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INSECTICIDE-INDUCED BEHAVIORAL RESPONSES OF *ANOPHELES MINIMUS,* A MALARIA VECTOR IN THAILAND

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ABSTRACT. This study was designed to determine the behavioral responses of 2 test populations of *Anopheles minimus* females to DDT at 2 g/m^2, deltamethrin at 0.0625 g/m^2, and lambdacyhalothrin at 0.0369 g/m^2 using an improved excito-repellency escape chamber. One test population was colonized in 1993 and referred to as a young colony. The 2nd field test population was collected from Ta-Soa County, Tri-Yok District, Kanchanaburi Province, in western Thailand and referred to as a wild population. Results showed that females of both young and wild test populations rapidly escaped from direct contact with DDT, deltamethrin, and lambdacyhalothrin. Lambdacyhalothrin exhibited the strongest irritant effect on female mosquitoes, followed by DDT and deltamethrin. Fewer females escaped from test chambers without direct contact with treated surfaces but the response was significantly different from that of the controls ($P < 0.05$). The noncontact response is indicative of a noncontact repellent action. Both contact irritancy and noncontact repellency are involved in *An. minimus* escape responses. Experimental hut studies that include monitoring of house-entering populations of *An. minimus* are needed for a meaningful assessment of noncontact repellent actions.

KEY WORDS Avoidance behavior, excito-repellency, malaria, vector

INTRODUCTION

Anopheles minimus Theobald is one of the most efficient malaria vectors in Southeast and East Asia (Reid 1968). In Thailand, *An. minimus* is considered to be a primary vector of malaria (Ayurakit-Kosol and Griffith 1956, Sucharit et al. 1988). One of the principal methods of malaria abatement has been through various methods of vector control to reduce transmission risk. Among these, extensive intradomicillary use of DDT has been conducted for chemical control once or twice a year (Prasittisuk 1995, Chareonviriyaphap et al. 1999). In spite of a long-term use of DDT, no record of physiologic resistance in *An. minimus* to DDT has been reported in Thailand. Well-documented behavioral responses of vectors to DDT raised the issue of avoidance behavior having a role in malaria prevention and in the suppression of insecticide resistance in malaria vectors (Roberts and Andre 1994, Roberts et al. 2000). Avoidance behavior is defined as the ability of insects to avoid insecticide-treated surfaces. Two forms of behavioral responses have been reported, as described in Chareonviriyaphap et al. (1997). The term avoidance behavior can also be used to describe the response that is stimulated by the combination of both irritancy and repellency (Chareonviriyaphap et al. 1999). No information on insecticide avoidance behavior of *An. minimus,* especially by noncontact repellency, has been previously reported.

In addition to DDT, insects also demonstrate behavioral responses to synthetic pyrethroids (Threlkeld 1985, Roberts and Andre 1994, Chareonviriyaphap et al. 1997). Several pyrethroids have been extensively introduced for malaria control in Thailand for impregnated bed-net and intradomicillary spraying, especially deltamethrin (Chareonviriyaphap et al. 1999). The continuing use of pyrethroids should be a major stimulus for extensive studies on the significance of pyrethroid avoidance behavior of *Anopheles* malaria vectors in Thailand. Moreover, avoidance behavior to insecticides by *An. minimus* is given little, if any, consideration. This is unfortunate because the role of irritant and repellent actions of pyrethroids should be clearly defined for malaria vectors before large-scale control programs are started and limited malaria control resources are expended.

Several test systems have been used in behavioral tests of insecticides against malaria vectors using the modified World Health Organization (WHO) excito-repellency test box (Bondareva et al. 1986, Quinones and Suarez 1989, Ree and Loong 1989), but no test system has been fully accepted (Roberts et al. 1984, Evans 1993). In recent years, Roberts et al. (1997) proposed a test system to discriminate between contact irritancy and noncontact repellency. The test system was standardized and used by Chareonviriyaphap et al. (1997) and subsequently by Bangs et al. (unpublished data). Unfortunately, this test system was cumbersome, and required much time for attaching test papers. Chareonviriyaphap and Aum-Aong (2000) developed an improved collapsible, metal excito-repellency test chamber for behavioral tests on mosquitoes (Fig. 1). As described in this report, the improved test system was used to study the behavioral responses of a young colony and a wild population of *An. minimus* against 3 different insecticides, with and without physical contact with insecticides.

MATERIALS AND METHODS

Anopheles minimus test populations

Young colony: This colony was maintained in the laboratory for 7 years. It was originally col-

