The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

"The susceptibility and behavioral response of *Anopheles albimanus* Weidemann and *Anopheles vestitipennis* Dyar and Knab (Diptera: Culicidae) to insecticides in northern Belize, Central America"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage that may arise from such copyright violations.

Michael J. Bangs
Department of Preventive Medicine & Biometrics
Uniformed Services University of the Health Sciences

ABSTRACT

The susceptibility and behavioral response of *Anopheles albimanus* Weidemann and *Anopheles vestitipennis* Dyar and Knab (Diptera: Culicidae) to insecticides in northern Belize, Central America

Michael John Bangs, Doctor of Philosophy, 1999.

Dissertation directed by: Donald R. Roberts, Professor, Department of Preventive Medicine and Biometrics

During a 9-month study period (1995-1996) in Caledonia Village, northern Belize, anopheline mosquitoes collected off human-bait and from experimental huts were evaluated for their susceptibility and behavioral responses to DDT and deltamethrin. Adult *Anopheles albimanus, Anopheles vestitipennis,* and *Anopheles crucians* were completely susceptible at diagnostic dosages for DDT (4.0%) and deltamethrin (0.025%) using the standard WHO susceptibility testing method. Dose response probit analysis indicated low heterogeneity for all 3 species populations' response to DDT and deltamethrin.

Behavioral responses were measured using excito-repellency (ER) boxes allowing either direct contact (irritancy) or noncontact (repellency) to be observed. Use of ER boxes produced strong behavioral avoidance responses with DDT and deltamethrin contact irritancy tests during 60-min exposure for *An. albimanus* and *An. vestitipennis* compared to controls and noncontact trials. A significant repellency response was noted for both species during the 5-hr noncontact tests compared to controls.

Six and 12-hour human-landing collections (HLC) within and outside 2 experimental huts, before and after DDT spraying, showed *An. albimanus* to be predominantly exophagic; whereas, *A. vestitipennis* more readily entered the huts to feed on humans. Five, 12-hr evening collections were carried out approximately 12 weeks post-spray. Early evening (1800-2100 hr) feeding patterns within the sprayed hut remained similar to the control, but resting behavior on wall surfaces was greatly diminished in the sprayed hut. Normal indoor entering females exited the sprayed hut sooner. Biting in the sprayed hut rapidly declined after 2300 hr. Overall, the number of entering and biting *Anopheles* over the 12-hr evening period was significantly less than that seen in the control. Epidemiologically, sporozoite ELISA, blood meal analysis, and HLCs incriminated *An. albimanus* and *An. vestitipennis* as the principal malaria vectors in Caledonia.

The behavioral effect of insecticides on mosquitoes has frequently been overlooked as an important means of reducing human-vector contact. This investigation supports the importance of avoidance behavior (excito-repellency), in particular with DDT, as

effective suppressive components in defense against malaria. These results indicate that the continued use of indoor residual insecticide spraying still bears merit as a means of decreasing transmission of malaria in northern Belize.

Key Words (Indexing): *Anopheles albimanus*, *Anopheles crucians*, *Anopheles gabaldoni*, *Anopheles punctimacula*, *Anopheles vestitipennis*, DDT, deltamethrin, insecticide susceptibility, excito-repellency, behavioral avoidance, experimental huts.

The susceptibility and behavioral response of *Anopheles albimanus* Weidemann and *Anopheles vestitipennis* Dyar and Knab (Diptera: Culicidae) to insecticides in northern Belize, Central America

