Contact irritancy and spatial repellency behaviors in Anopheles albimanus Wiedemann (Diptera: Culicidae) collected in Orange Walk, Belize, C.A

Isabelle Dusfour, Nicole L Achee, Donald R Roberts, John P Grieco

Contact irritancy and spatial repellency behaviors in *Anopheles albimanus* Wiedemann (Diptera: Culicidae) collected in Orange Walk, Belize, C.A.

Isabelle Dusfour✉, Nicole L. Achee, Donald R. Roberts, and John P. Grieco

Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, U.S.A.

Received 20 February 2009; Accepted 10 July 2009

ABSTRACT: *Anopheles albimanus* Wiedemann is a vector of malaria in northern Belize. Previous studies have identified behavioral responses in this species when exposed to certain chemicals. We used a high throughput screening system to evaluate the contact irritant and spatial repellency responses exhibited by *An. albimanus* collected from Orange Walk, Belize, Central America. *Anopheles albimanus* behavior was significantly altered by a number of key public health insecticides. Significant irritant responses to alphacypermethrin, deltamethrin, permethrin, DDT, and propoxur were recorded, while spatial repellency responses were only elicited by DDT. Malathion resulted in neither a contact irritant or spatial repellency response. These results are in congruence with behavioral patterns previously observed for *An. albimanus* and for *Aedes aegypti* in the same system. This study produced baseline data on the behaviors of *An. albimanus* and confirms that the majority of compounds used as indoor residual sprays may induce premature exiting by *An. albimanus* in addition to killing the mosquito. This effect would decrease contact with humans and thus disrupt malaria transmission. ***Journal of Vector Ecology* 34 (2): 232-237. 2009.**

Keyword Index: Anopheles albimanus, contact irritancy, spatial repellency, Belize.

INTRODUCTION

Anopheles albimanus Wiedemann is one of the primary malaria vectors found in northern Belize (Achee et al. 2000). This species is mainly exophilic, bites at dusk and dawn, and can be either exophagic or endophagic. The involvement of *An. albimanus* in malaria transmission is largely during their high population densities. Therefore, during the intensive malaria control program in the Americas between 1948 and 1992, Indoor Residual Spraying (IRS) using dichloro-diphenyl-trichloroethane (DDT) was successful in reducing vector density and malaria burden in northern Belize. Nowadays, deltamethrin is used as the primary IRS compound. Other insecticides, such as malathion and temephos, are also used for public health purposes.

Insecticides have been evaluated mainly for their toxic effects. However, some chemicals can affect adult mosquito behaviors and their role in disrupting the contact between humans and disease vectors is now well-described (Dethier et al. 1960, Grieco et al. 2000, Roberts et al. 2000, Grieco et al. 2007). These modified behaviors have been observed for several mosquito species, including *An. albimanus*. These behaviors include pesticide avoidance, excito-repellency, contact irritancy, irritability, and non-contact repellency. In light of the fact that a variety of terms have historically been used, we propose definitions of the two actions evaluated in this study: contact irritancy and spatial repellency. A contact irritant action is one that induces oriented movements of the insect away from a chemical only after making tarsal contact, and a spatial repellent action is one that produces oriented movements away from a chemical without making tarsal contact (Rutledge et al. 1999, Roberts et al. 2000). Irritability to DDT has traditionally been measured through the standard WHO conical chamber. Exposure to DDT induced *An. albimanus* to either elicit an irritant response (Brown 1958, Mancera and Hernandez 1960, Vargas 1961), or not (Quinones and Suarez 1989). Chareonviriyiaphap et al. (1997) also studied the effect of DDT and other pyrethroids on different populations of *An. albimanus*. Contact irritancy and non-contact repellency behaviors were measured separately using an excito-repellency box (Roberts et al. 1997, Chareonviriyaphap et al. 2002). Pyrethroids showed an action only after the mosquito made contact with the treated surface, while DDT exhibited both contact and non-contact activities. One exception was recorded in a population collected from Corozal, Corozal District in northern Belize, for which the non-contact behavior was not significant when exposed to DDT. Across the different studies, *An. albimanus* populations have exhibited variable behaviors while exposed to different insecticides used in vector control.