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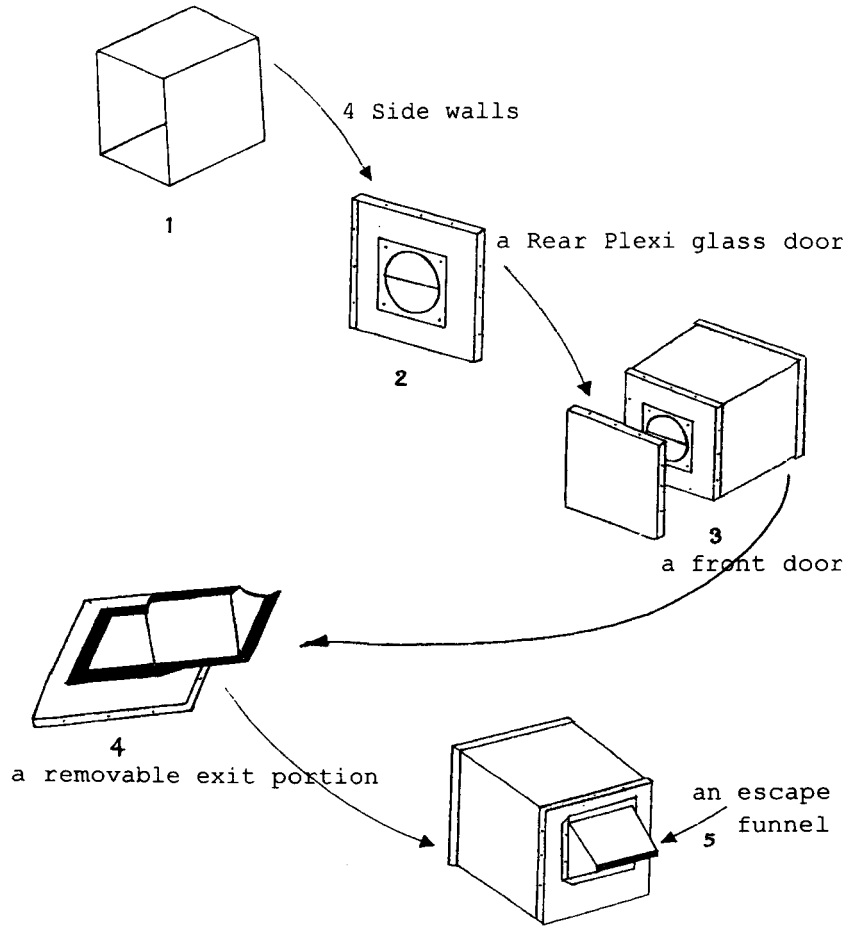

Fig. 1. An improved excito-repellency test chamber used to study the pesticide avoidance behavior of *Anopheles minimus*.

lected from animal quarters in Rong Klang District, Prae Province, northern Thailand, in 1993. The colony was maintained in laboratory-controlled conditions at the Malaria Division, Department of Communicable Disease Control (CDC), Ministry of Public Health, Nontaburi, Thailand, since 1995. The colony was received from the CDC and raised in the insectary at the Division of Biology, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaengsean Campus, Nakhon Pathom, Thailand, during these tests. This colony was physiologically susceptible to DDT, deltamethrin, and lambdacyhalothrin (Chareonviriyaphap et al., unpublished data).

Wild population: This population was obtained from human landing collections near a slow-running stream of Moo II, Ban Putuey, Ta-Soa County, Tri-Yok District, Kanchanaburi Province, western Thailand in March–December 1999. This population was physiologically susceptible to DDT, deltamethrin, and lambda-cyhalothirn (Chareonviriyaphap et al., unpublished data).

Mosquito rearing

Colony: The *An. minimus* colony was reared following the method of Chareonviriyaphap et al. (1997), with only minor modification. All life stages were maintained at 25 ± 5°C and 80 ± 10% relative humidity in the insectary at the Faculty of Liberal Arts and Science, Kasetsart University, Kamphaengsean Campus. Adults were provided with cotton pads soaked with 10% sugar solution from the day of emergence and adults were maintained in a 12 × 12 × 12-in. screened cage. Female mosquitoes were permitted to have a human blood meal on the 4th day after emergence. Two days after bloodfeeding, oviposition dishes were placed in the cage with the gravid females.

Wild population: Female *An. minimus* were collected as adults by human baits during the night (1800–2400 h). Behavioral tests were performed during the next day. Temperature and relative humidity were recorded during the tests. All mosquitoes were starved approximately 4 h before tests (Chareonviriyaphap et al., unpublished data).

Insecticide papers

The field dosage of DDT (2 g/m^2 of wall surface) was used in this investigation. Papers impregnated with deltamethrin and lambdacyhalothrin were dosed at established median lethal dose (LD_{50}) levels. Insecticide-impregnated papers with DDT at 2 g AI/m^2, deltamethrin at 0.0625 g AI/m^2, and lambdacyhalothrin at 0.0369 g AI/m^2 were prepared using diluent according to WHO protocol (Busvine 1958). Insecticide-impregnated test papers were treated at the rate of 2.75 ml of the insecticide solution per 180 cm^2.

Based on levels of DDT used in mosquito control, test papers (27.5 × 35.5 cm^2) were impregnated with DDT at 2 g AI/m^2. Papers impregnated with DDT were received from the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine (USACHPPM), Aberdeen Proving Ground, MD, in December 1998.

Based on the established LD_{50} dose for deltamethrin, test papers (27.5 × 35.5 cm^2) were impregnated with deltamethrin at 0.0625 g AI/m^2 (Chareonviriyaphap et al., unpublished data). Papers impregnated with deltamethrin were received from the USACHPPM, Aberdeen Proving Ground, MD, in December 1998.

