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PESTICIDE AVOIDANCE BEHAVIOR IN *ANOPHELES ALBIMANUS*, A MALARIA VECTOR IN THE AMERICAS[1]

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ABSTRACT. The behavioral responses of 4 populations of *Anopheles albimanus* females to DDT, permethrin, and deltamethrin were characterized in excito-repellency tests. One test population (ST) from El Salvador has been maintained as a colony for 20 years. A second population (ES) from Guatemala was colonized in 1992. Third and fourth populations consisted of field-caught specimens from Toledo District (TO) of southern Belize in 1994 and Corozal District (CO) of northern Belize, respectively. Females of ES, TO, and CO populations rapidly escaped from direct contact with treated surfaces for each of the 3 insecticides. Similarities in escape responses of insecticide-resistant (ES) versus insecticide-susceptible populations (TO, CO) suggest that there is no relationship between physiological and behavioral responses of *An. albimanus* populations to DDT, permethrin, and deltamethrin. Females from all but the ST colony escaped in greater numbers from chambers without direct contact with treated surfaces than from control chambers ($P < 0.05$). Few females from the ST colony escaped from test chambers, regardless of which insecticide was used or whether contact was allowed, indicating that the ST colony has lost its capability to respond to insecticides. Repellent responses were significant; but they were not pronounced in 30-min exposures, and they were very pronounced in 4-h exposures. We conclude that irritant and repellent responses of malaria vectors to insecticides are important components of malaria control operations.

INTRODUCTION

Anopheles albimanus Wiedemann is a primary vector of malaria in many areas of Central and South America (Breeland 1974). DDT has been used extensively to control malaria transmitted by this vector. Today, resistance of *An. albimanus* to DDT occurs in several countries (Brown 1986), but it does not occur in others in spite of regular DDT use (Roberts and Andre 1994). Behavioral avoidance of DDT has also been reported to occur in some *An. albimanus* populations (Rachou et al. 1963). In combination, findings of DDT avoidance and DDT resistance in conspecific populations raise questions about the role of avoidance behavior in preventing malaria transmission and in selecting for insecticide resistance in malaria vectors. Avoidance of DDT by malaria vectors has been recorded in the presence and absence of physiological resistance (Lockwood et al. 1984), but the relationships, if any, between physiological resistance and behavioral avoidance are unknown.

The term "avoidance behavior" will be used to describe behavior that is stimulated by some combination of irritancy and repellency, with irritancy occurring after physical contact and repellency occurring without physical contact with insecticide. Excito-repellency, like avoidance behavior, also is a broad classification of behavioral responses including both irritancy and repellency.

Pyrethroids elicit behavioral responses in insects (Threlkeld 1985). Mosquito control through the use of pyrethroid-impregnated bed nets and intradomiciliary spraying of pyrethroids has been initiated in some countries, including a few countries of Central and South America (Beach et al. 1989, Curtis et al. 1989, World Health Organization 1989). The increased use of pyrethroids should be a major stimulus for extensive tests and field studies on pyrethroid avoidance behavior in New World vectors of malaria.

The complexities of excito-repellency testing, including methods of analyzing and interpreting test data, have resulted in no test method being adequate or fully accepted. No test recommended by the World Health Organization will discriminate between contact irritancy and noncontact repellency. However, an experimental test system described by Roberts et al. (1997) addresses a number of deficiencies attributed to existing behavioral tests. The new test system was used in this series of studies on relationships of avoidance behavior and physiological resistance in colonized and wild-caught populations of *An. albimanus* mosquitoes from Central America. The *An. albimanus* populations were characterized for isozymes, for esterases, for insecticide susceptibilities, and for the irritancy and repellency effects of DDT, permethrin, and deltamethrin (Chareonviriyaphap et al., unpublished data). Behavioral responses of four *An. albimanus* populations were compared using three different in-

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secticides and with or without physical contact with insecticides.

MATERIALS AND METHODS

Anopheles albimanus test populations: 1. Santa Tecla colony (ST). This colony has been maintained in laboratory colonies for 20 years. It was originally collected from an animal stable in La Libertad Village along the Pacific Coast of El Salvador, Central America, in 1975. It was maintained at the U.S. Department of Agriculture, Gainesville, FL, and later at the Walter Reed Army Institute of Research (WRAIR), Washington, DC (Seawright, personal communication). The colony was obtained from the WRAIR and was maintained at the Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD, during these studies.

2. El Semillero colony (ES). This colony was originally collected from a cattle corral in El Semillero near the Pacific Coast of Guatemala in October 1992. It had been maintained in the Laboratory of Medical Entomology Research and Training Unit (MERTU/G) in Guatemala for 1 yr before we obtained colony specimens in 1993. This colony exhibited resistance to permethrin (C. Cordon-Rosales, personal communication).

3. Toledo field population (TO). This population was obtained from human-landing collections in a rice cultivation area of San Felipe Village, Toledo District, southern Belize, in September 1994. The field-caught females were susceptible to permethrin and deltamethrin but demonstrated resistance to DDT (Chareonviriyaphap et al., unpublished data).

4. Corozal field population (CO). This population was obtained from human-landing collections in a marsh area of Chan Chen Village, Corozal District, northern Belize, in February 1995. The wild-caught females were susceptible to DDT, permethrin, and deltamethrin (Chareonviriyaphap et al., unpublished data).

Insecticides: Three insecticides were used in behavioral tests.

1. Permethrin [3-phenoxybenzyl (IRS)-*cis-trans*-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate] (approximately 75% *trans,* 25% *cis* isomers) (94.7% purity). This chemical was received from AgrEvo Environmental Health (UK), United Kingdom, in January 1994. Based on established LD_{50} and LD_{90} doses for permethrin, test papers (12 × 15 cm^2) were impregnated with 0.0092 and 0.0462 g of active ingredient (AI)/m^2 (Chareonviriyaphap et al., unpublished data).

2. Deltamethrin [(*S*)-a-cyano-3-phenoxybenzl (*IR*)-*cis-trans*-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carboxylate] (99.7% purity). This chemical was obtained from the same company (AgrEvo Environmental Health [UK]) in January 1994. Based on established LD_{50} and LD_{90} doses for deltamethrin, test papers (12 × 15 cm^2) were impregnated with 0.0003 and 0.0019 g AI/m^2 (Chareonviriyaphap et al., unpublished data).

