STUDIES OF THE SUSCEPTIBILITY OF *ANOPHELES ALBIMANUS* AND *ANOPHELES VESTITIPENNIS* FROM DAJABON, DOMINICAN REPUBLIC, TO INSECTICIDES[1]

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ABSTRACT. Insecticide susceptibility of *Anopheles albimanus* and *An. vestitipennis* in Dajabon Province, Dominican Republic, was investigated. Only 74.3% of *An. albimanus* exposed to 4% DDT for 1 h died. The mortality in this species following exposure for 1 h to 0.25% permethrin was also 74.3%. However, this species was susceptible to malathion, fenitrothion and propoxur. The mortality obtained following exposure of *An. vestitipennis* to 4% DDT and 0.1% propoxur, both for 1 h, was 71% and 100%, respectively. However, the number of specimens exposed to propoxur was small.

INTRODUCTION

Studies on the bionomics, vector potential and susceptibility to insecticides of *Anopheles* mosquitoes were conducted in Dajabon Province of the Dominican Republic between July 1987 and October 1988. This paper presents the work on insecticide susceptibility of the 2 most abundant anopheline species in the area.

The history of early intervention measures against malaria and their outcomes are documented in unpublished reports and records of the Servicio Nacionál de Erradicación de la Malaria (SNEM)—the National Malaria Eradication Service, and only to a limited extent in published works (Pan American Health Organization [PAHO] 1984, Sulsona et al. 1985). Such measures in the form of larviciding, drainage and landfill were initiated as long ago as 1941. In 1948, DDT was used for the first time as an intradomiciliary residual spray for the control of malaria vectors. Antimalarial operations were gradually expanded, leading to the establishment of SNEM and the launching of a time-limited malaria eradication program in 1964.

Although *Anopheles* (*Nyssorhynchus*) *albimanus* Wied. was susceptible to DDT in the Dominican Republic during 1960 and 1962 (da Silva 1961,[5] Anonymous 1964[6]), it was found to be resistant to the insecticide in 1964 and later years (Martin et al. 1982,[7] Brown 1983). DDT- and fenitrothion-resistant populations of *An. albimanus* have been reported from Haiti (Brown 1983, Brown and Brogdon 1987). In Mexico and Guatemala (Brown 1986) DDT resistance is documented in *An.* (*Anopheles*) *vestitipennis* Dyar and Knab.

This study was aimed at understanding the current susceptibility status of anophelines to DDT, which is used by SNEM for malaria control, and to other insecticides that may be considered for use. The susceptibility of *An. albimanus* to 6 insecticides and of *An. vestitipennis* to 2 insecticides was investigated.

MATERIALS AND METHODS

Study area: The Municipio (District) of Dajabon, found in the province by the same name, was selected for the study. It lies in the northwest corner of the country, bounded on the west by the Rio Massacre, the river which forms the border between Haiti and the Dominican Republic at this point (Fig. 1, a and b). The study was centered at Dajabon Town, which is the main urban center of both the district and the province. Dajabon was selected because it is a frontier province in which malaria has not been successfully controlled and insecticide resistance in *An. albimanus* was previously detected there. In addition, the area offers a diversity of natural and man-made anopheline breeding habitats and is accessible by road throughout the year. Four sites: Bario La Fe, Calle Duarte, Colonia Japonesa and La Bomba (Fig. 1b) were selected for regular sampling of mosquitoes following a preliminary survey.

Test materials and procedures: Diagnostic test kits and procedures developed and issued by World Health Organization [WHO] (1981) were used for insecticide susceptibility tests. The di-

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[5] da Silva, O. J. 1961. DDT in cycles of 9 months, analysis of Dominican Republic, 9 pp. + 16 tables and 1 map. (Unpublished report).

[6] Anonymous 1964. An entomological study of the border between the Dominican Republic and the Republic of Haiti. 10 pp. + figures and tables. (Unpublished report).

[7] Martin, G. G., J. Ayalde, F. J. L. Antuñano, R. M. Rodríguez, D. Gañan, D. R. S. Cury, E. J. Medina and P. Mencia. 1982. Informe (general) de la evaluación del programa de malaria en la República Dominicana. 66 pp. + annexes. (Unpublished report).