Based on the established LD_{50} dose for lambdacyhalothrin, test papers (27.5 × 35.5 cm^2) were impregnated with lambdacyhalothrin at 0.0369 g AI/m^2 (Chareonviriyaphap et al., unpublished data). Papers impregnated with lambdacyhalothrin were purchased from WHO, Vector Control Unit, Penang, Malaysia, in January 1999.

Behavioral tests

Test chamber: In this study, we used the improved test chamber for all tests as described in a recent publication (Chareonviriyaphap and Aum-Aong 2000). Figure 1 shows the stainless steel, collapsible excito-repellency escape chamber (34 × 32 × 32 cm), facing the front panel and escape funnel. The box comprises 4 side walls, a rear Plexiglas inner door, a rear outer door cover and a front door, and a removable exit portal (an escape funnel). Each wall is constructed of stainless steel sheet (0.7-mm thickness), which has an aluminum sliding rib on each end and a socket, providing a surface for the test paper holder in the middle. The test paper holder has 2 sides; a sheet of fine mesh iron screen net is permanently attached on 1 side, and a panel to hold test papers to secure the panel on top is on the opposite side. A 0.8-cm gap between the test papers and screen prevents mosquitoes from making physical contact with the surface of test paper in the exposure windows during the noncontact repellency trials. The test paper holder is convenient and functions similarly under contact and noncontact conditions, depending on the purpose of the test. The holder simply has to be inverted to provide the proper conditions. A spring mechanism on 1 side of the test paper holder secures it tightly when putting the holder into the socket. The front door is constructed of a stainless steel frame with stainless steel sheet affixed on the front side. The steel sheet has a trough for sliding the exit funnel into place. Two screws at 1 end secure the funnel to the front panel. The inner rear door is constructed of a stainless steel frame and a transparent Plexiglas door that is attached to the frame. The Plexiglas door serves to seal the chamber and at the same time allow the investigator to look inside the exposure chamber before and after a test is conducted. A self-sealing 6-in. (15.5-cm)-diameter portal made of dental dam is used for placing test specimens inside the chamber and for removing the specimens from the chamber after each test. The outer rear door is constructed of stainless steel and is used to shut off all light inside the chamber when the test is being conducted. The last part is a removable exit funnel attached to the outside of the chamber. The escape funnel gap is a 20.5-cm-long and 1.5-cm-wide opening (a horizontal slit).

To assemble the collapsible excito-repellency chamber, all 4 side walls are put together by sliding the appropriate aluminum tongue and groove elements together to connect the 4 side walls of the chamber. The front door and the inner rear door are fixed to each side of the adjoining walls by screw nuts. To change the test papers, the nuts holding the transparent Plexiglas to screw in the metal frame are unscrewed, the Plexiglas door is removed, and the test paper holder is taken out from the chamber. When these changes are completed, the holder and Plexiglas door are put back in place.

For a complete test, 25 mosquitoes were introduced into each of 4 chambers using a mouth aspirator. After the mosquitoes were put in the chamber, the outer rear door was closed and secured. A receiving cage, a 6 × 6 × 6-cm paper box, was connected to the exit window for collecting escaping specimens. At the start of a test, a 3-min rest period was used to permit mosquitoes to adjust to test chamber condition (Busvine 1964, Chareonviriyaphap et al. 1997). After 3 min, the escape funnel was opened to initiate the observation period. Numbers of mosquitoes escaping from the exposure chamber into receiving cage were recorded at 1-min intervals (Chareonviriyaphap et al. 1997).

Tests performed: Unfed laboratory-reared *An. minimus* specimens were used in excito-repellency tests. The wild population was deprived of water for a minimum of 4 h before tests. Tests were performed during the day and each test was replicated at least 4 times. Tests were conducted to compare laboratory colony vs. wild populations and contact vs. noncontact and 3 insecticides that are currently used in malaria control in Thailand (DDT, deltamethrin, and lambdacyhalothrin). After a test was completed, the number of dead specimens was re-

Table 1. Mortalities of *Anopheles minimus* females after a 24-h holding period after exposures in contact trials of excito-repellency tests.

Condition	Population	Chemical[1]	Number		% mortality	
			Test	Escaped (%)	Escaped	Not escaped
Contact	Young colony	DDT	100	67 (67)	0	1
		DDT-C	99	18 (18)	1	2
		Del	126	107 (85)	3	2
		Del-C	127	10 (8)	0	1
		Lam	177	132 (75)	5	7
		Lam-C	171	32 (19)	1	0
	Wild population	DDT	100	67 (67)	1	0
		DDT-C	100	17 (17)	2	1
		Del	98	53 (54)	1	0
		Del-C	94	10 (10)	0	1
		Lam	96	91 (95)	1	0
		Lam-C	97	18 (19)	0	0
Noncontact	Young colony	DDT	101	25 (24)	1	0
		DDT-C	96	11 (11)	2	1
		Del	124	35 (28)	0	0
		Del-C	125	17 (14)	1	1
		Lam	174	27 (16)	1	0
		Lam-C	174	8 (5)	0	0
	Wild population	DDT	98	24 (24)	0	0
		DDT-C	97	10 (10)	0	0
		Del	98	24 (24)	0	0
		Del-C	100	11 (11)	0	1
		Lam	99	19 (19)	0	0
		Lam-C	95	15 (16)	0	0

[1] DDT, DDT at 2 g/m^2; Del, deltamethrin at 0.0625 g/m^2; Lam, lambdacyhalothrin at 0.0369 g/m^2; DDT-C, control (without DDT); Del-C, control (without deltamethrin); Lam-C, control (without lambdacyhalothrin).

corded separately for exposure and escape chambers. In addition, the escaped specimens and those remaining in the chamber, both controls and treatments, were maintained separately and 24-h mortalities were recorded.

