix of different physiological and nutritional states. All tests were performed during the day (0800–1630 h) based on availability of mosquitoes.

Each test series consisted of 2 insecticide test chambers and 2 paired control boxes. Mosquitoes were maintained in holding cups approximately 2–3 h before testing. For a complete test, 25 mosquitoes, 3–5 days old, were carefully introduced into each of 4 chambers by using a mouth aspirator, after which the outer rear door was closed and secured. A receiving cage (6 × 6 × 6-cm paper box) was connected to the exit portal for collecting any escaped mosquitoes. Mosquitoes were allowed a 3-min resting period to permit adjustment to test chamber conditions, after which the escape funnel was opened to begin the observation period. Mosquitoes escaping from the chamber into the receiving cage were recorded at 1-min intervals for a period of 30 min.

All trials were replicated 3 or more times for each particular test combination. Immediately after 30 min of exposure, the number of dead specimens remaining inside the chamber and those that had escaped to the receiving cage were recorded for treatment and control chambers. Additionally, all live specimens that had escaped or remained inside the chamber after 30 min were collected, provided sugar solution, and held in separate lots to record mortality during the 24-h postexposure period.

*Data analysis:* A survival analysis method described by Roberts et al. (1997) was used to analyze and interpret the behavioral response data (Chareonviriyaphap et al. 1997). The escape response data were subjected to Kaplan–Meier survival analysis as the preferred and most robust statistical treatment for excito-repellency data (Kleinbaum 1995). Unlike other methods of analysis that have attempted to quantitatively describe the behaviorial responses (irritability) to insecticide deposits, the generation of survival curves minimizes the loss of useful information and allows an estimation of mosquito escape probability over time of exposure. A log-rank method (Mantel and Haenzel 1959) was used to compare patterns of escape behavior within and between different treatment groups and biological conditions.

## RESULTS

Excito-repellency patterns of 4 important malaria vector species in Thailand exposed to field-rate deltamethrin (0.02 g/m$^2$) were performed in contact and noncontact exposure chambers. Overall percentage and rate of escape response was found to be higher in contact trials compared to noncontact and control trials in all test populations (Table 1). Contact rate of escape patterns from treated chambers allowing physical contact with residual deltamethrin were significantly higher than those from paired controls, although escape rates varied by test populations (Figs. 1 and 2). For example, a rapid escape response during the 30-min exposure was observed in populations MIRA (100%), MASB (99%), MISA (96%), and SASA (90%), whereas a more subdued response 70 and 80% escape, respectively, was observed in DISB and DISC test populations. Comparatively low numbers of female mosquitoes (≤25%) departed from the control chambers, with the exception of the DISB control where almost 60% escaped during the test time (Fig. 2). Unusually high escape patterns in control tests occur from time to time for reasons that are unclear. Repeated trials under the same or nearly identical conditions normally see these high rates of escape among controls as an unexplained anomaly.

In the noncontact trials, marked escape responses were observed in MISA (75%), DISB (72%), and DISC (58%) test populations, compared to the MASB (33%), SASA (49%), and MIRA (50%) populations after 30 min of exposure (Figs. 1 and 2). In some cases, a higher percentage of mosquitoes escaped from the control chambers, as observed in DISB (62%), MISA (58%), and SASA (25%) test populations when compared to MIRA

Table 1. Summary of escape response and mortality of female *Anopheles* species exposed to deltamethrin at 0.02 g/m² in contact and noncontact trials.

| Test[1] | No. observed | | | % mortality | |
|---|---|---|---|---|---|
| | Tested | Escaped | % escaped | Escaped[2] | Not escaped[3] |
| Contact | | | | | |
| DISB-C | 200 | 114 | 57 | 6.1 (7/114) | 0 |
| DISB | 200 | 140 | 70 | 1.4 (2/140) | 96.7 (58/60) |
| MISA-C | 100 | 21 | 21 | 0 | 0 |
| MISA | 100 | 98 | 98 | 3.1 (3/98) | 100 (2/2) |
| MIRA-C | 200 | 22 | 11 | 0 | 0 |
| MIRA | 200 | 200 | 100 | 2.0 (4/200) | 0 |
| MASB-C | 75 | 10 | 13 | 0 | 0 |
| MASB | 75 | 74 | 99 | 0 | 100 (1/1) |
| SASA-C | 200 | 50 | 25 | 0 | 0 |
| SASA | 200 | 180 | 90 | 0 | 100 (20/20) |
| DISC-C | 200 | 8 | 4 | 0 | 0 |
| DISC | 200 | 160 | 80 | 3.1 (5/160) | 100 (40/40) |
| Noncontact | | | | | |
| DISB-C | 200 | 124 | 62 | 1.6 (2/124) | 0.13 (1/76) |
| DISB | 200 | 144 | 72 | 0 | 0.053 (3/56) |
| MISA-C | 100 | 58 | 58 | 0 | 0 |
| MISA | 100 | 75 | 75 | 0 | 0 |
| MIRA-C | 200 | 28 | 14 | 0 | 0 |
| MIRA | 200 | 100 | 50 | 2.0 (2/100) | 0.04 (4/100) |
| MASB-C | 75 | 5 | 7 | 0 | 0 |
| MASB | 75 | 25 | 33 | 0 | 0 |
| SASA-C | 100 | 25 | 25 | 4.0 (1/25) | 0 |
| SASA | 100 | 49 | 49 | 2.0 (1/49) | 0 |
| DISC-C | 100 | 8 | 8 | 0 | 0 |
| DISC | 100 | 58 | 58 | 0 | 0 |