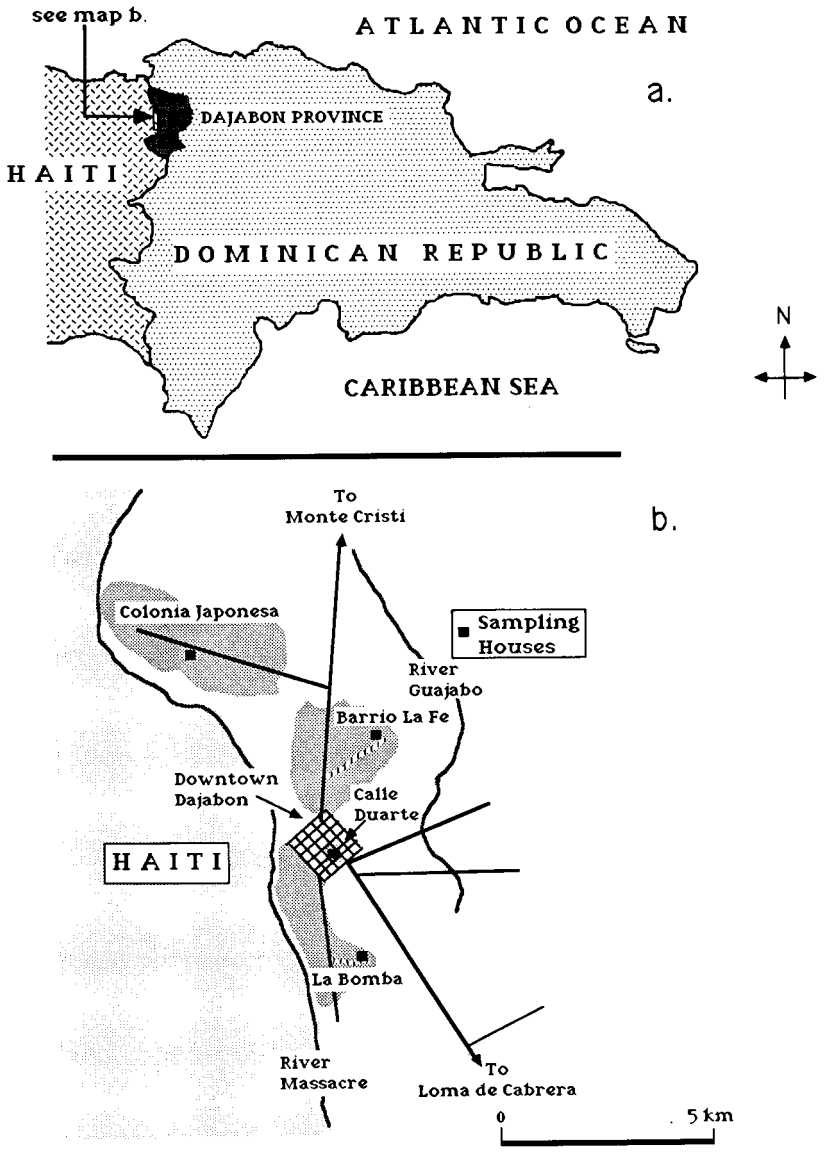

Fig. 1. a, The Dominican Republic and the Province of Dajabon where the study was conducted; b, Study sites in Dajabon Town and surrounding areas.

agnostic test is often used to detect the appearance and monitor the increase of resistance in mosquito populations (Davidson and Zahar 1973, Brown 1986, WHO 1986).

Specimens of *An. albimanus* were exposed for 1 h and 2 h to papers impregnated with 4% DDT, and for 1 h only to 0.25% permethrin, 5% malathion, 1.0% fenitrothion and 0.1% propoxur impregnated papers, although the diagnostic dose of fenitrothion is actually based on exposure for 2 h. In addition, papers treated with 2.13% resmethrin, 5% malathion and 0.1% propoxur produced and provided by the U.S. Army Environmental Hygiene Agency (USAEHA), Aberdeen Proving Ground, MD, were used. The 2.13% resmethrin test papers were a provisional product of USAEHA (B. C. Zeichner, personal communication, 1989). The susceptibility of *An. vestitipennis* was tested by exposure to 4% DDT from WHO for 1 h and 2 h, to 0.1% propoxur from WHO for 1 h and to 0.1% propoxur from the USAEHA for 1 h.