Data analysis: Behavioral response data were analyzed using a life table method (a survival analysis approach) to estimate the escape rate and compare differences in mosquito escape response among different populations and insecticides. The mosquito escape rate was estimated at 1-min intervals. Mosquitoes that escaped were treated as deaths and mosquitoes remaining in the test chamber were treated as survivals (Chareonviriyaphap et al. 1997). The time in minutes for 50 and 75% of the test population to escape (ET_{50} and ET_{75}, respectively) were estimated with the life table method. The log-rank method was used to compare patterns of escape responses (Mantel and Haenzel 1959). The statistical software package STATA was used for this analysis as described by Roberts et al. (1997).

RESULTS

This study was designed to compare behavioral responses of wild-caught and colonized *An. minimus* females when exposed to DDT at 2 g/m^2, deltamethrin at 0.0625 g/m^2, and lambdacyhalothrin at 0.0369 g/m^2. Percent mortalities and escape responses were recorded. Both field and laboratory trials were conducted during the day (0800–1600 h).

Escape responses of *An. minimus* females were tested in contact and noncontact exposure chambers. Mortalities of *An. minimus* females after a 24-h holding period after exposures in contact and noncontact trials are presented in Table 1. In contact trials, the percent mortalities of escaped and nonescaped specimens from 2 test populations were very low, ranging between 0 and 7%, for young colony and wild population specimens. Deltamethrin produced a larger number of test specimens escaping from exposure chambers by the young colony (85%) than by a wild population (54%). The wild population exhibited stronger responses to lambdacyhalothrin (95%) than did the young colony (75%). The number of escape responses from DDT was somewhat similar in both test populations (67% in both a young colony and a wild population). For controls, a comparatively low degree of contact irritant response was noted. Roughly 17–18% escaped from control chambers for DDT, 8–10% escaped for deltamethrin, and 19% escaped for lambdacyhalothrin (Table 1). In noncontact trials, percent mortalities of escaped and nonescaped specimens from treated chambers were also low (≤2%; Table 1). Twenty-four percent of test spec-

Table 2. Time in minutes for 50 (ET_{50}) and 75% (ET_{75}) of *Anopheles minimus* females to escape from exposure chambers (in excito-repellency tests) treated with DDT at 2 g/m², deltamethrin at 0.0625 g/m², and lambdacyhalothrin at 0.0369 g/m².

Population/ colony	DDT[1]		Del[2]		Lam[3]	
	ET_{50}[4]	ET_{75}[4]	ET_{50}	ET_{75}	ET_{50}	ET_{75}
Young	16	—[5]	6	15	6	29
Wild	15	—	26	—	4	10

[1] DDT at 2 g/m².
[2] Deltamethrin at 0.0625 g/m².
[3] Lamdacyhalothrin at 0.0369 g/m².
[4] Survival analysis was used to estimate the time in minutes for 50 and 75% of test populations to exit exposure chambers.
[5] Very few mosquitoes (<75%) escaped from exposure chambers, so that the ET_{75} estimates could not be calculated for a 30-min exposure period.

imens escaped from noncontact trials with DDT, for both young colony and wild population. Compared to controls, this was slightly more than a 2-fold increase in escape response over the number escaping from control chambers. With deltamethrin, the number of escaping mosquitoes from the treatment chamber was also approximately 2 times greater than from control chambers for both test populations (Table 1). With lambdacyhalothrin, escape responses were pronounced for the young colony, whereas a higher number escaped from control chambers for the wild population (Table 1).

Times in minutes for *An. minimus* females to escape from exposure chambers treated with DDT, deltamethrin, and lambdacyhalothrin were calculated (Table 2). The escape patterns from chambers treated with insecticides were defined as times for 50% (ET_{50}) and 75% (ET_{75}) of a test population to leave the treated chambers (Chareonviriyaphap et al. 1997). For a young colony, the ET_{50}s for DDT, deltamethrin, and lambdacyhalothrin were 16, 6, and 6 min, respectively. The ET_{75}s for deltamethrin and lambdacyhalothrin were 15 and 29 min, respectively, whereas the ET_{75} could not be estimated for DDT. For the wild population, the ET_{50} values for DDT, deltamethrin, and lambdacyhalothrin were 15, 26, and 4 min, respectively. The ET_{75} for DDT and deltamethrin could not be calculated, whereas the ET_{75} was 10 min for lambdacyhalothrin (Table 2).

