Identification of Elevated Esterase Activity in a Pyrethroid-Resistant Population of *Anopheles albimanus* Wiedemann

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ABSTRACT The standardized diagnostic World Health Organization (WHO) susceptibility test was used to evaluate DDT, permethrin, and deltamethrin on 2 laboratory colonized populations of *An. albimanus* from El Salvador (Santa Tecla) and Guatemala (El Semillero) and 2 field populations from northern (Corozal District) and southern (Toledo District), Belize. The Santa Tecla colony and Corozal field population were susceptible to all 3 compounds while the El Semillero colony showed resistance to all 3 compounds. The Toledo field population showed some resistance to DDT. The specific activity of esterase was measured in 5 populations of *An. albimanus*. These included Santa Tecla colony from El Salvador, El Semillero colony from Guatemala, Cayo population from Central Belize, Toledo population from Southern Belize, and Corozol population from Northern Belize. There was a 4 to 7 fold increase in the specific activity of esterase as measured by the hydrolysis of alpha- and beta- naphthylpropionate in the El Semillero colony compared to all the other populations, to include the TO population. This suggests that the development of physiological resistance to synthetic pyrethroids in the El Semillero colony from Guatemala may be related to increased esterase activity. Based on these overall results, permethrin and deltamethrin are potentially useful for *An. albimanus* control in Belize. The use of DDT in Toledo District seems effective, but warrants close monitoring in the future.

KEYWORDS: *Anopheles albimanus*, pyrethroid resistance, esterase activity.

INTRODUCTION

Resistance to insecticides has been recorded in 504 species of arthropods.[1] This includes *Anopheles albimanus* Wiedemann, one of the most important malaria vectors in Central and South America.[2] This species has demonstrated resistance to all major types of insecticides, including organochlorine compounds such as DDT; organophosphorus compounds such as malathion and fenitrothion; carbamates such as propoxur and bendiocarb; and synthetic pyrethroids such as permethrin and deltamethrin.[3]

The conventional method for measuring resistance is based on the World Health Organization (WHO) susceptibility test[4] which requires a comparatively high number of mosquitoes for testing. This susceptibility test can be complemented by biochemical assays that may give additional information on the underlying mechanisms of insecticide resistance. Two biochemical techniques, the microplate assay and the filter paper test, are often used to evaluate enzyme levels in field populations.[5] These tests are based on reactions that produce visual color differences. Biochemical tests can be used under field conditions without sophisticated equipment,[6] several insects can be evaluated simultaneously, and the same insect can be tested for other enzymes.[7] Esterase activity is often evaluated in organophosphate, carbamate-, and pyrethroid-resistant mosquitoes.[8] A microtiter plate technique was used to detect elevated levels in organophosphate and pyrethroid resistant *An. albimanus*.[3]

In this study, we conducted series of susceptibility tests using the standard WHO diagnostic test on the colonized populations from El Salvador and Guatemala, and 2 field populations of *An. albimanus* from Toledo and Corozal Districts, Belize. In addition, supplementary data were obtained by using the microtiter plate assay to measure the level of whole body esterases in *An. albimanus* obtained from 2 colonies (El Salvador and Guatemala) and 3 field populations from Belize.

Materials and Methods

Mosquito populations. Five different test populations of *An. albimanus* were used: 1) El Semillero colony (ES colony); 2) Santa Tecla colony (ST colony); 3) a field population from Cayo District, Belize (CA population); 4) a field population from Corozal District, Belize (CO population); and 5) a field population from Toledo District, Belize (TO Population). The origins and detailed backgrounds of these 5 *An. albimanus* populations are given in a recent publication.[9]

WHO diagnostic tests. Four populations of *An. albimanus* were exposed for 1 h to diagnostic dosages of DDT (4%), permethrin (0.25%), and deltamethrin (0.025%) according to the WHO protocol.[4,11,12] For each test, 5 cylinders (2 for the controls and 3 for the treatments) were used. Control cylinders contained filter paper impregnated with solvent; whereas, treatments contained paper impregnated with the diagnostic concentration of insecticide in solvent. Twenty mosquitoes were introduced into each cylinder. After 1 h, mosquitoes were transferred to holding containers, and a 10% sucrose solution was provided. Mortalities were recorded at 24 h, and each test was replicated 3 times.