3. DDT (Dichloro-diphenyl-trichloroethane) (99% purity). This chemical was purchased from the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine (USACHPPM), Aberdeen Proving Ground, MD, in October 1994. Based on established LD_{50} and LD_{90} toxicities for DDT, test papers (12 × 15 cm^2) were impregnated with 0.4069 and 0.7593 g AI/m^2 (Chareonviriyaphap et al., unpublished data). Additionally, papers were impregnated at levels of DDT used in malaria control, i.e., 2 g AI/m^2.

Insecticide-impregnated papers were received from the Entomological Sciences Division, USACHPPM, Aberdeen Proving Ground, MD. All papers were treated at the rate of 2.75 ml of the insecticide solution per 180 cm^2.

Mosquito rearing: Anopheles albimanus colonies were reared following the methods of Ford and Green (1972), with only minor modifications. All life stages were reared in an environmentally controlled (25 ± 5°C, 80 ± 10% relative humidity) insectary at USUHS. Adult mosquitoes were provided cotton pads soaked with 10% sugar solution from the day of emergence and adults were maintained in a 12 × 12 × 12-in. screened cage.

Behavioral tests: The test method consisted of enclosing 25 female mosquitoes in a chamber lined with insecticide-treated or untreated (control) test papers. Each chamber had a single portal for mosquitoes to escape to a receiving cage. The exposure chamber accommodated a screened insert (inner chamber) that, when placed in the first chamber, prevented the mosquitoes from making physical contact with test papers. Under test conditions, mosquitoes were enclosed within the chamber, and the only source of light came from the exit portal. A full test consisted of a pair of treatment chambers and a pair of control chambers.

One treatment chamber permitted tarsal contact with insecticide-treated papers, i.e., there was no inner chamber. The second treatment chamber included the inner chamber, so mosquitoes could not make contact with insecticide-treated papers. Treatment chambers were lined with test papers that were impregnated with insecticide and an oil-based carrier (risella oil). Control chambers were lined with papers that were impregnated with carrier alone. For brevity, tests with or without the inner chambers, for either treatment or control papers, will be referred to as contact trials (no inner chamber) or noncontact trials (with inner chamber). This test system, including methods of data analysis, has been described by Roberts et al. (1997). All cage components, with the exception of a rear panel, were constructed of metal so they could be chemically cleaned and used repeatedly with different chemicals and doses without risk of contamination.

A full test required 4 separate cardboard pint

(0.473 liters) cages of 25 mosquitoes (a test population) each. About 1 min was needed for 2 investigators, using oral aspirators, to introduce a test population into each of the 4 test chambers. After a 3-min holding period, the escape funnel in each chamber was opened to initiate observations. Numbers escaping from exposure chambers into 1-gallon (3.785 liters) cardboard receiving cages were recorded in 1-min intervals; after 5 min of observation, receiving cages were replaced with empty cages. The change of receiving cages facilitated the accurate counting of numbers of mosquitoes that escaped.

Tests performed: Only *An. albimanus* females were used in excito-repellency tests. Each test was replicated at least 3 times. To fulfill the goals of this research, tests were performed to compare the 3 insecticides, concentrations of insecticides, insecticide-resistant vs. insecticide-susceptible populations, colony vs. field-caught populations, insecticide contact vs. noncontact, and short-term (30 min) vs. long-term (4 h) noncontact exposure.

Observations on mortality of test populations were made immediately after completion of each test, i.e., the number of dead or knockdown specimens inside the exposure chamber was recorded. Additionally, specimens that escaped and the remaining test specimens collected from the exposure chamber were held separately for observation of mortalities after 24-h holding periods.

A shortage of CO specimens (Corozal, Belize) resulted in no test of DDT at 2.00 g/m^2. Likewise, a shortage of TO specimens (Toledo, Belize) resulted in no tests of DDT at 0.4060 and 0.7593 g/m^2 or of deltamethrin at 0.0003 g/m^2. The ES colony (Guatemala) was lost before tests of DDT at 0.4069 and 0.7593 g/m^2 could be conducted.

We were concerned that the 30-min exposure periods were insufficient for measuring noncontact behavioral responses (repellency). To increase the sensitivity of the test system, tests were conducted to compare noncontact treatments with noncontact controls with 4-h exposure periods. To prevent insecticide particles from falling from test papers above the exposure chamber, clean white paper was placed on top of the inner chamber for each test. Four-hour exposures were conducted with all 3 insecticides against the CO population only. Similar tests were not performed with the other 3 test populations since our goal was just to test for increased noncontact repellency during longer exposure periods.

Data analyses: We used test data in a life table survival analysis approach (Roberts et al. 1997) to estimate mosquito escape rates (or rates of mosquitoes staying in the chambers) and then compared differences in mosquito escape among populations, insecticides, and concentrations (doses) of insecticides. With this method, the mosquito escape rate was estimated at 1-min intervals. Mosquitoes that escaped were treated as "deaths," and those that remained in the exposure chamber were treated as "survivals." The time in minutes for 50 and 90% of the test population to escape was estimated with the life table method and these estimates were used as the "escape time" summary statistics (ET_{50} and ET_{90}). The log-rank method was used to compare patterns of escape behavior (Mantel and Haenzel 1959). A statistical software package, STATA®, was used for this analysis. These methods of analyzing excito-repellency data have been described by Roberts et al. (1997).

RESULTS

Escape responses of *An. albimanus* to DDT, permethrin, and deltamethrin were tested in contact and noncontact exposure chambers. Mortalities of females after a 24-h holding period, from contact and noncontact trials, are given in Tables 1 and 2. Higher mortalities were observed in contact trials than in noncontact trials, and higher mortalities were observed in noncontact trials than in controls. With 2.00 g/m^2 of DDT in contact trials, no mortality of escaped ES and TO females was observed, while 6.8% mortality was observed in ST females. A high mortality (38.5%) of nonescaped ST mosquitoes (96% remained in the chamber) was found compared to that of nonescaped ES and TO mosquitoes. With permethrin in contact trials (Table 1), 11% or less of the escaped ES and TO mosquitoes died, while almost 40% of the escaped ST mosquitoes died. No mortality occurred in contact trials with the CO mosquitoes or in any noncontact trials (Table 2). In contact trials with deltamethrin (Table 1), higher mortality was observed in ST specimens than in the other populations tested.