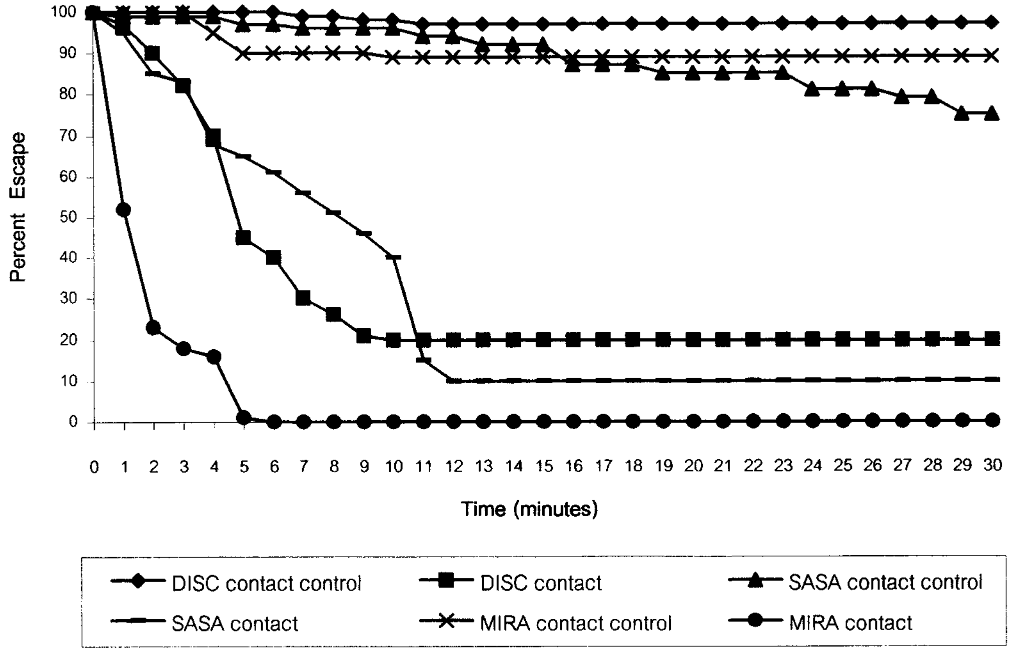
[1] DISB, *An. dirus* species B (laboratory population); MISA, *An. minimus* species A; MIRA, *An. minimus* species A; MASB, *An. maculatus* form B; SASA, *An. swadiwongporni*; DISC, *An. dirus* complex (field population); C, control test without insecticide.
[2] Dead/no. escaped given in parentheses.
[3] Dead/no. remaining inside chamber given in parentheses.

(14%), MASB (7%), and DISC (8%) populations. As noted in other studies, the repellent effect on mosquito behavior is far less evident compared to the irritant effect caused by direct physical contact with an insecticide in the pyrethroid class or DDT.

Times of escape from treated and control chambers, measured in 1-min intervals, were defined as escape time (ET), in terms of the time elapsed for 50% ($ET_{50}$) and 75% ($ET_{75}$) of the test population to depart the exposure chamber from the single exit aperture (Table 2). Because contact tests showed a greater and more rapid response than noncontact trials, escape patterns reflected this in time of escape. In contact trials, all 6 populations had $ET_{50}$ values of between 2 and 9 min, and an $ET_{75}$ of $\leq 13$ min (3–13 min) for 5 of the populations. As noted in the within and between population comparisons, DISB had the lowest percent escape and highest mortality in contact trials compared to the other populations. In the noncontact trial, the $ET_{50}$s for DISB, MISA, MIRA, and DISC are 7, 18, 5, and 12, respectively (Table 2). The $ET_{75}$ values for DISB could not be calculated. In noncontact trials, some $ET_{50}$ and $ET_{75}$ values for test populations could not be calculated for a 30-min exposure period because a few specimens escaped from the exposure chamber.