Comparisons between any 2 test populations of contact and noncontact trials against DDT, deltamethrin, and lambdacyhalothrin are given in Table 3 (data from Table 1). In contact trials with deltamethrin and lambdacyhalothrin, significant differences in escape responses were observed between the young colony and the wild population ($P < 0.05$). In noncontact trials with 3 compounds, no significant differences were found in escape responses between the young colony and the wild population ($P > 0.05$; Table 3).

Significant differences in escape patterns were

Table 3. Comparison of escape responses between 2 test populations of *Anopheles minimus* females in contact vs. noncontact trials by insecticides.

Insecticides[1]	Contact trial	Noncontact trial
DDT	Wild vs. young	Wild vs. young
Del	Wild vs. young*	Wild vs. young
Lam	Wild vs. young*	Wild vs. young

[1] DDT, DDT at 2 g/m²; Del, deltamethrin at 0.0625 g/m²; Lam, lambdacyhalothrin at 0.0369 g/m².
* Results of log-rank tests with statistically significant (0.05 level of probability) differences in patterns of escape behavior.

observed in comparisons of contact vs. noncontact, contact vs. control, and noncontact vs. control in tests with all 3 compounds against specimens of the young colony and a wild population ($P < 0.05$). Escape probabilities in contact trials were statistically higher than in controls for young colony and wild population ($P < 0.05$). Also, significantly stronger escape responses were found in noncontact trials than in controls against DDT, deltamethrin, and lambdacyhalothrin ($P < 0.05$; Table 4) (data from Table 1).

Figures 2–4 demonstrate the proportions of mosquitoes remaining in the exposure chambers treated with DDT, deltamethrin, and lambdacyhalothrin. These proportions are used to develop patterns of escape rates and demonstrate probabilities for escaping from exposure chambers in contact vs. noncontact (Fig. 2), contact vs. control (Fig. 3), and noncontact vs. control trials (Fig. 4) for both test populations. Significant differences were found in escape patterns for DDT, deltamethrin, and lambdacyhalothrin when contact trials were compared with noncontact and control trials and when noncontact trials were compared to control trials ($P < 0.05$). In contact trials with deltamethrin, the escape rate of populations from the young colony was significantly higher than that of wild-caught populations ($P < 0.05$; Figs. 2 and 3). This phenomenon was not observed in contact trials with lambdacyhalthrin. With lambdacyhalothrin, the escape rate was statistically higher for the wild population than

Table 4. Comparison of escape responses between contact vs. noncontact, contact vs. control, and noncontact vs. control for 2 test populations.[1]

Population	Contact vs. noncontact	Contact vs. control	Noncontact vs. control
Young	DDT*	DDT*	DDT*
	Del*	Del*	Del*
	Lam*	Lam*	Lam*
Wild	DDT*	DDT*	DDT*
	Del*	Del*	Del*
	Lam*	Lam*	Lam*

[1] DDT, DDT at 2 g/m²; Del, deltamethrin at 0.0625 g/m²; Lam, lambdacyhalothrin at 0.0369 g/m².
* Results of log-rank tests with statistically significant (0.05 level of probability) differences in patterns of escape behavior.