Times in minutes for mosquitoes to escape from treated chambers are given in Table 3. The escape patterns from chambers containing insecticides were defined as times for 50 and 90% of the population to leave the treated chambers (ET_{50} and ET_{90}). For DDT at 2.00 g/m^2, the ES population had an ET_{50} of 8 min and an ET_{90} of 19 min. The TO population had an ET_{50} of 2 min and an ET_{90} of 16 min. At the lower dose of permethrin, the ES, TO, and CO test populations had ET_{50} values of 2, 4, and 9 min, respectively. The ET_{90} values for ES and CO populations were 8 and 17 min, respectively. The ET_{90} of the TO population could not be calculated. Likewise, the numbers of ST specimens escaping from exposure chambers were low, and ET_{50} and ET_{90} values could not be estimated for any of the 3 insecticides tested (Table 3).

Multiple comparisons among 4 test populations in contact, noncontact, and control trials against 2.00 g/m^2 DDT, 0.0092 g/m^2 permethrin, 0.0462 g/m^2 permethrin, 0.0003 g/m^2 deltamethrin, and 0.0019 g/m^2 deltamethrin are shown in Tables 4–6. The patterns of escape behavior were tested with the log-rank method, and significance was established at the 0.05 level of probability. Marked differences in escape responses were found when the

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

Test population[1]	Insecticide/ dose[2]	Number (%)		% mortality[3]	
		Tested	Escaped	Escaped	Not escaped
ST colony, El Salvador	Per-1	100	23 (23)	39.1	58 (45/77)
	Per-C	100	3 (3)	0	0
	Per-2	100	13 (13)	7.6	12 (10/87)
	Per-C	100	0 (0)	0	0
	Del-1	150	28 (19)	17.8	27 (33/122)
	Del-C	150	1 (0.6)	0	0
	Del-2	75	7 (9.3)	0	3
	Del-C	75	1 (1.3)	0	0
	DDT-1	125	29 (23)	6.8	39 (37/96)
	DDT-C	125	7 (5.6)	0	0
ES colony, Guatemala	Per-1	100	97 (97)	11	0
	Per-C	100	0 (0)	0	0
	Per-2	150	150 (100)	8.6	0
	Per-C	150	9 (6)	0	0
	Del-1	75	71 (94.5)	5.6	0
	Del-C	75	3 (4)	0	0
	Del-2	75	75 (100)	2.6	0
	Del-C	75	6 (8)	0	0
	DDT-1	75	72 (96)	0	0
	DDT-C	75	4 (5.3)	0	0
TO population, Belize	Per-1	90	90 (100)	2.2	0
	Per-C	75	4 (5.3)	0	0
	Per-2	76	67 (88.1)	2.9	44 (4/9)
	Per-C	76	8 (10.5)	0	0
	Del-1	75	70 (93.3)	1.42	40 (2/5)
	Del-C	75	4 (5.3)	0	0
	DDT-1	115	113 (98)	0	50 (1/2)
	DDT-C	100	17 (17)	0	0
CO population, Belize	Per-1	75	74 (98.7)	0	0
	Per-C	75	6 (8)	0	0
	Per-2	75	75 (100)	0	0
	Per-C	75	3 (4)	0	0
	Del-1	75	75 (100)	0	0
	Del-C	75	2 (2.7)	0	0
	Del-2	75	75 (100)	0	0
	Del-C	75	7 (9.3)	0	0
	DDT-2	75	70 (93.3)	1.4	20 (1/5)
	DDT-C	75	8 (10.7)	0	0
	DDT-3	75	71 (94.7)	0	25 (1/4)
	DDT-C	75	2 (2.7)	0	0

[1] ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; TO: field population from Toledo District, Belize; CO: field population from Corozal District, Belize.

[2] Codes for insecticides and doses (g/m^2): Per = permethrin at doses of (1) 0.0462 and (2) 0.0092; Del = deltamethrin at doses of (1) 0.0019 and (2) 0.0003; DDT = DDT at doses of (1) 2, (2) 0.7593, and (3) 0.4069. Per-C, Del-C, and DDT-C are controls (without insecticides).

[3] Mortalities based on numbers dead following 24-h holding periods after specimens were removed from exposure chambers or after females escaped from exposure chambers.

ST test population was compared with the other test populations ($P < 0.05$) (Table 6). Significant differences in escape probabilities were seen for all compounds when the ST test population was compared to the ES population ($P < 0.05$).

Contact vs. noncontact responses of *An. albimanus* to 2.0 g/m^2 DDT, 0.0092 and 0.0462 g/m^2 permethrin, and 0.0003 and 0.0019 g/m^2 deltamethrin were compared. Escape probabilities in contact trials were significantly higher than in controls for all populations ($P < 0.05$). Significant differences ($P < 0.05$) were observed in ES, CO, and TO population escape responses between contact and noncontact trials (Table 4). There were no significant differences in the ST population responses in contact vs. noncontact exposures at lower doses of permethrin and deltamethrin ($P > 0.05$), but significant differences were found at higher doses ($P < 0.05$) (Table 5). Also, significant differences in escape responses were found in all four populations when contact responses were compared to noncontact responses (Tables 4 and 6).

Table 2. Mortalities of *Anopheles albimanus* females after a 24-h holding period following exposures in noncontact trials of excito-repellency tests.

Test population[1]	Insecticide/ dose[2]	Number (%)		% mortality[3]	
		Tested	Escaped	Escaped	Not escaped
ST colony, El Salvador	Per-1	100	1 (1)	0	0
	Per-C	100	3 (3)	0	0
	Per-2	100	4 (4)	0	0
	Per-C	100	1 (1)	0	0
	Del-1	150	3 (2)	0	0
	Del-C	150	0 (0)	0	0
	Del-2	75	2 (3)	0	0
	Del-C	75	3 (4)	0	0
	DDT-1	125	4 (3)	0	0
	DDT-C	125	5 (4)	0	0
ES colony, Guatemala	Per-1	75	8 (11)	0	2 (1/67)
	Per-C	75	2 (3)	0	0
	Per-2	125	24 (19)	4.1	1 (1/101)
	Per-C	125	4 (3)	0	0
	Del-1	75	15 (20)	0	0
	Del-C	75	4 (5)	0	0
	Del-2	75	14 (19)	0	0
	Del-C	75	3 (4)	0	0
	DDT-1	75	18 (24)	0	0
	DDT-C	75	6 (8)	0	0
TO population, Belize	Per-1	75	0 (0)	0	0
	Per-C	75	2 (3)	0	0
	Per-2	75	8 (11)	0	0
	Per-C	75	7 (9)	0	0
	Del-1	75	8 (11)	0	0
	Del-C	75	5 (7)	0	0
	DDT-1	100	24 (24)	0	0
	DDT-C	100	6 (6)	0	0
CO population, Belize	Per-1	75	6 (8)	0	0
	Per-C	75	4 (5)	0	0
	Per-2	75	6 (8)	0	0
	Per-C	75	3 (4)	0	0
	Del-1	75	7 (9)	0	0
	Del-C	75	5 (7)	0	0
	Del-2	75	10 (13)	0	0
	Del-C	75	3 (4)	0	0
	DDT-2	75	6 (8)	0	0
	DDT-C	75	3 (4)	0	0
	DDT-3	75	4 (5)	0	0
	DDT-C	75	1 (1)	0	0

[1] ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; TO: field population from Toledo District, Belize; CO: field population from Corozal District, Belize.