Female mosquito mortality from different test populations after a 24-h postexposure holding period in all contact and noncontact treatment and control trials are provided in Table 1. In general, low percent mortality was observed in females from all test populations managing to escape in both contact (0–6.1%) and noncontact trials (0–4%). All females that remained inside the chambers after 30 min of exposure in contact trials had died within 24 h, whereas most noncontact test specimens survived with a low percent mortality (0.04–0.13%) after the 24-h holding period.

Within-population comparisons of escape responses between contact trials and paired controls, contact and noncontact trials, and noncontact and paired controls for the 6 test populations are shown in Table 3. Significant differences were observed in all combination comparisons except in DISB. This population showed no difference when comparing irritancy and repellency responses ($P = 0.962$) or any differences between noncontact and paired control designs ($P = 0.07$), indicating that this long-



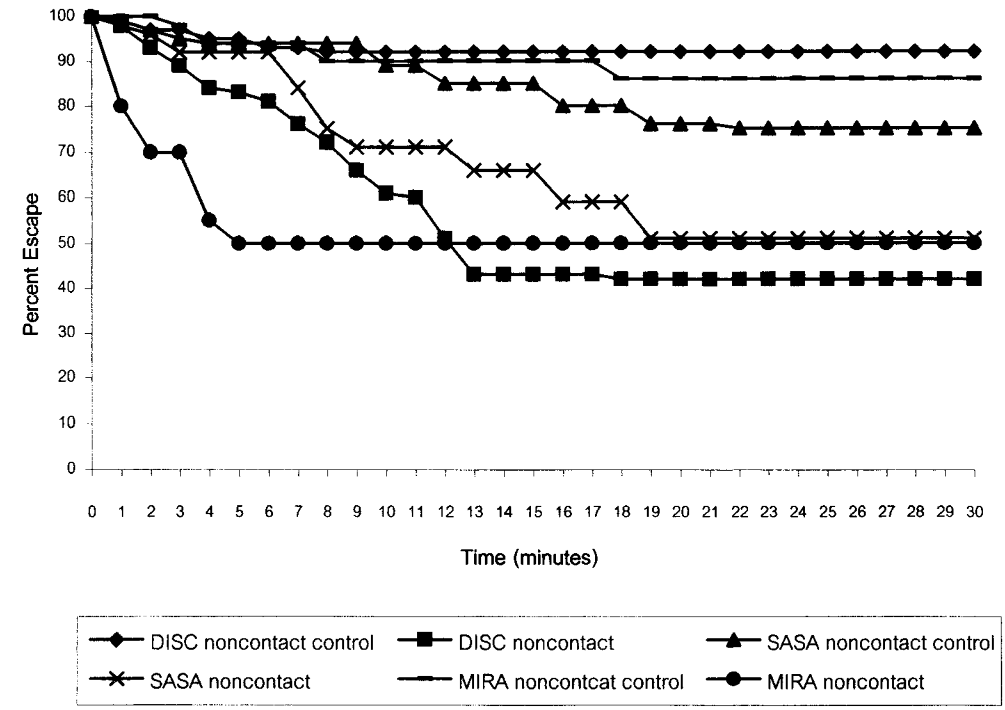




Fig. 1. Escape probability of *Anopheles dirus* complex (DISC), *An. swadiwongporni* (SASA), and resistant *An. minimus* species A (MIRA) mosquito populations in contact and noncontact trials and respective paired controls when using deltamethrin at 0.02 g/m².