Protein analysis. The total protein content of individual *An. albimanus* mosquitoes was determined using a BioRad protein assay system (Hercules, california). Individual mosquitoes were homogenized in 0.5 ml of phosphate buffer (0.2 mol, pH 7.0) using a plastic microcentrifuge tube and pestle. The homogenate was frozen at -70°C until the assay was performed. Five microliters were assayed using a microtiter plate technique. Results were compared to a standard curve.

Esterase enzyme assay. The methods[14,15]were used with the following modifications: alpha- and beta-naphthylpropionate was used in place of alpha- and beta- naphthylacetate. The quantity of naphthol produced from esterase reactions was calculated from standard curves of alpha- and beta-naphthol. Results were expressed as m-mol product/min/mg protein.

Data analysis. Abbott's formula was used to correct for control mortality. Larvae that pupated during any given test were subtracted from original numbers tested. A one way analysis of variance (ANOVA) was used to compare the protein content and esterase activity within and among populations. Significance was determined at $P < 0.05$.

Results

The percent mortality of adult *An. albimanus* at the single diagnostic dosage[15] is presented in Table 1. The ability of mosquitoes to survive the diagnostic dose after 24 h is indicative of resistance in the population; as defined by percent mortality in the test population. The ST colony and the CO field population were susceptible to both DDT and the 2 pyrethroids, as evidenced by 100% mortality to all 3 compounds. The mortality ranged between 45-50% for all 3 compounds in the ES colony, while the TO field population demonstrated some resistance to DDT (65% mortality), but complete susceptibility to the 2 pyrethroids.

Table 2 shows that there is no significant difference, as determined by an ANOVA, in the total protein content between ST and ES colonies ($P > 0.05$). However, a significant difference in the total body protein content was found when laboratory reared mosquitoes (ST and ES colonies) were compared with the 3 field populations ($P < 0.05$).

Esterase activity in 5 populations of *An. albimanus* was measured (Table 4). Elevated esterase was found only in the ES colony. The increase in esterase activity ranged from 4.7 to 7.2 times for alpha-naphthylpropionate and 3.3 to 3.9 times for beta-naphthylpropionate. An ANOVA was used to compared the esterase activity among the 5 populations for both alpha- and beta-naphthyl-propionate. ANOVA results indicated that esterase activity in the ES colony was significantly higher than the ST colony and the CA, CO, and TO field populations from Belize ($P < 0.05$). No statistical difference ($P > 0.05$) in esterase activity was seen among ST and the 3 field populations (Table 2).

The esterase activity between males and females in ST, ES colonies and CA field population was compared (Table 3). A significant difference in esterase activity between male and female mosquitoes (ANOVA, $P < 0.05$), was seen only in the ES colony. As shown in Table 3, total protein was significantly different between male and female mosquitoes in the ST and ES colonies ($P < 0.05$), but no significant difference was observed in the CA field population ($P > 0.05$).

Discussion

The pyrethroid resistance observed in the *An. albimanus* colony from Guatemala (ES colony) is consistent with the deltamethrin resistance reported by the others.[3,16] Frequently, pyrethroid resistance occurs in DDT resistant populations as reported for *Ae. aegypti* from Thailand[17] and Guyana. [18] Additionally, pyrethroid resistance in larvae was reported in a DDT selected strain of *An. stephensi* from Pakistan.[19] The WHO diagnostic test also indicated that the ES

Table 1. Percent mortality of adult *Anopheles albimanus* at the single diagnostic dosage based on three tests for each population and insecticide (susceptibility WHO test, 1975).

Mosquito population tested*	Permethrin (0.25%)	Deltamethrin (0.025%)	DDT (4.00%)
ST	100	100	100
ES	50	50	45
TO	100	100	65
CO	100	100	100

* 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.

Table 2. Comparison of specific activities of esterase for hydrolysis of alpha-and beta-naphthylpropionate between adult *Anopheles albimanus* populations.

Mosquito population tested*	mg protein/ ml (per mosquito) protein	m-mole-alpha-naphthol/min/mg protein	m-mole-beta naphthol/min/mg	n
ST	0.554±0.079a**	0.585±0.184c	0.711±0.198e	59
ES	0.558±0.093a	2.755±0.084d	2.382±0.427f	60
CA	0.413±0.071b	0.437±0.161c	0.588±0.159e	72
TO	0.435±0.076b	0.399±0.105c	0.607±0.187e	32
CO	0.436±0.068b	0.383±0.114c	0.623±0.158e	44

* ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; CA: Field population from Cayo District, Belize; TO; field population from Toledo District, Belize; CO: Field population from Corozal District, Belize.