by

Michael John Bangs

Dissertation submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy 1999

ACKNOWLEDGMENTS

I would like to thank all the members on the various committees for providing valuable guidance towards attainment of this prestigious degree: my major professor and mentor supreme, Dr. Donald R. Roberts, and the rest of the esteemed members, Drs. Richard G. Andre (Committee Chairman), Lance L. Sholdt, John H. Cross, Sylvie Manguin, Patrick Carney [Division of Tropical Public Health, Department of Preventive Medicine and Biometrics (PMB), Uniformed Services University of the Health Sciences (USUHS)], and Susan Langreth (Department of Microbiology/USUHS) who variously served on Research, Qualifying Exam and Dissertation Committees. Thanks are also extended to Dr. Harold H. Harlan for his support and suggestions, Dr. Paul B. Hshieh (Division of Biometrics, PMB/USUHS) for invaluable statistical advice and assistance, Mr. Brian C. Zeichner (USACHPPM) for the insecticide impregnated test papers, John F. Invest (Roussel Uclaf Environmental Health Ltd., U.K.) for providing experimental quantities of deltamethrin, Dr. Robert R. Wirtz, Walter Reed Army Institute of Research for the host-specific monoclonal antibodies (Mab) used in the bloodmeal ELISA, and *Plasmodium*-specific Mab for the sporozoite ELISA, Mr. Jim Pecor, Walter Reed Biosystematics Unit, for confirming the identification of submitted mosquito voucher specimens, Ms.Caroline Korves and Ms. Nicole Achee for conducting the sporozoite ELISA tests on the specimens captured in Caledonia, and Dr. Eliska Rejmankova

(University of California, Davis) for her valuable input on the descriptive ecology of the Caledonia study site in northern Belize.

Special thanks and appreciation are extended to Dr. Errol Vanzie, former Director of the Vector Control Programme (VCP) and Mr. Francis Westby, Chief of Operations, for permitting and supporting the field work to take place in Belize. Thanks also to Ms. Linda Reyes and Ms. Shilpa Hakre (Epidemiology Research Center, Belize City, Belize) for administrative assistance during my many stays in country. YNC Wayne Heideman and LtCol Olin Doss (DoD Military Liaison Office, Belize) also provided administrative support for in-country clearances and visa extensions. The gracious assistance of Mr. D. Deheaney (Corozal/Orange Walk Sector Malaria Supervisor) was instrumental in helping to select a suitable study site and providing the DDT and use of a hand-compression sprayer. Entomology technicians, Mr. Gustavo Escalante (Cayo) and Mr. E.R. Montejo (Corozal), providing assistance during the initial site surveys. Thanks are also extended to the Belize Defense Force for allowing me to room and store belongings in the Officers Quarters in Ladyville during my stays in Belize.

Most importantly, my sincerest and warmest appreciation to the people of Caledonia for graciously allowing me to come into their village, live and study over a 9-month period. The essential core effort, involving the tedious, sometimes agonizing, collecting of mosquitoes during the many long evenings, included Alex Rodriguez (Team Leader), Richard Rodriguez, Danny Cab, Mauricio Cab, Roque Teck, Ramiro Cab, Damian Cab, Lucilo Canto, Louis Howe and Aldo Cab, whom without their willing

assistance this investigation would not have been possible.

This dissertation reflects the results of research only. All quotes and figures from published and unpublished works used in this dissertation are hereby gratefully acknowledged, and with the clear understanding that there was no deliberate intention of infringement upon existing copyrights, if any. Mention of a pesticide or a commercial or proprietary product within does not constitute a recommendation or an endorsement of a particular product by the US Department of Defense. This research effort was supported by the Uniformed Services University of the Health Sciences, Grant No. RO87DB.

The answer is in nature

-T. Work

All truth passes through 3 stages.
First, it is ridiculed.
Second, it is violently opposed.
Third, it is accepted as self-evident

-Arthur Schopenhauer

The average Ph.D. thesis is nothing but
a transference of bones from one graveyard
to another

-J. Frank Dobie

Variety is wonderful....
until you have to analyze and interpret it!

-M.J. Bangs, 1996-1999

TABLE OF CONTENTS

APPENDICES Page

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

ATENCION !