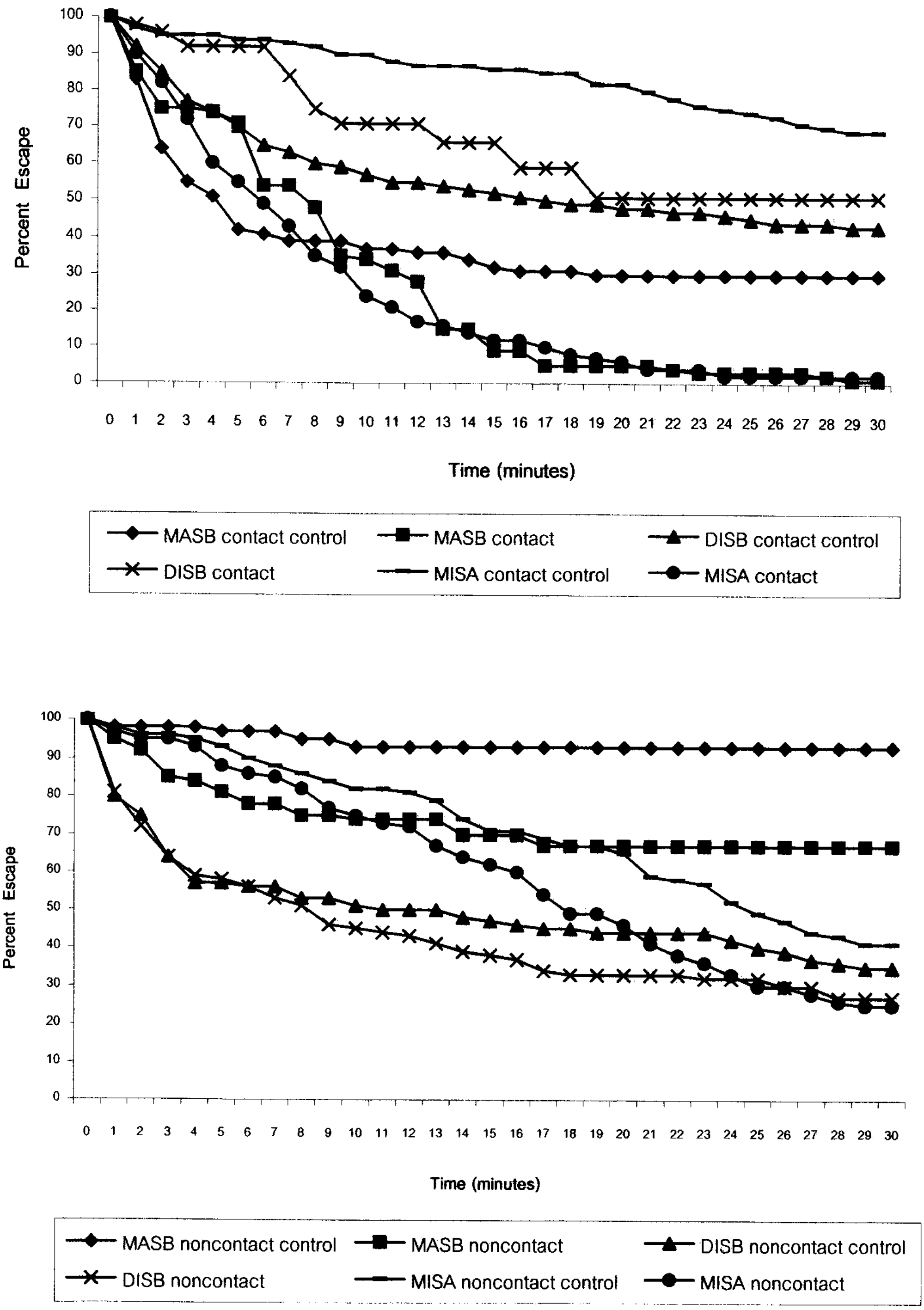



Fig. 2. Escape probability of *Anopheles dirus* species B (DISB), susceptible *Anopheles minimus* species A (MISA), and *Anopheles maculatus* form B (MASB) mosquito populations in contact and noncontact trials and respective paired controls when using deltamethrin at 0.02 g/m$^2$.

Table 2. Escape time in minutes for 50% ($ET_{50}$) and 75% ($ET_{75}$) of female *Anopheles* species to escape from exposure chambers treated with deltamethrin.[1]

| | DISB | | MISA | | MIRA | | SASA | | MASB | | DISC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test | $ET_{50}$ | $ET_{75}$ | $ET_{50}$ | $ET_{75}$ | $ET_{50}$ | $ET_{75}$ | $ET_{50}$ | $ET_{75}$ | $ET_{50}$ | $ET_{75}$ | $ET_{50}$ | $ET_{75}$ |
| Contact | 5 | — | 6 | 10 | 2 | 3 | 9 | 11 | 9 | 13 | 5 | 9 |
| Noncontact | 7 | — | 18 | — | 5 | — | — | — | — | — | 12 | — |

[1] DISB, *An. dirus* species B (laboratory population); MISA, *An. minimus* species A; MIRA, *An. minimus* species A; MASB, *An. maculatus* form B; SASA, *An. swadiwongporni*; DISC, *An. dirus* complex (field population). A dash indicates that too few specimens escaped from exposure chambers to allow calculation of an $ET_{50}$ or $ET_{75}$.

standing laboratory colony showed no marked repellent response to deltamethrin.