The WHO test and control papers used were well within the expiration dates indicated on the packages, and the USAEHA papers had been produced only a few months prior to their use.

Test specimens were collected at night by man-biting captures, aspiration from corrals and trapping with an animal-baited net trap. Mosquitoes that were not engorged at the time of collection were fed on the arm the same night. Tests were conducted in the field laboratory the following morning. Unless limited by paucity of specimens, 4 or more replicates were run at the same time, with ca. 25 specimens per replicate. We wished to expose at least 100 specimens to each insecticide used, as prescribed by the guidelines (WHO 1981); however, this was not always possible due to the limitation of available mosquitoes. Tests in which control mortalities were greater than 20% were discarded. If control mortalities ranged between 5 and 20%, the test mortalities were corrected by applying Abbott's formula (Busvine 1971).

Test results were statistically analyzed using the chi-square test. In cases where the exposure mortalities were corrected by using Abbott's formula (Busvine 1971), actual numbers of specimens observed to be dead (plus moribund) and alive were not used in chi-square tests performed. Instead, the number of exposed specimens was multiplied by the corrected mortality and rounded off to the nearest whole number to obtain the adjusted number of dead (plus moribund) specimens. This number and the corrected number of live specimens, which was found by subtracting it from the number of exposed specimens, were used in the statistical analyses.

RESULTS: TESTS ON *AN. ALBIMANUS*

DDT: The results of the series of tests on *An. albimanus* using 4% DDT are presented in Table 1. The exposure temperature ranged from 25 to 30°C, the exposure RH from 67 to 84% and the holding temperature from 23.3 to 31°C. The tests based on 1 h of exposure were repeated 3 times at intervals of approximately 6 months. A chi-square test on the results obtained on the 3 occasions showed the mortality rates to be not statistically significant ($\chi^2_2 = 0.620$, $P = 0.73$). The results of tests based on 2 h of exposure were analyzed in the same manner as the results of the 1 h tests, and the weighted mean mortality of 81.0% was computed after statistical tests showed exposure mortalities of the 3 test dates not to be significantly different ($\chi^2_2 = 2.001$, $P = 0.37$). Further, mortalities were compared based on the duration of exposure. The difference between the 74.3% and 81.0% mortalities, obtained after 1 h and 2 h exposure, respectively, was not significant at the 0.05 significance level ($\chi^2_1 = 3.445$, $P = 0.06$).

Pyrethroids: The tests using permethrin and resmethrin were done simultaneously using subsamples of field-collected mosquitoes. The exposure temperature was 27.5°C, the exposure RH 80% and the holding temperature 24–30°C. The results, presented in Table 2, show that the *An. albimanus* population had the same level of resistance to permethrin as for DDT (Table 1). Resmethrin, however, killed 100% of the mosquitoes. It should be noted that WHO has not established a diagnostic dose for this insecticide.

Table 2. Results of susceptibility tests on *Anopheles albimanus* populations from Dajabon, Dominican Republic, using 0.25% permethrin from WHO and 2.13% resmethrin from the USAEHA and an exposure period of 1 h, in October 1988.

	Number[a]		Mortality (SE) %	
Insecticide	Exposed	Control	Exposed	Control
Permethrin	100 (4)	125 (5)	74.3 (4.4)	14.4 (3.1)
Resmethrin	100 (4)	125 (5)	100.0 (0.0)	12.8 (3.0)

[a] Numbers in parentheses represent the number of replicates.

Table 1. Results of susceptibility tests on *Anopheles albimanus* populations from Dajabon, Dominican Republic, using 4% DDT and exposure periods of 1 h (upper half of table) and 2 h.