SE LE SUPLICA

MANTENGASE ALEJADO

SONA DE

EXPERIMENTO DE MALARIA

Ministry of Health
Belize

CHAPTER 1

General

Introduction

GENERAL INTRODUCTION

"*Residual insecticides are used in the fight against malaria, to interrupt transmission of the parasite and not purely to control the vectors..., it seems that quite often it is forgotten that the true objective of applying these insecticides is to reduce or eradicate the disease*" **A. Gabaldon, 1953.**

In 1939, Dr. Paul Müller first described the insecticidal properties of DDT. He was awarded the Nobel Prize in Medicine in 1948 for his revolutionary discovery, underscoring DDT's most recognized and beneficial use was and is in public health, for malaria control. However, malaria remains a serious health problem throughout many of the tropical and subtropical regions of the world, including the Americas (PAHO, 1994a; WHO, 1997b). Before the impressive insecticidal property of DDT was discovered, malaria suppression programs relied on detailed epidemiological and ecological information to formulate control strategies (Wright, et al. 1972). The pre-DDT era control measures were directed at reducing vector density or human-vector contact, most often by modification of larval habitats. DDT changed the antimalarial strategy dramatically, from an ecological-based to a chemical-based campaign. Over the last 2 decades, the resurgence of malaria has renewed interest in *Anopheles* biology, ecology and behavior as hopeful avenues of study for developing more sound and effective control. Likewise; the dramatic increase in malaria incidence in Belize, beginning in 1991, generated interest in identifying and obtaining detailed descriptions of the vector

roles and biology of the native *Anopheles* species (Roberts, 1996). Unfortunately, relative to many regions of the world, the literature on the bionomics of malaria vectors in the Neotropics remains scanty and geographically fragmented (Elliott, 1969; Tonn, 1983; Zimmerman, 1992). The investigation as described herein was designed to concentrate on a very limited, ecologically defined area, with the hopes of describing a small portion of the complex biology of the indigenous anophelines and their response to insecticides. Emphasis was placed on observing both physiological and behavioral responses of natural *Anopheles* populations to 2 different chemicals and classes of compounds currently in use worldwide for the control of malaria and other vector-borne diseases, namely, DDT (a chlorinated hydrocarbon/organochlorine) and deltamethrin (a synthetic pyrethroid). Accurate knowledge of the influence of insecticides on vectors and a clearer understanding of the local malaria ecology and epidemiology should enable vector control efforts to be more selective and cost-effective. Additionally, this study would also provide important new information for comparison with other malaria vector populations in Latin America.

General study design

This field study took place in the village of Caledonia, located near the New River, in the northern coastal plain of Belize, Central America. Caledonia was chosen as a study site based on several criteria, including a recent history of relatively high malaria incidence compared to most villages in the northern health sector. The Ministry of Health considered the village a priority area for continued malaria control, receiving

yearly DDT spraying despite the significant reduction of vector control activities countrywide beginning in 1989. Other important factors included the presence of suitable larval habitats (Rejmankova, et al. 1993) and acceptable base-line adult mosquito densities, the relative ease of year-round road access to the village, and the availability of sufficient human labor for evening mosquito collections. The study site has been described is detail later in this chapter.

At various periods during 1995-96, three primary interrelated topics of study, concentrated on particular epidemiological parameters important in the transmission and control of malaria (Fig. 1). The primary objective was to quantify, in a standardized way, the toxic and behavioral responses of wild-caught populations of anophelines to DDT and deltamethrin. This integrated approach focused primarily on the behavioral effects of insecticides on the local anopheline species compared to normal feeding behavior and activity patterns. Observations involved: 1) the susceptibility status of wild-caught adult anophelines to DDT and deltamethrin, using the standard WHO contact susceptibility test protocol and supplies; 2) the behavioral response (avoidance or excito-repellency) of *Anopheles* using DDT and deltamethrin in specially constructed response test boxes and, 3) behavioral responses of vectors to DDT-sprayed and unsprayed huts. Two identical experimental huts were constructed to accommodate these tests. Huts were equipped with 4 entry or exit window traps to measure, as near as possible, the natural house-frequenting response of the local vectors. Observations in experimental huts included the use of evening human-bait captures, the use of various colored fluorescent powders for mark-recapture studies of mosquitoes of different physiological conditions, and determination of the differences in indoor resting sites between treated and untreated

huts. Observations were also made of the peridomiciliary (outdoor) human-biting activity using human-landing collections (HLC), and resting patterns of anophelines during the evening hours. After normal patterns of house entering, exiting and biting were documented, the internal walls of one hut were sprayed with 4% DDT. This represents the first known use of experimental huts to study vector behaviors and responses to insecticides in Belize.