Multiple comparisons of escape patterns (rate of escape) between the 6 test populations of female *Anopheles* in contact and noncontact trials were analyzed with the log-rank method at the 0.05 level of probability (Table 4). In contact trials, significant differences were found in all cases, except for DISB vs. SASA, DISB vs. DISC, and MISA vs. DISC population comparisons. For noncontact trials, only 2 paired population comparisons failed to show a significant difference (MIRA vs. MASB and MASB vs. SASA). In this study, only *An. minimus* (MIRA) was found to be physiologically resistant to deltamethrin and significant differences were found in escape responses between MIRA and the 5 deltamethrin-susceptible populations in contact tests.

## DISCUSSION

The mathematical framework for understanding the repellent, irritant, and toxic properties of insecticides on mosquitoes and how they function in control of malaria has been proposed by Roberts et al. (2000). This work, along with other related studies, has clearly suggested that the excito-repellent and toxicological actions must be accurately assessed by using different vectors and chemical insecticides throughout malaria-endemic areas (Chareonviriyaphap et al. 2001, Sungvornyothin et al. 2001). This study observed the behavioral responses of 4 important malaria vectors from Thailand to the standard operational field dose of residual deltamethrin, the currently approved indoor residual insecticide for malaria control in Thailand. These results contribute to the ongoing work to optimize and standardize an excito-repellency test system that is deemed an essential component for assessing public health insecticides and their mode of action in disease vector and transmission control. We also compared the behavioral responses between a deltamethrin-susceptible and deltamethrin-resistant laboratory population of *An. minimus* species A.

Significant avoidance responses were observed in contact trials, compared to noncontact and control trials, and significant differences in escape responses were documented between noncontact trials and contemporaneous paired controls. The most dramatic behavioral avoidance response after phys-

Table 3. Within-population comparison of escape response between paired control and contact trials, contact and noncontact trials, and paired noncontact and control trials for 6 test populations of female *Anopheles* against deltamethrin at the field rate of 0.02 g/m$^2$.[1]

| No replicates contact/noncontact | Test population | Control vs. contact (*P*) | Contact vs. noncontact (*P*) | Noncontact vs. control (*P*) |
|---|---|---|---|---|
| 8/8 | DISB | 0.0120 | NS | NS |
| 4/4 | MISA | 0.0001 | 0.0001 | 0.0080 |
| 8/8 | MIRA | 0.0001 | 0.0001 | 0.0001 |
| 3/3 | MASB | 0.0001 | 0.0001 | 0.0001 |
| 8/4 | SASA | 0.0001 | 0.0001 | 0.0010 |
| 8/4 | DISC | 0.0001 | 0.0001 | 0.0001 |

[1] DISB, *An. dirus* species B (laboratory population); MISA, *An. minimus* species A; MIRA, *An. minimus* species A; MASB, *An. maculatus* form B; SASA, *An. swadiwongporni*; DISC, *An. dirus* complex (field population); $P < 0.05$ indicates log-rank tests with significant differences in avoidance behavior patterns; NS = $P > 0.05$.

Table 4. Comparison of escape patterns between test populations of female *Anopheles* in contact and noncontact trials with deltamethrin.[1]

| Test population comparisons | Contact trial (*P*) | Noncontact trial (*P*) |
|---|---|---|
| DISB vs. MISA | 0.0001 | 0.0001 |
| DISB vs. MIRA | 0.0001 | 0.0004 |
| DISB vs. MASB | 0.0001 | 0.0001 |
| DISB vs. SASA | NS | 0.0001 |
| DISB vs. DISC | NS | 0.0009 |
| MISA vs. MIRA | 0.0001 | 0.0001 |
| MISA vs. MASB | 0.0001 | 0.0121 |
| MISA vs. SASA | 0.0469 | 0.0032 |
| MISA vs. DISC | NS | 0.0001 |
| MIRA vs. MASB | 0.0020 | NS |
| MIRA vs. SASA | 0.0001 | 0.0287 |
| MIRA vs. DISC | 0.0001 | 0.0001 |
| MASB vs. SASA | 0.0001 | NS |
| MASB vs. DISC | 0.0001 | 0.0001 |

[1] DISB, *An. dirus* species B (laboratory population); MISA, *An. minimus* species A; MIRA, *An. minimus* species A; MASB, *An. maculatus* form B; SASA, *An. swadiwongporni*; DISC, *An. dirus* complex (field population); $P < 0.05$ indicates log-rank tests with significant differences in avoidance behavior patterns; NS = $P > 0.05$.

ical contact with deltamethrin was observed in MIRA, followed by MASB, and SASA test populations. A colonized population DISB demonstrated the weakest responses to deltamethrin. Noncontact repellency was detected at significant levels compared to paired controls, except in the DISB colony. Strong repellency was observed in MIRA and DISC, with more than 50% of the test population escaping from the test chambers within 30 min. Repellency was less pronounced in MASB, SASA, and MISA but remained significant compared to the controls. These observations on repellency action are in agreement with the results from previous studies (Chareonviriyaphap et al. 1997, 2000; Sungvornyothin et al. 2001), which reported an intermediate avoidance response compared to irritancy, yet significant overall repellency effects of deltamethrin to *Anopheles albimanus* Wiedemann from Central America and *An. minimus* from Thailand, respectively. Mortality was low in mosquitoes escaping the treated chambers in contact and noncontact trials, an indication that behavioral avoidance greatly reduces the opportunity for residual insecticides to impact survival through toxicity.