[2] Codes for insecticides and doses (g/m^2): Per = permethrin at doses of (1) 0.0462 and (2) 0.0092; Del = deltamethrin at doses of (1) 0.0019 and (2) 0.0003; DDT = DDT at doses of (1) 2, (2) 0.7593, and (3) 0.4069. Per-C, Del-C, and DDT-C are controls (without insecticides).

[3] Mortalities based on numbers dead following 24-h holding periods after specimens were removed from exposure chambers or after females escaped from exposure chambers.

Statistical comparisons between 2 doses, LD_{50} and LD_{90}, of DDT, permethrin, and deltamethrin, in contact and noncontact trials showed significantly stronger escape responses for higher doses of permethrin in contact trials for the ES, CO, and TO populations (Table 5). The escape response for the CO population was also significantly greater in contact trials with the higher dose of deltamethrin. All other differences in escape responses between dosage levels were not significant ($P > 0.05$), and there were no significant differences between 2 doses of the 3 chemicals in noncontact trials.

Figures 1–4 show the proportions of mosquitoes remaining in the exposure chambers under different test conditions. These proportions are used to show patterns of escape rates. The patterns are indicative of escape probabilities between contact and noncontact trials (Figs. 1 and 3) and between noncontact and control trials (Figs. 2 and 4) with 4 populations of *An. albimanus*. Significant differences

Table 3. Time in minutes for 50 (ET_{50}) and 90% (ET_{90}) of *Anopheles albimanus* females to escape from exposure chambers (in excito-repellency tests) treated with DDT, permethrin, or deltamethrin.

Population/ colony[1]	DDT[2]		Per-1[3]		Per-2[4]		Del-1[5]		Del-2[6]	
	ET_{50}[7]	ET_{90}	ET_{50}	ET_{90}	ET_{50}	ET_{90}	ET_{50}	ET_{90}	ET_{50}	ET_{90}
ST colony	nc[8]	nc	nc	nc	nc	nc	nc	nc	nc	nc
ES colony	8	19	2	8	1	7	7	11	2	6
TO population	2	16	4	nc	1	7	—[9]	—	1	6
CO population	—	—	9	17	4	11	9	19	4	10

[1] ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; TO: field population from Toledo District, Belize; CO: field population from Corozal District, Belize.
[2] DDT at 2 g/m^2.
[3] Permethrin at 0.0462 g/m^2.
[4] Permethrin at 0.0092 g/m^2.
[5] Deltamethrin at 0.0019 g/m^2.
[6] Deltamethrin at 0.0003 g/m^2.
[7] Survival analysis was used to estimate the time in minutes for 50 and 90% of test populations to escape from exposure chambers.
[8] Very few specimens (0–23%) escaped from exposure chambers, so the ET_{50} and ET_{90} estimates could not be calculated for a 30-min exposure period. For the TO population, the ET_{90} could not be calculated because only 88% escaped during the 30-min exposure period.
[9] Mosquitoes were not available for testing.

in escape patterns were seen when contact trials were compared with control and noncontact trials ($P < 0.05$). The patterns of escaping females from contact vs. noncontact trials and from noncontact vs. control trials for deltamethrin (not illustrated) were similar to escape responses for permethrin (Figs. 3 and 4). Escape patterns of the ES, TO, and CO test populations in noncontact trials were higher than those from control trials. Significant noncontact repellency to DDT and synthetic pyrethroids ($P < 0.05$) was seen in trials with the ES test specimens.

Table 4. Comparison of escape responses (in excito-repellency tests) between contact and control trials, contact and noncontact trials, and noncontact and control trials for 4 colonies or populations of *Anopheles albimanus* mosquitoes.

Chemical	Population/ colony[1]	Contact vs. control dose (g/m^2)	Contact vs. noncontact dose (g/m^2)	Noncontact vs. control dose (g/m^2)
DDT	ST	2.0000*[2]	2.0000*	2.0000
	ES	2.0000*	2.0000*	2.0000*
	CO	0.4069*	0.4069*	0.4069*
	CO	0.7593*	0.7593*	0.7593
	TO	2.0000*	2.0000*	2.0000*
Permethrin	ST	0.0092*	0.0092	0.0092
	ST	0.0462*	0.0462*	0.0462
	ES	0.0092*	0.0092*	0.0092*
	ES	0.0462*	0.0462*	0.0462*
	CO	0.0092*	0.0092*	0.0092*
	CO	0.0462*	0.0462*	0.0462*
	TO	0.0092*	0.0092*	0.0092
	TO	0.0462*	0.0462*	0.0462
Deltamethrin	ST	0.0003*	0.0003*	0.0003
	ST	0.0019*	0.0019*	0.0019
	ES	0.0003*	0.0003*	0.0003*
	ES	0.0019*	0.0019*	0.0019*
	CO	0.0003*	0.0003*	0.0003*
	CO	0.0019*	0.0019*	0.0019*
	TO	0.0019*	0.0019*	0.0019

[1] ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; TO: field population from Toledo District, Belize; CO: field population from Corozal District, Belize.
[2] The * identifies results of log-rank tests with statistically significant (0.05 level of probability) differences in patterns of escape behavior.

Tests for increased levels of escape response with 4-h exposures in noncontact trials were conducted with CO populations (Fig. 5). In 4-h noncontact tests, the CO test populations showed statistically significant responses for all insecticides and doses compared to the controls ($P < 0.05$). Four-hour contact trials were not performed, since all CO test specimens in contact trials escaped from the treated chambers within the standard (30-min) exposure period.