This study was similar to objectives given by Rachou et al. (1973), namely, the identification of the anopheline species in the study area, evaluation of the epidemiological importance of the species present, and define the behavior and response of vectors to DDT-sprayed surfaces. However, in El Salvador, Rachou et al. (1966, 1973) conducted far more detailed, longitudinally-based studies than described herein. Both studies other measured other parameters, including: observations of seasonal variations in anopheline densities using evening human-landing collections; DDT susceptibility tests, bioassays of DDT residues on sprayed wall surfaces in experimental huts, and excito-repellency tests of wild-caught adult mosquitoes. Additionally, in the course of my study, I was able to briefly investigate natural daytime resting sites of adult *Anopheles*. Blooded mosquitoes captured from outdoor resting sites were assayed for blood meal origin using an ELISA technique (Chow, et al. 1993). Additionally, anophelines captured by human-bait were tested using an ELISA to detect circumsporozoite (i.e., sporozoites) antigen (Beach, et al. 1992). Unfortunately, because of time and personnel limitations, measures of other important entomological parameters (e.g., adult dispersion, longevity, gonotrophic cycle, etc.) were not possible.

This investigation concentrated on the effects of DDT and deltamethrin. Despite the negative image DDT has maintained since the 1960s (Chapin & Wasserstrom, 1983; Ware, 1994), the decision to assess DDT was justifiable. First, DDT remains the most cost-effective and widely used residual insecticide in the world for the control of malaria (Service, 1992; Roberts, et al. 1997a). Second, the country of Belize continues to use DDT on a limited (focal) priority basis and it remains a valuable tool in the Vector Control Programme's (VCP) arsenal when used to prevent malaria transmission. It was of interest to the Belize VCP to reaffirm the useful status of DDT and clarify its true effects on local vectors. Third, there is a tremendous amount of conflicting information regarding DDT's effectiveness to control vectors and malaria transmission. In particular, the issue of behavioral responses (i.e., excito-repellency), reported over 50 years ago, continues to spark debate and misunderstanding regarding its true implications for malaria control (Kennedy, 1946; Roberts & Andre, 1994). Deltamethrin was selected for assessment and comparison based on a VCP decision to use this compound as an alternative chemical in the event DDT was no longer available (Vanzie, 1995).

This study took place entirely in a field setting. Inherent in such studies is a plethora of issues and obstacles not generally encountered in laboratory-based work. Controlled field experiments investigating aspects of mosquito ecology and behavior are particularly difficult and time consuming (Service, 1993a). Likewise, it is known that mosquito behavior and population dynamics can vary temporally and spatially (Service, 1989). Studies are at the mercy of meteorological vagaries, terrain features, human activities and other uncontrollable local conditions that can, and often do, greatly influence vector distribution and outcome measures (Read, et al. 1978; Wolda & Galindo,

1981). More frustrating are the problems in sampling bias and interpretation because of the degree of biological and behavioral variance living organisms possess superimposed on variations in place, time and environment (Mattingly, 1962; Garrett-Jones, 1970; Bidlingmayer, 1985). The use of capture, trapping and other sampling methods play an essential part in all studies on the ecology and behavior of mosquitoes. The influence of trap design, timing, location and experience can greatly affect capture efficiency (Gillies, 1970). Usually a combination of sampling techniques is required to supplement the limitations of individual sampling methods (Muirhead-Thomson, 1968). Moreover, imponderable aspects of vector biology that cannot be accurately measured, and the complete ignorance of other influences, results in difficult and unproven assumptions being invoked, compounding problems of analysis and interpretation. The inherent heterogeneity in biological measures and responses of individuals and populations, often requiring sufficiently large numbers of experimental replication of sampling and testing, complicates field studies further. Despite these apparent weaknesses, the tradeoff and strength of fieldwork is the ability to investigate biological events under the influence of natural heterogeneity- that which only occurs in Nature. Conversely, extrapolation of observations from laboratory-controlled, often genetically homogenous populations, to situations occurring under natural conditions is generally not allowed