Of the mosquitoes under study, DISB produced higher numbers of escaped mosquitoes from the control chambers compared to the other 5 test populations. The reason for this is unclear. Because this colony has been maintained in the laboratory for more than 16 years, it may have lost some ability to respond normally to insecticides. A similar phenomenon was observed in the 20-year-old colony of *An. albimanus* from the Walter Reed Army Institute of Research (WRAIR). The WRAIR colony showed virtually no response to all chemicals tested, and all mosquitoes that remained in the treated test chamber did not survive past the 24-h postexposure holding period (Chareonviriyaphap et al. 1997). The poorer escape response compared to the other populations appears to be colony-related, because DISB has been continuously maintained under artificial conditions in the laboratory for nearly 2 decades before this study. Long-term colonization possibly has unintended effects on normal behavioral patterns in mosquitoes, although this would not appear to be the case with *An. dirus* when comparing contact escape responses between colony and field populations ($P = 0.56$).

Irritancy, a result of physical contact with insecticide-treated surfaces, by mosquitoes were recognized even before the early stages of broad-scale use of insecticides to control vector mosquitoes (Kennedy 1946). Subsequent observations indicated that some insecticides also could induce a repellent effect, without actual physical contact with a treated surface. Repellency effects to insecticides used in malaria control have been reported in several anopheline species (Roberts and Alecrim 1991, Roberts et al. 1997, Chareonviriyaphap et al. 1997). One of the 1st species of mosquitoes to demonstrate a repellent effect, *Anopheles culicifacies* Giles from India, provided further evidence of noncontact repellency in mosquito vectors, a phenomenon that has been long been ignored or discounted as important in malaria control. One of the reasons for the poor understanding of avoidance behavior in mosquitoes was the lack of an adequate test system to measure both irritancy and noncontact repellency, which eventually was satisfied by Roberts et al. (1997) with the development of a true excito-repellency test system. When properly configured, this test system allows observations that distinguish irritancy and repellency, and was 1st used to measure behavioral responses of *An. albimanus* to DDT and some pyrethroids under laboratory and natural field conditions (Chareonviriyaphap et al. 1997). Subsequently, improved excito-repellency escape chambers have been developed that provide information on both irritant and repellent responses (Chareonviriyaphap and Aum-Aung 2000; Chareonviriyaphap et al. 2002). Improved test systems have been used to quantify the insecticide-induced behavioral responses of wild-caught *An. minimus* in Thailand (Chareonviriyaphap et al. 2001).

Our findings on behavioral responses of malaria vectors to insecticides are similar to those of previous studies (Ree and Loong 1989; Evans 1993; Chareonviriyaphap et al. 1997, 2001; Bangs 1999). The behavioral responses to deltamethrin by female mosquitoes from different test populations varied depending on innate characteristics of each test population. Although the nutritional and physiological status of laboratory mosquitoes was carefully controlled, field-caught mosquitoes were naturally heterogeneous in age and nutritional status. Because avoidance behavior is significantly influenced by the nutritional and physiological condition of the mosquito, the interpretation of avoidance responses to insecticides derived from field populations should be interpreted with caution (Sungvornyothin et al. 2001).

Pyrethroid-class insecticides have long been known to elicit excito-repellent responses in insects (Threlkeld 1985). The combined effects of irritancy and repellency produced in the presence of an insecticide can have a dramatic impact on the effectiveness of chemical control of mosquito vectors, thus profoundly impacting the local transmission of disease. Behavioral avoidance of treated surfaces, especially irritancy, generally prevents sufficient contact with a residual insecticide, thus greatly reducing the risk of premature mortality in bloodseeking anophelines. However, a reduction in the toxic effects of a chemical may not necessarily equate to an increase in risk of human–vector contact inside houses (Roberts et al. 2000). We believe a convincing argument exists that the consequence of the combined effect of repellency and irritancy in reducing house-entering mosquito densities and interrupting patterns of bloodfeeding behavior exerts a profound influence on transmission, likely overriding the influence of contact toxicity. The implications of these and other findings that describe the dramatic display of mosquito avoidance of insecticide-treated surfaces (wall surfaces and impregnated bed-nets) warrant continued study. We believe excito-repellency assays of the type described in this study should become an integral part of the overall assessment of an insecticide's ability to control disease transmission.