The current study aimed to obtain data on behavioral modifications of *An. albimanus* in response to exposures to different insecticides. Three pyrethroids (alphacypermethrin, deltamethrin, permethrin), an organochlorine (DDT), an organophosphate (malathion), and a carbamate (propoxur) were selected because they were either used in public health or because the action elicited on *An. albimanus* was not yet known. We used a HIgh Throughput Screening System (HITSS) developed by Grieco et al. (2005). To date, the HITSS has proved its efficacy in measuring the contact irritancy and spatial repellency in *Aedes aegypti* populations (Grieco et al.

2005, Achee et al. 2009). A better understanding of these effects on a wider range of insect vectors could have a role in improving vector control strategies by more efficiently disrupting contact between humans and vectors.

MATERIALS AND METHODS

Mosquitoes

Behavioral assays were performed with female *Anopheles albimanus* F1 generation, progeny collected in the area of Orange Walk, Orange Walk District, Belize C.A. (88°33'38.6W, 18°04'46.5N) and in the locality of San Roman, Orange Walk District, Belize C.A. (88°30'33.055W, 18°17'39.036N). Collections of females were conducted in October, 2007, during the rainy season. Eggs were harvested from field populations and the F1 progenies reared to the adult stage. Females used in the assays were from four to seven days-old, non-mated, and starved from sugar for 24 h prior to testing.

Behavioral tests were first performed on the population from San Roman. However, technical problems due to heavy rain prevented further collections at this location. The Orange Walk Town population was then collected. Based on CDC protocol, resistance levels in the larval stage were performed for the insecticides used in the study. Contact irritancy and spatial repellency responses for DDT at a dose of 250 nmoles were measured following protocols described below. No differences were recorded between the two populations, allowing us to treat the two populations as the same (data not shown).

Behavioral assays

Contact irritancy and spatial repellency behaviors were evaluated with the High-Throughput Screening System (HITSS) developed by Grieco et al. (2005) and followed the protocol described at www.usuhs.mil/pmb/TPH/index.html. Nylon strips were treated with 1.5 ml of acetone-based-insecticide-solution and were allowed to dry for at least 15 min prior to being placed into the HITSS metal test cylinder. Controls were treated using the same protocol but with acetone only. All chemicals were acquired as technical grade neat material and were purchased from Sigma-Aldrich (St. Louis, MO): DDT (organochlorine (OC) CAS 50-29-3), malathion (organophosphate (OP) CAS 121-75-5); Chem Service Inc. (West Chester, PA): deltamethrin (pyrethroids (Py), CAS 52918-63-5), permethrin (pyrethroids (Py) CAS 52645-53-1); Ultra (Kingston, RI): propoxur (carbamate (CB) CAS 114-26-1). Alphacypermethrin (pyrethroids (Py), CAS 67375-30-8) were provided by BASF-The Chemical Company (Florham Park, NJ).

Contact irritancy assay

In the evaluation of contact irritancy, the assay configuration consisted of a clear cylinder connected to a metal test chamber. A single replicate of the assay consisted of ten females being introduced into the metal test chamber which contained the treated substrate. The mosquitoes were allowed to rest for 30 s after which time a butterfly valve separating the two ends of the chamber was placed in the open position for 10 min. The gate was then closed and the number of mosquitoes exiting into the clear chamber, as well as those remaining in the metal test chamber and those that were knocked-down, were recorded for each test system. A total of six to eight replicates were performed for each insecticide and dose.

The number of mosquitoes escaping in the treatment was corrected by the number of mosquitoes escaping into the control chamber as well as the number found knocked-down before escaping (in the metal chamber) per dose and insecticide. Percentages of females escaping were then calculated. The significance of the response was evaluated with a nonparametric rank test (PROC NPAR1WAY), comparing the number of mosquitoes in the clear chamber between treated and control assay systems.

Spatial repellency assay

To evaluate the spatial repellency response, the assay was configured so that a central clear cylinder was connected to two metal test chambers (i.e., control and treatment). Groups of 20 female mosquitoes were introduced into the clear cylinder and allowed to rest for 30 s, after which time the butterfly valves were placed in the open position for 10 min. The gates were then closed and the number of specimens entering into either the metal test chamber containing the treatment or control substrate, along with any knock-down, was recorded for each test chamber. Nine replicates of each chemical and dose were conducted. A nonparametric signed-rank test (PROC UNIVARIATE) was used to determine if a spatial activity index (SAI) was significantly different from zero. The SAI values range from −1 (highly attractant) to 1 (highly repellent), with zero indicating no response. This value has been modified from the oviposition index of Kramer and Mulla (1979), and represents the proportion of females in the control chamber divided by the proportion in the treated chamber after correcting for the proportion of females in the control chamber (Grieco et al. 2005).