This dissertation does not attempt a complete review of research on public health insecticides or the many malaria vector studies on bionomics or insecticides that have taken place in the Neotropics or the world, in general. Nor does this study intend to generalize other findings with the results presented herein. This study presents results and interpretations that were temporally and geographically limited. Caution should be

exercised extrapolating results beyond the site examined to other regions having the same vector species and seemingly similar epidemiological or ecological characteristics. Nevertheless, the success or failure of any control approach, whether standard (e.g., insecticides, chemotherapy) or emerging technologies (e.g., vaccines, biological/genetic control) will depend ultimately on a better understanding of the natural dynamics of malaria transmission in the field (Collins and Paskewitz, 1995).

Increased Global Malaria: The Vector Control Dilemma

Malaria remains one of the most important infectious diseases throughout the tropical and subtropical world. Annually, between 300-500 million clinical cases and an estimated 1.5 to 2.7 million die from the disease (WHO, 1997a). Malaria greatly undermines the health and welfare of inhabitants, endangering the lives of millions and is a major obstacle to social and economic development.

Malaria is a remarkable disease. Despite nearly a century of monumental effort to control this malady, malaria remains one of the most important diseases of humans, worldwide. In terms of shear morbidity and resultant mortality, the unrelenting misery caused by this parasite throughout recorded human history has been enormous (Harrison, 1978). Overall, progress against malaria has been significant despite a dramatic resurgence of the disease today (Campbell, 1997; Krogstad, 1996; Olliaro, et al. 1996). Beginning in the 1950s, the wide-scale use of residual insecticides and the availability of effective antimalarial therapeutics, successfully eradicated endemic malaria from many temperate regions (e.g., United States, most of Europe) where it was once prevalent

(Brown, et al. 1976; Bruce-Chwatt, 1970; Wright, et al. 1972; Gabaldon, 1978; Haworth, 1988). Indoor residual spraying with DDT is considered the major reason for the overall success of malaria control in the 1950s and 1960s (WHO, 1995). These much-heralded efforts effectively cleared malaria from the more temperate regions; but it remains entrenched in many subtropical and tropical countries.

As malaria has resurged in modern times, its incidence in some areas has attained unprecedented and alarming levels and continually threatens expansion into regions considered malaria-free (WHO, 1997a). Despite years of continued intervention, the disease continues to threaten or afflict nearly half of the world's population. In the 1990s, the overall picture of malaria is one of frustration, neglect and monumental disappointment.

At the beginning of the 20th century, one of the principal methods of malaria eradication and control had been environmental modification and source reduction of larval habitats, followed 50 years later with the use of residual insecticides inside houses against adult mosquitoes (Russell, et al. 1963; Pant, 1988). In other words, a tactic of direct assault on the reduction of vectors and/or human-vector contacts. However, despite the documented and lasting successes in eliminating or reducing disease throughout many parts of the world, recent efforts by international organizations and others have advocated a significant reduction in the use of vector control as a principal intervention method. Instead, greater emphasis has been given to increasing early disease diagnosis and prompt treatment strategies (Najera, 1989; Trigg & Kondrachine, 1998). In some areas, increased use of synthetic pyrethroids and insecticide-impregnated bednets have over-optimistically replaced indoor residual spraying programs (Miller, 1988).

However, compared to indoor residual spraying, the overall effectiveness of this strategy regarding actual protection afforded has been remarkably varied from study to study (Rozendaal, 1989).