	Number[a]		Mortality (SE) %[b]	
Test date	Exposed	Control	Exposed	Control
July 1987	106 (5)	88 (5)	76.3 (4.1)	12.5 (3.5)
Dec. 1987	100 (4)	100 (4)	74.7 (4.3)	5.0 (2.2)
July 1988	114 (5)	67 (3)	72.1 (4.2)	9.0 (3.5)
Totals and weighted means of 1 h tests	320	255	74.3 (2.4)	8.6 (1.8)
Sep. 1987	26 (2)	25 (2)	87.5 (6.5)	8.0 (5.4)
July 1988	69 (3)	48 (2)	83.8 (4.4)	10.4 (4.4)
Oct. 1988	109 (6)	110 (6)	77.6 (4.0)	10.0 (2.9)
Totals and weighted means of 2 h tests	204	183	81.0 (2.7)	9.8 (2.2)

[a] Numbers in parentheses represent the number of replicates.

[b] Mortalities were corrected by using Abbott's formula (Busvine 1971) in cases where control mortalities were between 5 and 20%, in all tables.

Organophosphorus and carbamate insecticides: *Anopheles albimanus* was fully susceptible to malathion and fenitrothion as well as to propoxur (Table 3). All 3 have been determined by WHO (1984) to be suitable for use as residual insecticides for the control of mosquito vectors of disease. In this series of tests the exposure temperature was 25.6–30°C, the exposure RH 56–68% and the holding temperature 24–30°C.

TESTS ON *AN. VESTITIPENNIS*

The exposure temperature was 25–30°C, the exposure RH 60–77% and the holding temperature 23.3–29.4°C. The results of tests on *An. vestitipennis* using 1 h and 2 h exposures to DDT and 1 h exposure to propoxur are presented in Table 4. The population had a significant level of resistance to DDT. Although adequate numbers were not tested in the 2 h exposure period, chi-square test showed no significant difference when 1 h and 2 h mortalities were compared ($\chi_1^2 = 2.453$, $P = 0.12$). Although propoxur killed 100% of the *An. vestitipennis*, the results are based on low numbers of mosquitoes tested.

DISCUSSION

Insecticide resistance is an important factor in the control of insect pests and vectors of disease. According to Brown (1986), 56 *Anopheles* species were resistant to DDT, which was and probably still is, the most widely used insecticide for malaria control (PAHO 1988). *Anopheles albimanus* is one of the few species that were found resistant to all 4 groups of insecticides: to DDT in at least 14 countries, to organophosphorus compounds in 6 countries, to carbamates in 5 countries and to pyrethroids in 3 countries (Brown 1986, WHO 1986, Malcolm 1988).

The DDT resistance detected in *An. albimanus* in Dajabon was not a unique finding. In an unpublished report, Feinstein (1987)[8] recorded high levels of DDT resistance in the species in several localities in the Elias Piña and other areas in the southwestern part of the country. All the populations tested by him were susceptible to malathion as were those of Dajabon, as shown by this study. Less than 100% (80–98%) mortality was obtained by him on exposure of *An. albimanus* to the WHO diagnostic dose of the pyrethroid, deltamethrin. This is higher mortality than that obtained in the present study using permethrin, another pyrethroid. Although high levels of resistance to DDT had

[8] Feinstein, B. 1987. Informe de viaje a la Republica Dominicana por Sr. B. Feinstein del 4 de Octubre al 31 de Octubre, 1987. 2 pp. + 3 tables and 2 charts. (Unpublished report).

Table 3. Results of susceptibility tests on populations of *Anopheles albimanus* from Dajabon, Dominican Republic, using organophosphorus and carbamate insecticides and an exposure period of 1 h.

Test date	Insecticide (source)[a]	Number		Mortality (SE) %	
		Exposed	Control	Exposed	Control
Mar. 1988	5% malathion (WHO)	97 (6)	49 (3)	100.0 (0.0)	6.1 (3.4)
Oct. 1988	5% malathion (USAEHA)	118 (6)	82 (6)	100.0 (0.0)	7.3 (2.9)
Oct. 1988	1% fenitrothion (WHO)	110 (5)	75 (4)	100.0 (0.0)	2.7 (1.9)
Oct. 1988	0.1% propoxur (WHO)	121 (8)	106 (7)	100.0 (0.0)	0.9 (0.9)
Oct. 1988	0.1% propoxur (USAEHA)	108 (8)	90 (7)	100.0 (0.0)	4.4 (2.2)

[a] WHO = World Health Organization; USAEHA = United States Army Environmental Hygiene Agency.