RESULTS

Contact irritancy assay

Anopheles albimanus showed a significant contact irritancy response to most of the insecticides tested. Pyrethroids at 25 nmoles/cm^2 and propoxur at 2.5 nmoles/cm^2 were strongly irritant (Table 1). The corrected percent escaping ranged from 57.5 to 96.3%. Lower concentrations of these compounds elicited weaker or non-significant responses except for deltamethrin. Thirty-nine percent of the mosquitoes escaped when exposed to alphacypermethrin at 2.5 nmoles/cm^2, whereas no significant movement was observed in response to permethrin at 2.5 nmoles/cm^2 and propoxur at 0.25 nmoles/cm^2. A moderate behavioral response was observed for DDT at both 25 and 250 nmoles/cm^2, with percent escaping equal to 10.41 and 20.27%, respectively. At all doses of malathion, no significant irritancy was observed for *An. albimanus*. In addition, high

Table 1. Responses of *Anopheles albimanus* females in the contact irritancy assay.

Chemical		Dose (nmoles/cm2)	Number of replicates	Mean number escaping (SE)		Corrected % escaping (SE)	Mean % KD in the treated chamber	P†
Family	Name			Treated	Control			
Pyrethroid	α-cypermethrin	2.5	8	2.00 (0.26)	0.62 (0.26)	39.86 (4.62)	27.5 (3.23)	0.0003
		25	8	3.37 (0.41)	0.37 (0.18)	79.44 (4.14)	67.5 (7.05)	0.0001
Pyrethroid	Deltamethrin	2.5	6	6.66 (0.91)	0.33 (0.21)	70.18 (9.93)	5.00 (3.41)	0.0021
		25	6	4.16 (0.98)	0.16 (0.16)	57.5 (10.62)	18.33 (4.01)	0.0043
Pyrethroid	Permethrin	2.5	7	0.71 (0.47)	0.28 (0.18)	2.69 (4.81)	0 (0)	0.7062
		25	6	3.16 (1.04)	0.33 (0.21)	96.29 (2.34)	64.44 (9.87)	0.0021
Organochlorine	DDT	25	8	1.50 (0.18)	0.50 (0.18)	10.41 (1.90)	0 (0)	0.0108
		250	8	2.62 (3.13)	0.75 (0.16)	20.27 (3.13)	0 (0)	0.0010
Organophosphate	Malathion	25	6	0 (0)	0 (0)	0 (0)	0 (0)	1
		250	6	0.83 (3.37)	0 (0)	8.33 (3.37)	0 (0)	0.1818
Carbamates	Propoxur	0.25	6	3.16 (1.07)	0.83 (0.30)	28.70 (13.37)	0 (0)	0.0541
		2.5	6	5.66 (0.76)	0.83 (0.30)	83.70 (6.19)	31.66 (10.13)	0.0021

†*P* values are from Wilcoxon two-sample test for difference between the number escaping in a chemical treatment chamber and a control chamber.

Table 2. Responses of *Anopheles albimanus* females in the spatial repellency assay.

Chemical		Dose (nmoles/ cm2)	Number of replicates	Mean percent responding (SE)	Mean SAI (SE)	SR	P > S
Family	Name						
Pyrethroid	α-cypermethrin	2.5	9	26.11 (6.65)	0.11 (0.20)	3	0.6718
		25	9	32.77 (7.59)	0.21 (0.13)	9.5	0.1250
Pyrethroid	Deltamethrin	2.5	9	25.24 (5.18)	0.05 (0.25)	2.5	0.8359
		25	9	21.67 (2.50)	0.10 (0.17)	5.5	0.4844
Pyrethroid	Permethrin	2.5	9	16.11 (3.89)	0.32 (0.24)	8.5	0.2969
		25	9	7.77 (2.90)	0.05 (0.17)	0.5	1
Organochlorine	DDT	25	9	29.44 (2.27)	0.25 (0.08)	19.5	0.0195
		250	9	43.88 (2.16)	0.79 (0.06)	22.5	0.0039
Organophosphate	Malathion	25	9	18.48 (4.39)	0.42 (0.22)	12	0.1015
		250	9	12.25 (2.64)	0.20 (0.28)	5	0.5703
Carbamates	Propoxur	0.25	9	13.88 (2.32)	0.41 (0.16)	-11.5	0.0625
		2.5	9	12.77 (2.64)	0.40 (0.22)	-9	0.140625

P values are from Wilcoxon two-sample test for difference between the number escaping in a chemical treatment chamber and a control chamber.

numbers of knocked-down mosquitoes were recorded in the treated chambers at the highest doses of the pyrethroids and propoxur (Table 1). Exposure to malathion and DDT did not result in this activity even after a ten-min exposure.