The reasoning behind the changes in the control strategy is the still widely held belief that insecticide resistance (i.e., reduced susceptibility, behavioral avoidance) and innate vector behavior (exophagy and exophily) in a number of important anopheline species negates any significant effects that could be achieved by standard methods of vector control, including the use of indoor residual spraying (Gillies, 1956; Busvine & Pal, 1969; Metcalf, 1983; Bang, 1985; Chapin & Wasserstrom, 1983). However, in those countries continuing to use DDT, in spite of high levels of physiological resistance present, this insecticide still continues to reduce disease incidence (WHO, 1986; Haworth, 1988; Roberts & Andre, 1994). The reduction of vector control programs has been deemed the most important cause for increasing malaria in Africa, Asia and the Americas (Farid, 1991; Roberts, et al. 1997a; Mouchet, et al. 1998). The last several decades have seen a number of countries, for one reason or another, experience dramatic increases in malaria after having stopped routine spray programs. Likewise, there are examples of striking reductions in malaria incidence after resumption of spraying with DDT (Roberts, et al. 1997a). Madagascar, which has witnessed large periodic epidemics of malaria in the 1980's, finally resumed a DDT house spraying program in 1988 that drastically reduced the incidence of disease by more than 90 per cent by 1993 (Mouchet, et al. 1998). Fifty years ago, Gabaldon (1949a) stated ..."that the action of DDT residual spraying should be measured only in terms of malaria reduction and not deduced *a priori* from studies of its effects on mosquitoes." The repercussion from stopping vector

control activities has been an increase in disease incidence. The justification for the continued use of insecticides, under any circumstances, is simply the reduction in disease incidence.

More recently, international calls have been made for the complete elimination of DDT for use in public health programs (Curtis, 1994; PAHO, 1994b; WHO, 1994; Lopez-Carrillo, et al. 1996) because of environmental and human health concerns and the perceived poor effectiveness in the control of vectors. This runs counter to the compelling evidence and rationale for continued use of DDT and other chemicals to control vectors (Brown, 1972; Brown et al., 1976; Service, 1992, Roberts & Andre, 1994; Roberts et al. 1997a). Moreover, public policy decisions on pesticide use and regulation in developed countries, especially the United States, continue to influence pesticide usage in less developed nations were legitimate public health needs still exist (Higley, et al. 1992). Much of the blame for the present resurgence of malaria could be justifiably directed at logistical and operational problems, not technical ones (Davidson, 1989). Shrinking preventive medicine health budgets for operational costs and an indifference by governments and international bodies appear most to blame for many of the re-emerging vector-borne diseases worldwide (Brown, et al. 1976; Gabaldon, 1978; Bruce-Chwatt, 1979; Farid, 1991; Service, 1992; Arata, 1994).

The resurgence of malaria worldwide, including the Americas, has renewed interest in *Anopheles* biology (Tonn, 1983; Service, 1989; Zimmerman, 1992). It is well known that there are considerable variations in malaria epidemiology, even within small areas, due to differences in geography, ecology and human activities. The bionomics and behavior of *Anopheles* vectors (e.g., feeding activity, host preference, longevity, resting

habits, flight range, malaria susceptibility, etc.) varies among and within particular species in response to specific ecological, seasonal and meteorological conditions (Gillies, 1956, 1988; Elliott, 1969; Zimmerman, 1992). Evidence also suggests intraspecific genetic differences over a species' range may account for some of the observed biological variations (Smits, et al. 1996). As malaria transmission is strongly linked to climate and landscape and other ecological conditions, detailed studies on vector biology and vector responses to environmental conditions must continue to play a major role in understanding the epidemiology and risk of malaria transmission.

No single control tool or approach is appropriate for malaria control in all epidemiological situations (WHO, 1995). Rather than planning malaria control for broad geographic areas, consideration must be given to transmission characteristics within defined ecological zones, as well as variations that occur at the district and village level (Service, 1989; 1993a). This philosophy and a greater understanding of vector bionomics has gradually been incorporated into an Integrated Pest Management (IPM) approach to vector control. Various methods of vector reduction, such as environmental modification or elimination of larval habitats, release of biological control agents (e.g., larvivorous fish), community health education (e.g., bednet use), improved housing (e.g., walls, window screening) and other methods are included under IPM. Furthermore, within IPM, selective chemical control methods, when appropriate, should be used (Brown, 1983). An integral part of the decision process about control strategy requires accurate identification and surveillance of the ecological and entomological characteristics of areas with defined risks (WHO, 1975a).