In conclusion, deltamethrin exerted remarkable excito-repellency in 4 species of *Anopheles,* all of which are regarded as important vectors of malaria in Thailand. All 6 populations showed vigorous contact irritancy to the operational dosage of deltamethrin compared to paired controls and noncontact repellency trials, in both controlled laboratory colonies and field-caught populations, regardless of nutritional and physiological status of the test populations. However, the degree of repellency was less profound than that of irritancy,and in most cases produced a significant avoidance response compared to paired controls. The differences in escape responses between the long-standing colony of *An. dirus* and the other species tested appear to be a consequence of prolonged colonization and isolation from varying natural stimuli. Additional efforts are currently underway to promote development of standardized excito-repellency response tests.

## ACKNOWLEDGMENTS

We thank the staff of the Malaria Division, CDC, Ministry of Public Health, for providing the colonies of *An. minimus* and *An. dirus.* Special thanks are extended to the Department of Entomology, AFRIMS, for kindly providing *An. maculatus* form B. We are grateful to all anonymous reviewers for comments and suggestions on this manuscript. This project was funded by the Thailand Research Fund, Kasetsart University Research and Development Institute, and Center of Agricultural Biotechnology, Kasetsart University, Bangkok, Thailand.

## REFERENCES CITED

Baimai V. 1989. Speciation and species complexes of the *Anopheles* malaria vector in Thailand. In: Proceedings of The Third Conference on Malaria Research, Thailand, 1989 October 18–20; Chiang Mai, Thailand. Bangkok, Thailand: Malarial Division. p 146–162.

Bangs MJ. 1999. The susceptibility and behavioral response of *Anopheles albimanus* Weidemann and *Anopheles vestitipennis* Dyar and Knab (Diptera: Culicidae) to insecticides in northern Belize, Central America. Ph.D. dissertation. Uniformed Services University of the Health Sciences, Bethesda, MD.

Bondareva NL, Artem'ev MM, Gracheva GV. 1986. Susceptibility and irritability caused by insecticides to malaria mosquitoes in the USSR. Part 1. *Anopheles pulcherrimus. Med Parazitol Parazit Bolezni* 6:52–55.

Brown AWA. 1964. Experimental observations governing the choice of test method for determining the DDT-irritability of adult mosquitoes. *Bull WHO* 30:97–111.

Chareonviriyaphap T, Aum-Aung B. 2000. Improved excito-repellency escape chamber for behavioral tests on mosquitoes. *Mekong Malaria Forum* 5:82–86.

Chareonviriyaphap T, Aum-Aung B, Ratanatham S. 1999. Current insecticide resistance patterns in mosquito vectors in Thailand. *Southeast Asian J Trop Med Public Health* 30:184–194.

Chareonviriyaphap T, Bangs MJ, Ratanatham S. 2000. Status of malaria in Thailand. *Southeast Asian J Trop Med Public Health* 31:225–237.

Chareonviriyaphap T, Prabaripai A, Sungvornyothin S. 2002. An improved excito repellency for mosquito behavioral test. *J Vector Ecol* 27:250–252.

Chareonviriyaphap T, Roberts DR, Andre RG, Harlan H, Bangs MJ. 1997. Pesticide avoidance behavior in *Anopheles albimanus* Wiedemann, a malaria vector in the Americas. *J Am Mosq Control Assoc* 13:171–183.

Chareonviriyaphap T, Sungvornyothin S, Ratanatham S, Prabaripai A. 2001. Pesticide-induced behavioral responses of *Anopheles minimus,* a malaria vector in Thailand. *J Am Mosq Control Assoc* 17:13–22.

Coluzzi M. 1963. Studies on irritability of DDT to anopheline mosquitoes. *WHO Vector Control* 33:1–22.

Evans RG. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrin, and DDT to *Anopheles gambiae. J Am Mosq Control Assoc* 9:285–293.

Kennedy JS. 1946. The excitant and repellent effects on mosquitos of sublethal contacts with DDT. *Bull Entomol Res* 37:593–607.

Kleinbaum DG. 1995. *Survival analysis* New York: Springer-Verlag.

Mantel N, Haenzel W. 1959. Statistic aspects of the analysis of data from retrospective studies of diseases. *J Natl Cancer Inst* 22:719–748.

Ministry of Public Health. 2000. *Annual malaria report 2000* Available from Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Nontaburee, Thailand. 186 p.