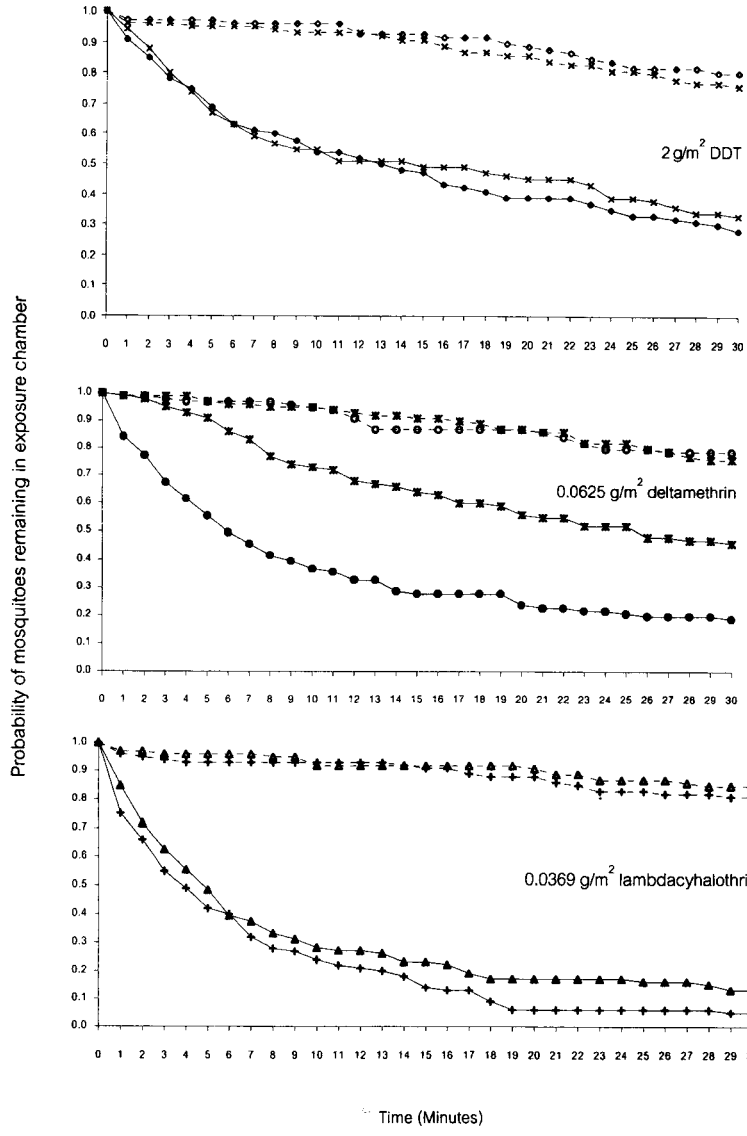

Fig. 2. Escape probability of *Anopheles minimus* females remaining in exposure chambers in contact vs. noncontact trials with DDT at 2 g/m², deltamethrin at 0.0625 g/m², and lambdacyhalothrin at 0.0369 g/m². Young, contact DDT (◆——◆); young, noncontact DDT (◇– – –◇); wild, contact DDT (×——×); wild, noncontact DDT (×– – –×); young, contact deltamethrin (●——●); young, noncontact deltamethrin (○– – –○); wild, contact deltamethrin (✱——✱); wild, noncontact deltamethrin (✱– – –✱); young, contact lambdacyhalothrin (▲——▲); young, noncontact lambdacyhalothrin (△– – –△); wild, contact lambdacyhalothrin (+——+); wild, noncontact lambdacyhalothrin (+– – –+).

for the young colony ($P < 0.05$; Figs. 2 and 3). No significant differences in escape rate were observed between wild and young test populations when tested against DDT (Figs. 2 and 3). In noncontact trials with DDT, deltamethrin, and lambdacyhalothrin, no significant difference patterns were found between the young colony and the wild population ($P > 0.05$). However, escape rates of the 2 test populations for all 3 compounds in noncontact trials were statistically higher from treatment chambers than from control chambers (Fig. 4).

DISCUSSION

Behavioral responses of malaria vectors to insecticides have long been recognized. In the past, behavioral responses were generally overlooked in national malaria control programs, which focused entirely on toxicologic responses to insecticides. Today, the development of insecticide resistance in insect pests and disease vectors occurs in some countries. Resistance has been very limited in many areas, including Thailand, in spite of extensive use of chemicals to control