DISCUSSION

Two forms of "behavioral avoidance," contact irritability and noncontact repellency, have been described (Davidson 1953, Rawlings and Davidson 1982). Irritability occurs when an insect is stimulated to move away from an insecticide-treated surface after direct physical contact with the insecticide residue. In contrast, repellency occurs when the insect detects and avoids treated surfaces without physical contact (Roberts and Andre 1994). In this study, both contact irritability and noncontact repellency were documented to occur with *An. albimanus* in the presence of DDT, permethrin, and deltamethrin.

Our laboratory and field results demonstrated that *An. albimanus* females from Guatemala (ES population) and from Belize (TO and CO populations) showed dramatic escape responses from exposure chambers that permitted direct contact with insecticide-treated surfaces. This irritancy response was not observed in mosquitoes that had been in colony for 20 years (ST population). For all 4 test

Table 5. Comparison of escape resonses for 2 doses (LD_{50} and LD_{90}) of different insecticides and populations of *Anopheles albimanus* mosquitoes in contact and noncontact trials.

Insecticide	Type of trial	Population/colony[1]	Compared dose level
DDT	Contact	CO	0.4069 vs. 0.7593
	Noncontact	CO	0.4069 vs. 0.7593
Permethrin	Contact	ST	0.0092 vs. 0.0462
	Contact	ES	0.0092 vs. 0.0462*[2]
	Contact	CO	0.0092 vs. 0.0462*
	Contact	TO	0.0092 vs. 0.0462*
	Noncontact	ST	0.0092 vs. 0.0462
	Noncontact	ES	0.0092 vs. 0.0462
	Noncontact	CO	0.0092 vs. 0.0462
	Noncontact	TO	0.0092 vs. 0.0462
Deltamethrin	Contact	ST	0.0003 vs. 0.0019
	Contact	ES	0.0003 vs. 0.0019
	Contact	CO	0.0003 vs. 0.0019*
	Noncontact	ST	0.0003 vs. 0.0019
	Noncontact	ES	0.0003 vs. 0.0019
	Noncontact	CO	0.0003 vs. 0.0019

[1] ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; TO: field population from Toledo District, Belize; CO: field population from Corozal District, Belize.

[2] The * identifies results of log-rank tests with statistically significant (0.05 level of probability) differences in patterns of escape behavior between doses of the insecticides.

Table 6. Comparison of escape responses between populations of *Anopheles albimanus* females in contact and noncontact trials by dose of insecticide.

Insecticide	Dose (g/m^2)	Contact trial (population or colony)	Noncontact trial (population or colony)
DDT	2.0000	ST vs. ES*[2]	ST vs. ES*
		ST vs. TO*	ST vs. TO*
		ES vs. TO*	ES vs. TO
Permethrin	0.0092	ST vs. ES*	ST vs. ES*
		ST vs. CO*	ST vs. CO
		ST vs. TO*	ST vs. TO
		ES vs. CO*	ES vs. CO
		ES vs. TO*	ES vs. TO
		CO vs. TO	CO vs. TO
	0.0462	ST vs. ES*	ST vs. ES*
		ST vs. CO*	ST vs. CO
		ST vs. TO*	ST vs. TO
		ES vs. CO	ES vs. CO*
		ES vs. TO*	ES vs. TO*
		CO vs. TO*	CO vs. TO
Deltamethrin	0.0003	ST vs. ES*	ST vs. ES*
		ST vs. CO*	ST vs. CO*
		ES vs. CO	ES vs. CO
	0.0019	ST vs. ES*	ST vs. ES*
		ST vs. CO*	ST vs. CO*
		ST vs. TO*	ST vs. TO
		ES vs. CO	ES vs. CO*
		ES vs. TO*	ES vs. TO*
		CO vs. TO	CO vs. TO

[1] ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; TO: field population from Toledo District, Belize; CO: field population from Corozal District, Belize.

[2] The * identifies results of log-rank tests with statistically significant (0.05 level of probability) differences in patterns of escape behavior between populations and trials.

populations, there was a lower escape rate from chambers that did not permit physical contact with treated surfaces. Regardless, the numbers escaping from noncontact test conditions were significantly greater than the numbers escaping from control chambers. This suggests that both contact irritancy

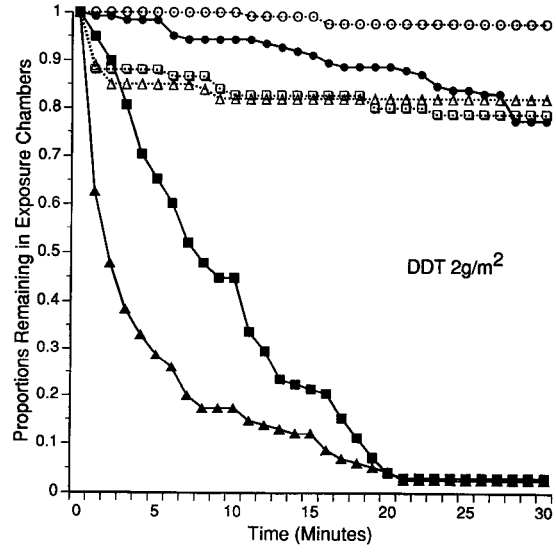

Fig. 1. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 2 g/m^2 DDT. (ST: Santa Tecla colony; ES: El Semillero colony; TO: field populations from Toledo District, Belize; —●—: contact ST; · · · ○ · · ·: noncontact ST; —■—: contact ES; · · · □ · · ·: noncontact ES; —▲—: contact TO; · · · △ · · ·: noncontact TO.)

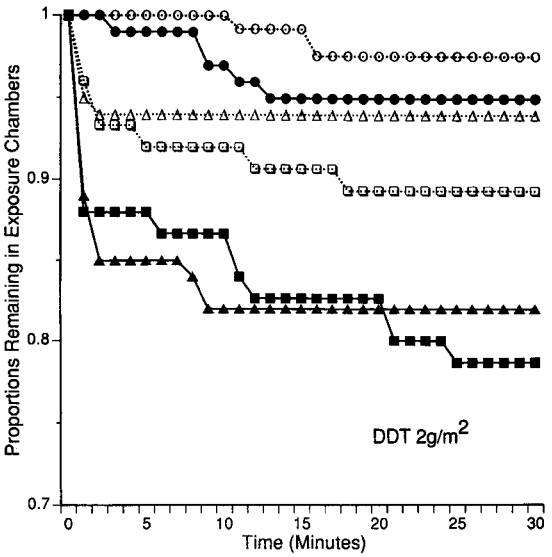

Fig. 2. Proportions of *Anopheles albimanus* females remaining in exposure chambers in noncontact vs. control trials with 2 g/m² DDT. (ST: Santa Tecla colony, ES: El Semillero colony, TO: field populations from Toledo District, Belize; —●—: noncontact ST; · · · o · · ·: control ST; —■—: noncontact ES; · · · □ · · ·: control ES; —▲—: noncontact TO; · · · △ · · ·: control TO.)

and noncontact repellency are involved in *An. albimanus* escape responses.