Spatial repellency assay

A significant spatial repellency action ($P < 0.05$) was observed only in response to DDT (Table 2). *Anopheles albimanus* was repelled at 29.44 and 43.88% at doses of 25 and 250 nmoles/cm^2, respectively. Other compounds provoked non-directional responses ranging from 7.77% of activity for permethrin at 25 nmoles/cm^2 to 32.77% for alphacypermethrin at 25 nmoles/cm^2.

DISCUSSION

Anopheles albimanus were exposed to five insecticides from three chemical families in order to establish contact irritancy and spatial repellency. All of these compounds have been associated with vector control programs and are known for their killing effect. Our work demonstrated that a panel of insecticides at different doses also alter *An. albimanus* behaviors.

Anopheles albimanus is irritated by the pyrethroids, DDT, and propoxur, but each chemical elicits varying degrees of response. Among those compounds, permethrin and propoxur at low doses did not significantly irritate *An. albimanus*. A spatial repellency was only observed with DDT. This compound functioned primarily as a repellent and secondarily as a contact irritant. In addition to eliciting behavioral responses, pyrethroids and propoxur induced a rapid knock-down effect.

Our results are congruent with behavioral parameters measured within an excito-repellency box in which *An. albimanus* populations were exposed to deltamethrin, permethrin, and DDT. This excito-repellency assay also revealed a significant but weak response to the pyrethroids in the non-contact repellent configuration after a 30-min exposure, not seen with the HITSS (Chareonviriyaphap et al. 1997). This could be due to differences inherent in the two assay systems rather than differences in the mosquito populations. In addition, our study measured for the first time the effects of malathion and propoxur on *An. albimanus* behaviors. All the behavioral changes measured in this study are also consistent with the ones observed for *Ae. aegypti* under the same chemical exposure, using the HITSS system (Achee et al. 2009). The current laboratory findings with permethrin, propoxur, malathion, and DDT must be confirmed in the field, but there is no indication that these results will not translate to real-world experimental hut studies (Grieco et al. 2000, Grieco et al. 2007). Indeed, the HIgh Screening Throughput System has also demonstrated congruent results with experimental hut studies carried out with *Ae. aegypti* in Thailand (Grieco et al. 2007). Therefore, we can assume that deltamethrin used in Belize as an indoor residual spray could result in a premature exiting of *An. albimanus* from the house in addition to knocking them down. Other pyrethroids, DDT, and propoxur also demonstrated an irritant activity which induce premature exiting if used for IRS. The combined irritant and repellent actions of DDT would explain its success in malaria control in Northern Belize (Roberts et al. 2000). Such results have a direct implication in field and vector control strategy. *Anopheles albimanus* is mainly exophilic but enters the house to bite at dusk and dawn. Preventing the vector from entering the house by repellent action or inducing a premature exiting by an irritant effect will play a role in reducing human-vector contact and thus malaria transmission.

For decades, the search for novel compounds for use as indoor residual sprays (IRS) has focused solely on the toxic actions of these chemicals. Beyond toxicity, chemicals may have irritant and repellent actions, which alter vector behavior to reduce host-vector contact. (Cullen and DeZulueta 1962, Hamon et al. 1970, Chareonviriyaphap et al. 1997, Grieco et al. 2000). This study produced baseline data on the behaviors of *An. albimanus* and confirms that the majority of compounds used as indoor residual sprays may induce premature exiting by *An. albimanus* in addition to killing the mosquito. These results will also serve as a baseline for the evaluation of new chemicals and their selection based on characteristics other than toxicity.

Acknowledgments

The opinions and assertions are the private views of the authors and are not to be construed as official or reflecting the view of the Uniformed Services University of the Health Sciences. We acknowledge the members of the field team, Ireneo Briceno and Russell King, for their time and effort in collecting and preparing mosquito samples. We also thank Dr. Mike R. Sardelis for his assistance in statistical analysis. This work is supported by the "behavior modifying compounds" grant #5U01AI054777-02 funded by the National Institutes of Health.