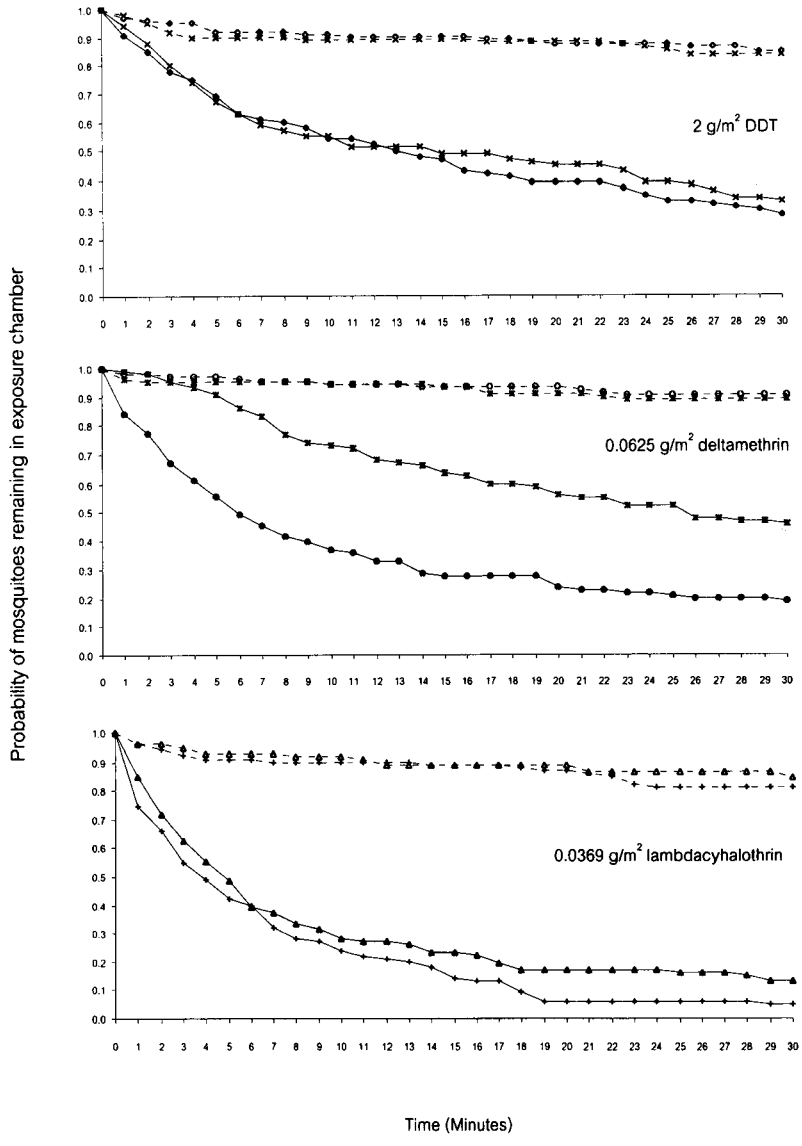

Fig. 3. Escape probability of *Anopheles minimus* females remaining in exposure chambers in contact vs. control trials with DDT at 2 g/m², deltamethrin at 0.0625 g/m², and lambdacyhalothrin at 0.0369 g/m². Young, contact DDT (♦——♦); young, control DDT (◇– – –◇); wild, contact DDT (×——×); wild, control DDT (×– – –×); young, contact deltamethrin (●——●); young, control deltamethrin (○– – –○); wild, contact deltamethrin (✱——✱); wild, control deltamethrin (✱– – –✱); young, contact lambdacyhalothrin (▲——▲); young, control lambdacyhalothrin (△– – –△); wild, contact lambdacyhalothrin (+——+); wild, control lambdacyhalothrin (+– – –+).

insect pests and disease vectors (Roberts and Andre 1994; Chareonviriyaphap et al. 1997, 1999). This phenomenon suggests that behavioral avoidance could be more important than killing vectors to reduce human vector contact. The important role of behavioral avoidance has now been quantified in a probability model (Roberts et al. 2000).

At least 2 different categories of behavioral responses of mosquito vectors to insecticides are believed to exist (Davidson 1953, Rawlings and Davidson 1982, Roberts and Andre 1994, Chareonviriyaphap et al. 1997, Rutledge et al. 1999). Irritability occurs when insects make physical contact with insecticides and may cause vectors to exit treated areas, whereas repellency acts from a distance and deters insects from entering treated areas (Roberts et al. 2000). In this present study, both contact irritability and noncontact repellency were documented. Clear behavioral responses to all 3 compounds were observed with *An. minimus* test populations, with greater responses resulting from contact with treated surfaces.

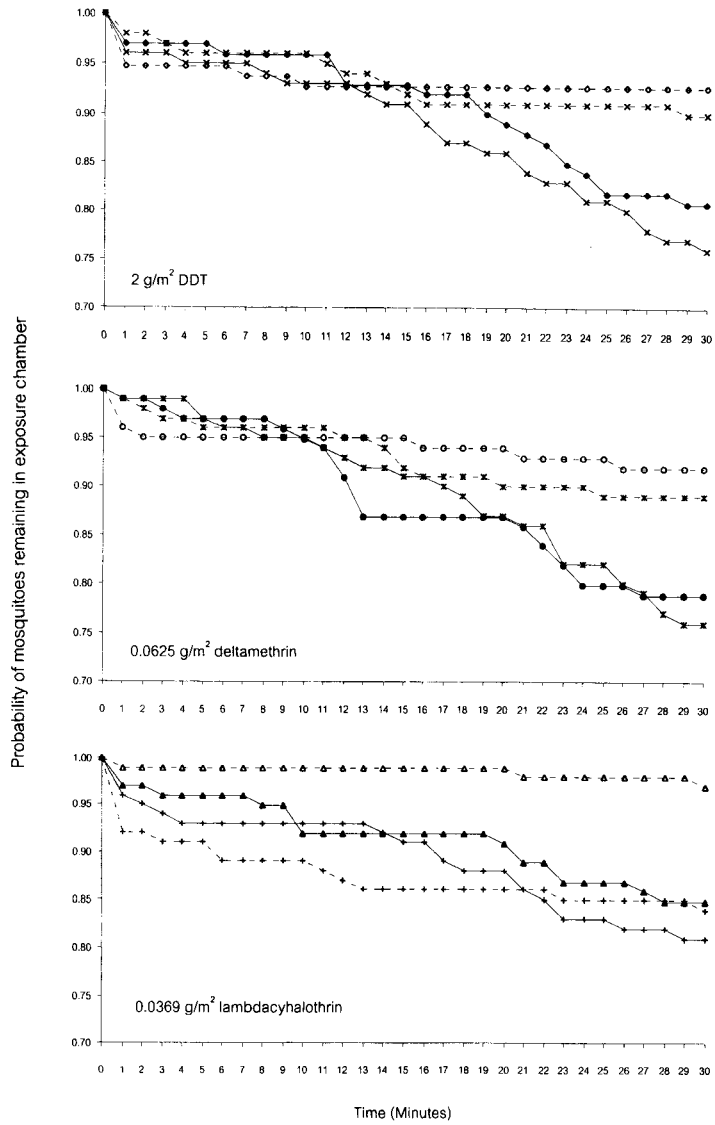

Fig. 4. Escape probability of *Anopheles minimus* females remaining in exposure chambers in noncontact vs. control trials with DDT at 2 g/m², deltamethrin at 0.0625 g/m², and lambdacyhalothrin at 0.0369 g/m². Young, noncontact DDT (♦——♦); young, control DDT (◊– – –◊); wild, noncontact DDT (×——×); wild, control DDT (×– – –×); young, noncontact deltamethrin (●——●); young, control deltamethrin (○– – –○); wild, noncontact deltamethrin (✱——✱); wild, control deltamethrin (✱– – –✱); young, noncontact lambdacyhalothrin (▲——▲); young, control lambdacyhalothrin (△– – –△); wild, noncontact lambdacyhalothrin (+——+); wild, control lambdacyhalothrin (+– – –+).