Smyth and Roys (1955) and Soliman and Cutkomp (1963) reported that DDT had a specific effect on antennal chemoreceptors and on sensory hairs of tarsal segments. The effect of DDT on sensory hairs of tarsi could form the basis of irritability (Soliman and Cutkomp 1963). In combination, the function of antennal chemoreceptors and sensory hairs on tarsal segments probably results in avoidance behaviors that involve both contact irritability and noncontact repellency.

Physiological resistance and behavioral avoidance are considered to be products of selective pressure from insecticide use (Lockwood et al. 1984). As derived characteristics, the presence and level of one response in a population is thought to influence selective pressure for the presence and level of the other. However, identical behavioral responses in both resistant and susceptible populations indicate that there is no relationship between these 2 response variables in *An. albimanus* populations. This finding suggests 2 hypotheses, one being that behavioral avoidance and physiological resistance are controlled by different genes. As a second hypothesis, we propose distinctly different origins for physiological vs. behavioral responses of *An. albimanus* populations to the 3 insecticides. It is probable that physiological resistance is a recent development due to selective pressures from agricultural uses of insecticides. Alternatively, the behavioral responses of *An. albimanus* populations may have evolved gradually as adaptations for avoiding classes or families of toxic chemicals produced by plants.

In contrast to the behavioral responses of field-caught or recently colonized populations, an older colonized population (ST colony) showed little to no behavioral response to the insecticides. Large numbers of ST test specimens died in contact trials. After 2 decades of laboratory maintainance, the ST colony has lost its genetic variability (Chareonviriyaphap et al., unpublished data) and, as a consequence, its natural ability to respond behaviorally to the 3 insecticides. A similar phenomenon was seen in earlier studies with a Gorgas Panama laboratory population of *An. albimanus* that was maintained as a colony for 20 years. The Panama mosquitoes showed excitation times (minutes to first flight following DDT exposure) that were 2 times longer than those of field populations (Brown 1958). In combination, these results caution against the use of colony populations as "standards" for studying behavioral responses of mosquitoes to insecticides.

In our studies, ES, TO, and CO test specimens quickly escaped exposure chambers without receiving a lethal dose of DDT, demonstrating strong natural behavioral avoidance of DDT. Additionally, the ES, TO, and CO test specimens were able to escape unharmed from permethrin-treated chambers. Populations of *An. albimanus* from Guatemala (ES) and Belize (CO and TO) that escaped from deltamethrin-treated surfaces were still alive 24 h later. Two out of 5 nonescaped TO test specimens died within 24 h, but escaped and nonescaped ES mosquitoes showed very low mortality. Bown et al. (1987) reported that *An. albimanus* departed deltamethrin-treated huts in Mexico with low mortality. The escape patterns of the ES and TO test populations in the presence of DDT were totally different from escape patterns of the ST test population, as well as being different from all control chamber escape patterns. The rate of escape of TO specimens exposed to DDT was quicker than that of the ES population (Fig. 2). This may be due to differences in test conditions, i.e., laboratory vs. field conditions, and age of mosquitoes at the time of testing. Roberts et al. (1984) reported that freshly fed *An. darlingi* Root females showed lower rates of escape than did unfed or late fed females when exposed to DDT. Hamon and Eyraud (1961) found that older *An. gambiae* and *An. funestus* Giles mosquitoes demonstrated less irritability than young mosquitoes. In our studies, laboratory tests were conducted with 3- to 5-day-old unfed female mosquitoes (ST and ES colonies), while the physiological age of field mosquitoes was unknown. Hecht et al. (1960) reported that *An. albimanus* is more active at higher temperatures. Tests conducted in the field were performed at higher ambient temperatures and humidities, which are conditions that might favor greater avoidance of DDT.