Patipong S. 2000. Protecting against mosquito bites. *Mekong Malaria Forum* 5:86–88.

Pell JK, Spinney MA, Ward KJ. 1989. Observations on the behavior of adult *Anopheles gambiae* encountering residual deposits of lambda-cyhalothrin compared with the other major classes. In: Fourth Annual Confererence of the Society for Vector Ecology European Region. p 1–18.

Pinichpongse S, Bullner GR. 1967. The current status of malaria entomology in Thailand. *Warasan Malar* 11: 43.

Quinones ML, Suarez MF. 1989. Irritability to DDT of natural populations of the primary malaria vectors in Colombia. *J Am Mosq Control Assoc* 5:56–59.

Rachou RG, Moura LM, Duret JP, Kerr JR. 1963. Experiences with the excito-repellency test box—model OPS. In: Proceedings of the 50th Annual Meeting of the New Jersey Mosquito Exterminators Association and the 19th Annual Meeting of the American Mosquito Control Association. p 442–447.

Rattanarithikul R, Green CA. 1986. Formal recognition of the species of the *Anopheles maculatus* group (Diptera: Culicidae) occurring in Thailand, including the description of two new species and a preliminary key to females. *Mosq Syst* 18:246–278.

Rattanarithikul R, Panthusiri P. 1994. Illustrated keys to the medically important mosquitoes of Thailand. *Southeast Asian J Trop Med Public Health* 25:60–183.

Ree HI, Loong KP. 1989. Irritability of *Anopheles farauti,* *Anopheles maculatus*, and *Culex quinquefasciatus* to permethrin. *Jpn J Sanit Zool* 40:47–51.

Roberts DR, Alecrim WD. 1991. Behavioral response of *Anopheles darlingi* to DDT sprayed house walls in Amazonia. *PAHO Bull* 25:210–217.

Roberts DR, Alecrim WD, Tavares AM, McNeill KM. 1984. Influence of physiological condition on the behavioral response of *Anopheles darlingi* to DDT. *Mosq News* 4:357–361.

Roberts DR, Chareonviriyaphap T, Harlan HH, Hshieh P. 1997. Methods for testing and analyzing excito-repellency responses of malaria vectors to insecticides. *J Am Mosq Control Assoc* 13:13–17.

Roberts DR, Alecrim WD, Hshieh P, Grieco J, Bangs M, Andre RG, Chareonviriyaphap T. 2000. A probability model of vector behavior: effects of DDT repellency, irritability, and toxicity in malaria control. *J Vector Ecol* 25:48–61.

Rutledge LC, Echana NM, Gupta RK. 1999. Responses of male and female mosquitoes to repellents in the World Health Organization insecticide irritability test system. *J Am Mosq Control Assoc* 15:60–64.

Shalaby AM. 1966. Observations on some responses of *Anopheles culicifacies* to DDT in experimental huts in Gujarat State, India. *Ann Entomol Soc Am* 59:938–944.

Subbarao SK. 1998. *Anopheline species complexes in South-East Asia* Technical Publication, Southeast Asia Regional Office 18. p 82. Available from the World Health Organization, New Delhi, India.

Sungvornyothin S, Chareonviriyaphap T, Prabaripai A, Trirakhupt T, Ratanatham S, Bangs MJ. 2001. Effects of nutritional and physiological status on behavioral avoidance of *Anopheles minimus* (Diptera: Culicidae) to DDT, deltamethrin and lambdacyhalothrin. *J Vector Ecol* 26:202–215.

Suwonkerd W, Aum-Aong B, Rimwangtrakul K, Wongkattiyakul S, Kattiyamongkool B, Chiprarop U, Takagi M. 1990. A field study on response of *Anopheles dirus* to DDT and fenitrothion sprayed to huts in Phetchabun Province, Thailand. *Trop Med (Nagasaki)* 32:1–5.

Threlkeld SFH. 1985. Behavioral responses in *Drosophilia melanogaster* associated with permethrin and ectiban. In: Proceedings of the 32nd Annual Meeting, Canadian Pest Management Society. 1985 June 24–26; Charottetown, Prince Edqard Island, Canada.

WHO [World Health Organization]. 1970. Insecticide resistance and vector control (17th report of the WHO Expert Committee on Insecticides). Instructions for determining the irritability of adult mosquitoes to insecticide. *WHO Tech Rep Ser* 433:158–163.

WHO [World Health Organization]. 1975. Manual on practical entomology in malaria. Part II. Methods and techniques. *WHO Offset Publ* 13:1–191.

WHO [World Health Organization]. 1992. Vector resistance to pesticides. *WHO Tech Rep Ser* 818. p. 2–50.