REFERENCES CITED

Achee, N.L., C.T. Korves, M.J. Bangs, E. Rejmankova, M. Lege, D. Curtin, H. Lenares, Y. Alonzo, R.G. Andre, and D.R. Roberts. 2000. *Plasmodium vivax* polymorphs and *Plasmodium falciparum* circumsporozoite proteins in *Anopheles* (Diptera: Culicidae) from Belize, Central America. J. Vector Ecol. 25: 203-211.

Achee, N.L., M.R. Sardelis, I. Dusfour, K.R. Chauhan, and J.P. Grieco. 2009. Characterization of spatial repellent, contact irritant and toxicant chemical actions of standard vector control compounds. J. Am. Mosq. Contr. Assoc. 25: (in press).

Brown, A. W. A. 1958. Laboratory studies the behaviouristic resistance of *Anopheles albimanus* in Panama. Bull. Wld. Hlth. Org. 19: 1053-1061.

Chareonviriyaphap, T., A. Prabaripai, and S. Sungvornyothrin. 2002. An improved excito-repellency test chamber for mosquito behavioral tests. J. Vector Ecol. 27: 250-252.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.J.

Harlan, S. Manguin, and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus,* a malaria vector in the Americas. J. Am. Mosq. Contr. Assoc. 13: 171-183.

Cullen, J. R. and J. DeZulueta. 1962. Observations on the irritability of mosquitoes to DDT in Uganda. Bull. Wld. Hlth. Org. 27: 239-250.

Dethier, V.G., L.B. Browne, and C.N. Smith. 1960. The designation of chemicals in terms of the responses they elicit for insects. J. Econ. Entomol. 53: 134–136.

Grieco, J.P., N.L. Achee, R.G. Andre, and D.R. Roberts. 2000. A comparison study of house entering and exiting behavior of *Anopheles vestitipennis* (Diptera: Culicidae) using experimental huts sprayed with DDT or deltamethrin in the southern district of Toledo, Belize, C.A. J. Vector Ecol. 25: 62-73.

Grieco, J.P., N.L. Achee, T. Chareonviriyaphap, W. Suwonkerd, K. Chauhan, M.R. Sardelis, and D.R. Roberts. 2007. A new classification system for the actions of IRS chemicals traditionally used for malaria control. PLoS ONE. 2: e716.

Grieco, J. P., N.L. Achee, M.R. Sardelis, K. Chauhan, and D.R. Roberts. 2005. A novel high throughput screening system to evaluate the behavioral response of adult mosquito to chemicals. J. Am. Mosq. Contr. Assoc. 21: 404-411.

Hamon, J., J. Mouchet, J. Brengues, and G. Chauvet. 1970. Problems facing anopheline vector control: vector ecology and behavior before and after application of control measures. Conference on Anopheline Biology and Malaria Eradication. Misc. Publ. Entomol. Soc. Am. 7: 28-41.

Kramer, W.L. and M.S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: oviposition response of *Culex* mosquitoes to organic infusions. Environ. Entomol. 8: 1111-1117.

Mancera, V.O. and J. Hernandez. 1960. El efecto irritante mosquitos de algunas insecticidas de contacto. Pruebas *Anopheles albimanus* Wied. CNEP Bol. (Mexico). 4: 82-85.

Quinones, M.L. and M.F. Suarez. 1989. Irritability to DDT of natural populations of the primary malaria vectors in Colombia. J. Am. Mosq. Contr. Assoc. 5: 56-59.

Roberts, D. R., W. D. Alecrim, P. Hshieh, J. P. Grieco, M. Bangs, R.G. Andre, and T. Chareonviriphap. 2000. A probability model of vector behavior: effects of DDT repellency, irritancy, and toxicity in malaria control. J. Vector Ecol. 25: 48-61.

Roberts, D.R., T. Chareonviriyaphap, H.H. Harlan, and P. Hshieh. 1997. Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. J. Am. Mosq. Contr. Assoc. 13: 13-17.

Rutledge, L.C., N.M. Echana, and R.K. Gupta. 1999. Responses of male and female mosquitoes to repellents in the World Health Organization insecticide irritability test system. J. Am. Mosq. Contr. Assoc. 15: 60-64.

Vargas, V. 1961. Algunas observaciones sobre el efecto irritante del DDT cepas de *Anopheles (Nyssorhynchus) albimanus* y *Anopheles (Anopheles) punctimacula* Costa Rica. Rev. Biol. Trop. Costa Rica. 95: 97-105.