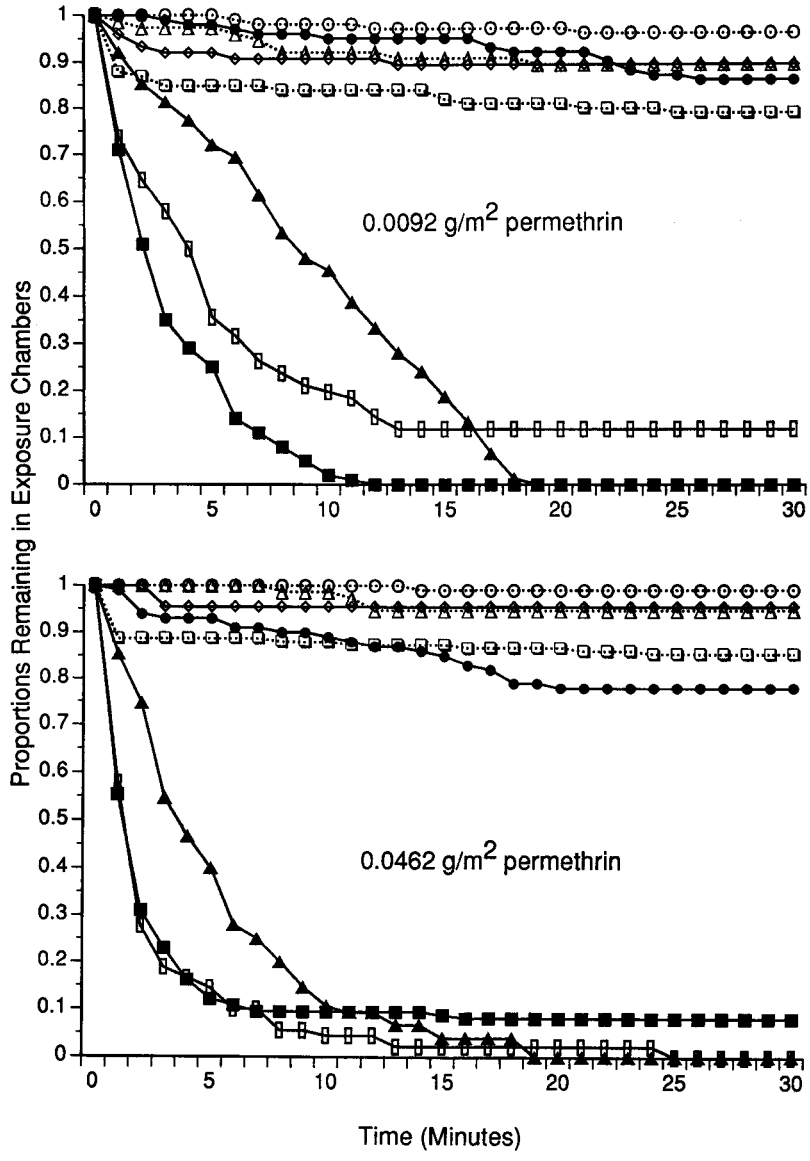

Fig. 3. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact and noncontact trials with 0.0092 and 0.0462 g/m² permethrin. (ST: Santa Tecla colony; ES: El Semillero colony; TO: wild-caught from Toledo District, Belize; CO: field populations from Corozal District, Belize; —●—: contact ST; · · · ○ · · ·: noncontact ST; —■—: contact ES; · · · □ · · ·: noncontact ES; —▲—: contact CO; · · · △ · · ·: noncontact CO; —◆—: contact TO; · · · ◇ · · ·: noncontact TO.)

A more gradual escape response to permethrin and deltamethrin was observed in CO test specimens compared to that in ES and TO test specimens (Table 3). However, all CO test specimens eventually escaped from contact exposure chambers. The differences in escape patterns among these populations may have been influenced by differences in physiological age or gonotrophic status of female mosquitoes, as described by Busvine (1964), or due to ambient test conditions. Some of the field mosquitoes were bloodfed, which may have caused delayed escape patterns.

In contact trials, more mosquitoes escaped at higher concentrations of insecticides. At lower concentrations, synthetic pyrethroids produced poor escape responses in ST test specimens. This relationship between concentration of insecticide and degree of behavioral response was consistent with findings of Ree and Loong (1989), who reported an increased irritability response of *An. maculatus* Theobald with in-

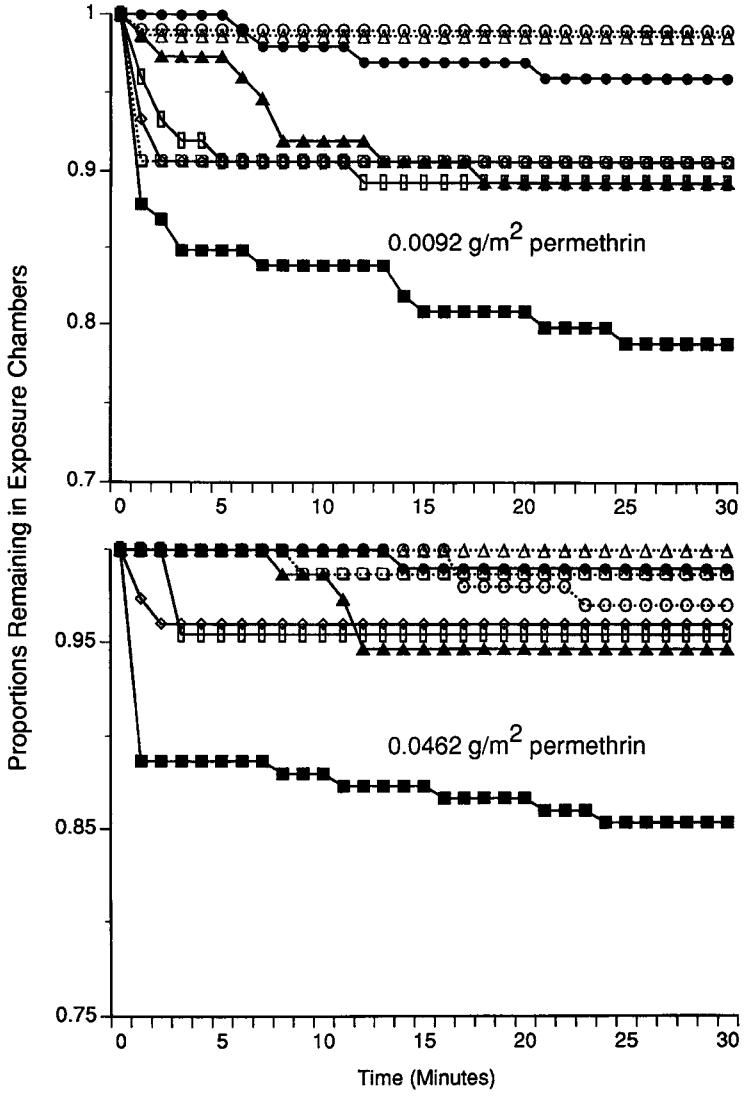

Fig. 4. Proportions of *Anopheles albimanus* females remaining in exposure chambers in noncontact and control trials with 0.0092 and 0.0462 g/m² permethrin. (ST: Santa Tecla colony; ES: El Semillero colony; TO: wild-caught from Toledo District, Belize; CO: field populations from Corozal District, Belize; —●—: noncontact ST; · · · ○ · · ·: control ST; —■—: noncontact ES; · · · □ · · ·: control ES; —▲—: noncontact CO; · · · △ · · ·: control CO; —◆—: noncontact TO; · · · ◇ · · ·: control TO.)

creasing concentrations of permethrin. Brown (1958) also found that time to first flight of *An. albimanus* after exposure to DDT was short at higher concentrations compared to that at lower concentrations.

Noncontact repellency of DDT may play a role in reducing human–vector contact, as shown by studies on *An. culicifacies* Giles in India (Shalaby 1966). In our tests, the ES and CO test populations showed noncontact repellency to DDT, even though the exposure period was only 30 min. This suggests that the repellency effect of DDT is also involved in *An. albimanus* escape responses. In short-term exposures, no repellency to synthetic pyrethroids was seen in TO test populations since numbers escaping from the treated chambers were relatively low and similar to those of the controls.

Different insecticide concentrations appeared to have no influence on escape patterns in short-term exposures with noncontact trials. As seen in the ES, TO, and CO test specimens, irritancy contributed to a strong and immediate response, while short-term exposure in noncontact trials (repellency) produced a weak, but statistically significant, escape response. Both irritancy and repellency were presumably additive properties that produced an overall stronger avoidance response.