** Results of a and b test for protein, c and d for hydrolysis alpha naphthylpropionate, and e and f for beta-naphthylpropionate. No significant difference at 0.05 level in the same latter (within the column).

Table 3. Comparison of the specific activities of esterase for hydrolysis of alpha- and beta-naphthypropionate between male and female adult *Anopheles albimanus* populations

Mosquito population tested*	mg protein/ ml (per mosquito) protein	m-mole-alpha-naphthol/min/mg protein	m-mole-beta naphthol/min/mg	n
ST				
Female	0.596±0.069**	0.619±0.183	0.634±0.180	36
Male	0.488±0.039	0.532+±0.178	0.596±0.222	23
ES				
Female	0.598±0.100**	3.188±0.739**	2.530±0.485**	35
Male	0.522±0.043	2.028±0.354	2.173±0.042	60
CA				
Female	0.415±0.068	0.471±0.172	0.612±0.186	35
Male	0.410±0.073	0.422±0.148	0.564±0.124	37

* ST: Santa Tecla colony from El Salvador; ES: El Semillero colony from Guatemala; CA: field population from Cayo District, Belize.

** Significant difference between male and female *An. albimanus* at 0.05 level.

colony was resistant to DDT, suggesting that resistance to synthetic pyrethroids may have arisen from previous use of DDT.

Results of WHO susceptibility test indicated that the CO field population from northern Belize was susceptible to DDT, but the TO field population from southern Belize expressed some resistance to DDT. This insecticide has been used for malaria control in Belize until 1990 when most anti-malarial activities in the country was suspended due to a shortage of government funds for insecticide purchase. However, DDT is still being used for malaria control along the Mexican-Belizean border in northern Belize (*ie*, Corozal District) through an inter-country agreement in which the insecticide is donated (Vanzie per comm). Additionally, a stratified program of house spraying was restarted in mid 1996 for all of Belize.

Pyrethroids are insecticidal esters derived from primary alcohols and are susceptible to hydrolysis by esterases.[20] Elevated esterase levels have been reported in the pyrethroid-resistant southern army worm, *Spodopera eridania*,[21] and the pyrethroid-resistant Egyptian cotton leafworm, *Spodoptera littoralis*.[22] Similarly, 3 deltamethrin resistant populations of *An. albimanus* from Guatemala have demonstrated elevated esterase level.[3] Our study indicated that there was a 4-7 fold increase in hydrolysis of alpha- and beta-naphthylpropionate to naphthol in whole body homogenates from the ES colony when compared to the ST colony and to the CA, CO, and TO field populations from Belize that were susceptible to pyrethroids.

In both ST and ES laboratory colonies, whole body extracts from females contained significantly more protein than extracts from males. This was not true of the Belizean field populations tested. Additionally, these field-caught mosquitoes were consistently smaller in size than laboratory-reared mosquitoes. External factors such as availability and quantity of food during larval development, larval density, and temperature during larval development, can affect mosquito.[23] Any or all of these factors are more likely to impact on field populations than on laboratory colonies.

A significantly higher esterase activity was found in organophosphate-resistant *Cx. quinquefasciatus* males than in females[24]; however, susceptible females demonstrated a significantly higher esterase activity than susceptible males. In our study, ES females had a significantly higher esterase activity compared to ES males. While the reason for difference is unknown, it could possibly be related to the foraging

of females for oviposition sites or for bloodmeals. It has been shown that selection by toxic substances can increases the amount of enzymes that are responsible for detoxification.[24] Increase in the quantity of enzymes is often associated with gene amplification. Ferrari and Georghiou (1990) reported amplification of an esterase B1 gene in *Cx. quinquefasciatus* that had high titers of an esterase that confers resistance to organophosphate insecticides. Furthermore, gene amplification appeared to be the mechanism causing protein overproduction when an organism is under environmental stress.[25] Female mosquitoes from Guatemala, before colonization, may have been under continual selection pressure when seeking blood meals, nectar or plant fluids, resting sites, or oviposition sites in areas sprayed by pyrethroids or other insecticides, *eg*, females may have visited oviposition sites that were associated with insecticide-treated crops. Indeed, these categories of selection pressure may have played a significant role in the higher esterase activity observed in the female mosquitoes from Guatemala.

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