However significant, issues of multi-drug resistant malaria parasites, insecticide and 'behavioral' resistant vectors, the rising costs to maintain even rudimentary control efforts and exponential human population growth and expansion have all contributed to the current malaria crisis. The fact that populations and countries at highest risk are often the poorest and most disadvantaged in allocated resources only serves to compound the malaria control problem.

In recent decades there has been a dramatic shift in research dollars and emphasis given to molecular and genetic approaches for understanding and controlling malaria. Support for vector studies, particularly fieldwork on mosquito biology, has greatly diminished in comparison (El-Mallakh, et al. 1998). Significant advances in knowledge have been made through this effort, but little has translated into operationally relevant control outside the laboratory. Annually, millions of dollars are expended for vaccine research alone, so far with minimum success. Unfortunately, workers handling any vaccine will face the unenviable task of wide scale distribution at reasonable cost to the poorest, most inaccessible, malaria-endemic areas. The logistics for doing so are daunting (Oaks, et al. 1991). In all, the tenacious ability of this small assemblage of microscopic organisms to thrive, in spite of a seemingly tenuous life cycle and the persistent human attempts to control it, has made malaria all the more remarkable a disease.

Aspects of toxicity and biochemical/physiological resistance

Pesticides are a pervasive component of our modern existence. Without them, the general lifestyle we enjoy today with regards to our health and nutrition would probably be different in many respects. The price we pay for pesticide application is one of its most vexing evolutionary products: resistance. However, the operational significance of resistance can vary greatly from one location and situation to the next. Resistance to insecticides by mosquitoes dramatically illustrates the selection principal of evolution. Resistance is simply the result of applying a stringent biological (genetic) selection mechanism, such as a pesticide, over a number of generations (Georghiou, 1972a; Georghiou & Saito, 1983).

It is estimated that over 90% of all pesticides are used in agriculture and less than 10% in public health (Shidrawi, 1990). The conservative number of arthropods resistant to pesticides stands at around 500, most being major pests to agricultural (~75%) and public health (Georghiou, 1986). Brown (1986) listed 109 mosquito species as resistant to organochlorines (primarily DDT and/or dieldrin). A WHO database on resistance from 1947-85 includes 186 arthropods of medical and veterinary importance as resistant to one or more insecticides, including a disproportionate share (63%) of mosquitoes (117 species). At least 67 species of anophelines (nearly 20% of described species) are known resistant to one or more pesticides (Shidrawi, 1990). An increasing number of *Anopheles* have also developed resistance to newer, synthetic pyrethroids (Malcolm, 1988). Fourteen species, including *An. albimanus*, show resistance to 3 or 4 chemical groups. In particular, the intense use of agricultural insecticides has been strongly correlated with

selection of high levels of resistance to multiple classes of compounds by some vector species (Lines, 1988). This has been documented extensively in *An. albimanus* from Central America (Georghiou, 1972b; Bown, 1987). Where insecticide resistance in disease vectors has had real or apparent epidemiological, economic or political impact, this has often led to a change in control strategies (Metcalf, 1983).

The occurrence of insecticide resistance in medically important arthropods has received considerable attention beginning shortly after the discovery of resistant house fly and mosquito populations in the 1940s (Hoskins & Gorden, 1956; Busvine, 1956; Davidson, 1958, de Zulueta, 1959; Brown and Pal, 1971). The measurement, interpretation, significance and proposed alternative measures to counteract resistance has generated much review and debate (Davidson & Zahar, 1973; Molineaux, et al. 1979; Brown, 1981; Brown, 1986; Georghiou, 1986; Plapp, 1986; WHO, 1992; Roberts & Andre, 1994; Brogdon & McAllister, 1998).

In my research, two different classes of compounds were evaluated for toxicity and behavioral response of wild-caught mosquitoes to contact (irritancy) and non-contact (repellency) designs. DDT and synthetic pyrethroids (deltamethrin) are neurotoxins acting upon the peripheral nerves and on the central nervous system affecting nerve transmission. These insecticides interact at the molecular level with sodium ion channels by delaying sodium inactivation and suppressing potassium permeability across the nerve phospholipid cell membrane. (O'Brien, 1978; Ware, 1994). A change in binding affinities results in continuous hyperexcitability producing tremors, convulsions and paralysis (prostration) resulting in eventual death. Because of the similarity of target site

receptors and biochemical/genetic mechanisms, pyrethroid cross-resistance with DDT is common (Prasittisuk & Busvine, 1977; WHO, 1986; Zerba, 1988).