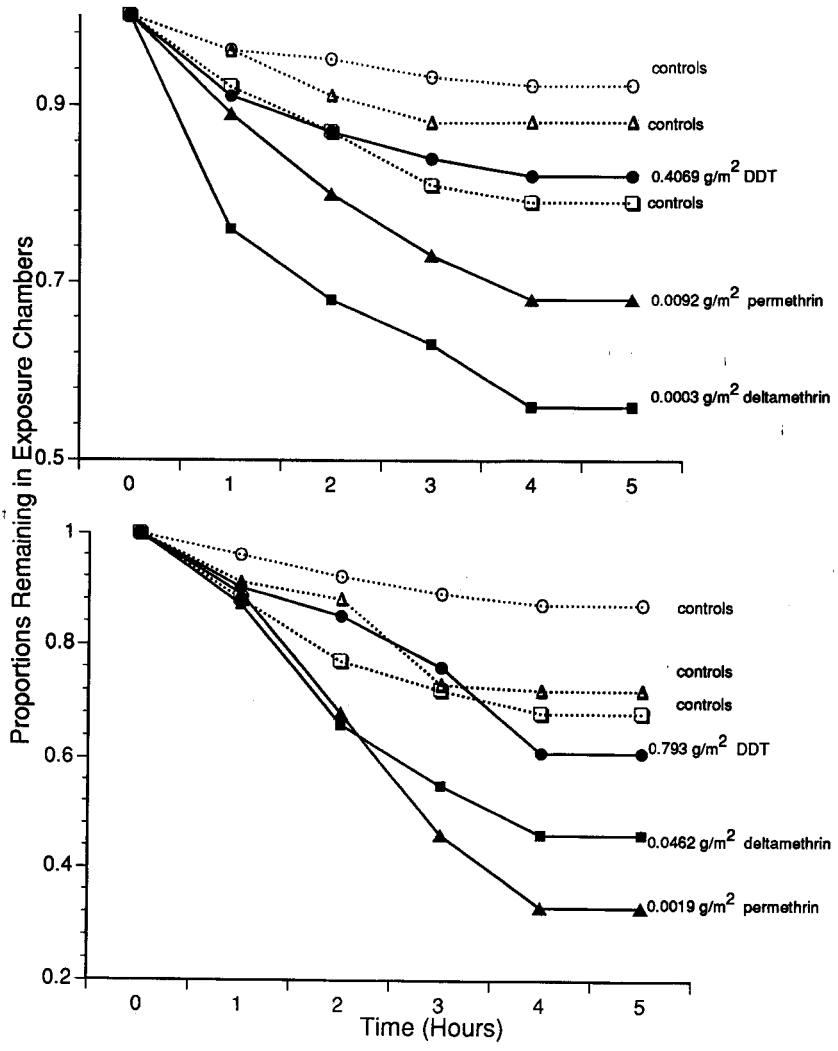

Fig. 5. Proportions of *Anopheles albimanus* females remaining in exposure chambers in noncontact vs. control trials for DDT, permethrin, and deltamethrin. Test specimens were caught in Corozal (CO) District, Belize. (—●—: DDT treatment; · · · ○ · · ·: DDT control; —■—: deltamethrin treatment; · · · □ · · ·: deltamethrin control; —▲—: permethrin control; · · · △ · · ·: permethrin treatment.)

Results from long-term exposure (4 h) to each of the 3 insecticides suggest an even more important role for noncontact repellency in *An. albimanus* avoidance of insecticide residues. Overall, deltamethrin was the most repellent insecticide, followed by permethrin and DDT. Unlike with short-term exposures in noncontact trials, greater escape activity was seen at higher doses of all 3 compounds with 4-h exposures. These differences seem to indicate that a 30-min exposure is not adequate for a meaningful test of noncontact repellency.

Our study showed that DDT, permethrin, and deltamethrin irritate and repel *An. albimanus* females and that most specimens escaping insecticide exposure will survive. Our findings are in agreement with the results of field studies by Roberts and Alecrim (1991), who reported a strong repellent action of DDT residues in houses. A repellent exerts an area effect, and if it is sufficient to reduce indoor biting then it will also reduce indoor transmission of malaria. However, other investigators have proposed that the irritant properties of permethrin and deltamethrin in treated huts have an unsatisfactory impact on malaria vectors (e.g., Rishikesh et al. 1978). Similar reasoning led to the termination of DDT use in many countries in Soviet Central Asia, Asia, and South Africa after DDT was shown to produce irritant effects in vectors (Bondareva et al. 1986, Sharp et al. 1990). Unfortunately, the use of this chemical may have been stopped because of the very property that made it historically effective in malaria control, namely, strong excito-repellency

action. Our tests suggest that behavioral responses elicited by DDT, permethrin, or deltamethrin might interrupt indoor *An. albimanus*–human contact.

In this study, we used survival analysis techniques for treatment of the data, as described by Roberts et al. (1997). The power of this analysis relates to the use of escape probabilities over time for comparing the responses of different test populations. The escaped mosquito was classified as "dead," while the nonescaped mosquito was classified as a "survivor." We believe that survival analysis minimizes the loss of valuable information and is the method of choice for a biological interpretation of excito-repellency test results.

Busvine (1964) reported that the degree of irritability in mosquitoes varies with the type of insecticide. Both DDT and pyrethroids generally cause mosquitoes to leave treated surfaces before being knocked down. However, in our tests, the pyrethroids produced a more immediate irritant effect than DDT. For these comparisons, the ET_{50} and ET_{90} values proved to be powerful estimators of insecticidal effects on vector escape behavior and served as useful summary statistics for comparing insecticides and doses. Earlier laboratory tests with this vector (Brown 1958, Rachou et al. 1963) in Panama and El Salvador showed that DDT has pronounced behavioral effects. In our comparisons of ET values, DDT at 2 g/m^2 (the field dose) again showed a powerful behavioral effect with *An. albimanus* populations from Guatemala and Belize. The lower ET values, i.e., shorter escape times, for *An. albimanus* when tested against the 2 pyrethroids at LD_{50} and LD_{90} dosage levels were remarkable. If DDT elicits an important behavioral response from *An. albimanus* females by reducing man–vector contact inside houses, then we can assume that this might also be the primary action of permethrin and deltamethrin when used in malaria control applications. Frequently, mosquitoes do not enter sprayed houses, or, if they enter, they often escape before feeding on humans. Consequently, a strong avoidance behavior can reduce human–vector contact and disease transmission.

In conclusion, behavioral responses of malaria vectors to insecticides are important components of the insecticide–malaria control equation. More field research is needed on the behavioral responses of vector populations from different geographic locations to various insecticides.

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