Both young and wild test populations of female *An. minimus* demonstrated tremendous irritancy responses to DDT, deltamethrin, and lambdacyhalothrin, and most specimens took off from the treated chambers without receiving a lethal dose. In this study, DDT was dosed at 2 g/m², whereas deltamethrin (0.0625 g/m²) and lambdacyhalothrin (0.0369 g/m²) were dosed at established LD_{50} levels. Based on dosages employed, DDT could produce higher percent mortality compared with deltamethrin and lambdacyhalothrin. However, in our study, low percent mortalities were observed in escaped and nonescaped specimens, indicating strong natural behavioral avoidance of all 3 compounds. In noncontact trials with 3 compounds, female mosquitoes demonstrated significant avoidance responses ($P < 0.05$). Surprisingly, percent mortality in nonescaped mosquitoes (those remaining in the chamber through the 1-h exposure period) from the treated chamber were low. Perhaps low mortalities resulted from mosquitoes avoiding the treated surfaces by hanging or resting on untreated corners

inside the chamber. Some areas of the exposure chambers were free from insecticides (details on chamber design were given by Chareonviriyaphap and Aum-Aong [2000]). In this present study the wild population showed much quicker escape responses to the chambers treated with DDT and lambdacyhalothrin than to those treated with deltamethrin. The young colony exhibited stronger responses to the 2 pyrethroids than to DDT. The comparatively weaker response to deltamethrin by test specimens from the wild population vs. test populations from a young colony was unclear. However, age composition and physiologic status of wild specimens could play a role in this result. Evans (1993) reported a strong irritant effect of lambdacyhalothrin and DDT with test populations from a laboratory colony of *Anopheles gambiae* Giles. Similar results were observed in a recently colonized population of *Anopheles albimanus* Wied. from Guatemala and field populations from Belize by Chareonviriyaphap et al. (1997) and in a wild population of *Anopheles vestitipennis* Dyar and Knab from Belize by Bangs et al. (unpublished data).

Lambdacyhalothrin produced a greater contact irritant response in both young and wild test populations than did DDT, as evidenced by numbers of escapees and lower ET_{50} and ET_{75} values. In comparison, test specimens from a laboratory colony of *An. gambiae* showed a stronger irritant response to DDT than to lambdacyhalothrin (Evans 1993). In Thailand, lambdacyhalothrin has not been widely researched and is being used exclusively on a small scale (Chareonviriyaphap et al. 1999). As a consequence, no recommended dosage has been established. In this study, we found that the impact of lambdacyhalthrin at 0.0369 g/m^2 on *An. minimus* females was strong and that might be an adequate dosage for a surface spray; as reported by Evans (1993), the recommended dosage for lambdacyhalothrin is 0.03 g/m^2 for *An. gambiae.*

In Thailand, *An. minimus* is endophagic (bites indoors) and endophilic (rests on walls after biting) in unsprayed houses (Nutsathapana et al. 1986; Poolsuwan 1995; Chareonviriyaphap, unpublished data). Huts treated with insecticides almost completely repelled *Anopheles dirus* Peyton and Harrison (Suwonkerd et al. 1990). We believe that residual wall treatment with insecticides is still very useful against those vectors that bite and rest inside the house. From the results of this study, we concluded that the primary impact of DDT-, deltamethrin-, and lambdacyhalothrin-sprayed house walls is excito-repellency, not toxicity, and that these insecticides are still effective in combating *An. minimus* in Thailand. These findings are in agreement with those of the study of Evans (1993) that high proportion of *An. gambiae* had not received a lethal dose and were unharmed after leaving a chamber treated with DDT and lambdacyhalothrin.

We now have a mathematical framework for understanding how repellent, irritant, and toxic actions of chemicals function to control malaria. However, we still need to test the major elements of this model with different vectors and chemicals from many malaria endemic areas. Additionally, for multiple comparisons of excito-repellency test results with experimental hut studies under field conditions are needed. The goal should be to more carefully evaluate the role of repellency in malaria control.

In conclusion, without a clearer understanding of the dynamics between insecticide residues and vector behavior, vector control activities will continue to be hampered by a failure to understand the impact of behavioral avoidance behaviors on mosquito populations and malaria transmission. A better understanding of behavioral responses of vectors to various chemicals will allow for greater efficiency in program design and strategies for targeting appropriate vectors. Careful targeting of applications of vector control measures will allow for effective vector control and will minimize the amount of insecticides used in the malaria control program. Clearly, more field research is needed on the behavioral responses of vector populations from different geographical areas in Thailand. Chemically induced avoidance behaviors by malaria vector mosquitoes should be defined using standardized methods (e.g., excito-repellency boxes and experimental huts) to determine the exact impact of chemicals on malaria transmission and malaria control (Roberts et al. 2000). This present investigation, along with standardized methods for excito-repellency testing of mosquitoes (Chareonviriyaphap et al., unpublished data), contributes a more complete test system.

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