Table 4. Results of susceptibility tests on *Anopheles vestitipennis* from Dajabon, Dominican Republic, using 4% DDT and 0.1% propoxur.

Test date[a]	Insecticide (source)[b]	Number[c]		Mortality (SE) %	
		Exposed	Control	Exposed	Control
Dec. 1987/1	DDT (WHO)	100 (4)	100 (4)	71.0 (4.5)	4.0 (2.0)
Sep. 1987/2	DDT (WHO)	33 (2)	17 (2)	84.9 (6.2)	0.0 (0.0)
Oct. 1988/1	Propoxur (WHO)	33 (7)	23 (7)	100.0 (0.0)	0.0 (0.0)
Oct. 1988/1	Propoxur (USAEHA)	29 (7)	64 (7)	100.0 (0.0)	7.8 (3.4)

[a] Numbers after slashes represent the number of h of exposure.
[b] WHO = World Health Organization; USAEHA = United States Army Environmental Hygiene Agency.
[c] Numbers within parentheses represent the number of replicates.

been detected in *An. albimanus* from Dajabon during the late 1970s (Martin et al. 1982),[7] the 25.7% survival on exposure to the diagnostic dose of this insecticide in 1987 and 1988 shows that resistance still persists. This is in spite of DDT or any other insecticide not having been used in the area for public health purposes since 1983. DDT was also not used for agricultural purposes since 1984 or perhaps even earlier. Similar persistence of DDT resistance, in the absence of any known selection pressure by the insecticide, was reported by Herath et al. (1988) in *An. culicifacies* Giles and *An. subpictus* Grassi, in Sri Lanka. It is often asserted that insecticide resistance in a mosquito population is gradually lost and reversion to susceptibility ensues in a matter of months or years after the selection pressure is eased or removed (Abedi and Brown 1960, Pal 1974, Brown 1983).

Selection for resistance in malaria vectors can result from agricultural application of insecticides (Georghiou 1972, Davidson 1982, Brown 1986). Information obtained from the records of Centro de Ventas de Materiales Agropecuarios (CVMA), the governmental agency in Dajabon Town that distributes pesticides to farmers, revealed that in 1987 it sold one organochlorine (only 250 lb of heptachlor bought by one person), 9 types of organophosphorus and 3 types of pyrethroid insecticides. One of the pyrethroids was permethrin. The same source revealed that no other sale of organochlorines has been made since 1984; however, they did not have records of sales made prior to that year.

Because resistance to DDT is known to result in cross-resistance to pyrethroids and vice-versa (Leahey 1985, WHO 1986), it is not surprising that *An. albimanus* was found to be equally resistant to DDT and permethrin. Since pyrethroids are used for crop protection in Dajabon, it is possible that they continue to exert some selection pressure.

The reasons for exposure to resmethrin resulting in 100% mortality in a population of *An. albimanus* that contains a relatively high proportion of permethrin-resistant members may lie in the difference in the chemistry of the 2 compounds and on the concentrations used for testing. One way in which resmethrin differs chemically from DDT and permethrin is in its lack of halogens (Thomson 1986). Toxicologically, it is less active on insects than permethrin. On *An. stephensi* Theobald, for example, permethrin is 1.5 times more toxic than resmethrin (Zerba 1988). Based on these relative toxicity values, it appears that the test concentration of resmethrin was too high.

Despite a brief use of malathion for malaria control in 1981 and the current use of a number of organophosphorus compounds for crop protection, no indication of resistance to this group of insecticides or to carbamates was encountered in *An. albimanus* or *An. vestitipennis*. These insecticides may thus be the chemicals of choice if indoor spraying is considered for malaria control.

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