Two general types of responses to insecticides are recognized: physiological, often involving biochemical mechanisms, and behavioral avoidance (Roberts & Andre, 1994). Physiological resistance to pesticides is well known and can be defined as an inherited characteristic imparting the development of an ability in a population of insects (or any pest) to tolerate doses of one or more pesticides that would prove lethal to individuals in a 'normal' population of the same species (WHO, 1995). In contrast, insecticide 'tolerance' is the ability of an individual organism to withstand a dose of poison that would have been lethal at some point earlier in its lifetime. Resistance is a process of genetic selection, the inheritable ability to withstand toxicants, that results in a percentage of individuals (strains) in the general population being resistant. The frequency of a gene or genes that code for a particular resistance mechanism, conferring resistance, will vary by geographic location and can increase or decrease over time depending on selection pressure (Abedi & Brown, 1960; Brown, 1981). DDT resistance in *Anopheles* is considered monofactorial, with incomplete dominance, that is responsible for a fluctuating percentage of resistant mosquitoes in a population over time. The development of resistance is dependent on the genetic variability present in the target population either before or arising during the period of selection (Oppenoorth, 1985). Sometimes the level or degree of resistance (proportion of resistant homozygotes) can be quite high and develop over a short period of time (Davidson & Zahar, 1973; Brown, 1986). Resistance in a species is rarely universal or fixed (constant) so that extrapolating resistance patterns from one population to other geographical populations is inappropriate

(Georghiou & Mellon, 1983). Populations, separated in time and space, must be tested as discrete units to determine susceptibility status and its possible implications on control (Brent, 1986).

Resistance can be understood as the result of changes in regulatory genes controlling the amount and nature of target proteins or enzymes synthesized (Plapp, 1986). A variety of single and multiple cross-resistance mechanisms have been described in arthropods depending on the class of compounds involved (Georghiou & Saito, 1983; Oppenoorth, 1984; WHO, 1986; Miller, 1988). The two recognized mechanisms of resistance are grouped as target site resistance/insensitivity and metabolic resistance/detoxification (Devonshire, et al. 1992). For DDT (organochlorines) and/or pyrethroids, the most prominent mechanisms include the selection for the *kdr* (knockdown resistance) gene and sodium channel target-site insensitivity (Narahashi, 1983). Resistance is mediated also by qualitative and quantitative changes in proteins that result in insecticide detoxification, including multi-function oxidases (monooxygenases) and increased general esterases (for pyrethroids), increased metabolism by glutathione *S*-transferase (DDT-ase or DDT-dehydrochlorinase) for DDT, metabolic hydrolases (for pyrethroids), and reduced cuticular penetration (Kerkut & Gilbert, 1985; Zerba, 1988). Simple biochemical assays have been developed to detect various enzyme-based resistance mechanisms in mosquitoes (Brown & Brogdon, 1987; Brogdon, 1989). These assays have an advantage of allowing detection of low levels of (incipient) resistance. Biochemical assays were not used in this study because standard contact assays indicated that the study populations were highly susceptible to DDT and deltamethrin.

The standard WHO adult mosquito susceptibility test method (WHO, 1981a,b) was used in this study, primarily as a commonly recognized means of comparability with results from other regions (Rouch & Miller, 1986; Shidrawi, 1990). This test uses standardized insecticide-treated papers of varying concentrations, placed in holding tubes, on which groups of mosquitoes are allowed contact over a set period of time. Knockdown response and mortality are measured during and after exposure for up to 24 hours. Tests can be altered to reflect response of mosquitoes to different chemical doses over a particular time period or use of a single dose (usually a designated diagnostic concentration) comparing varying exposure times (Ariaratnam & Brown, 1969). As originally intended, this test was designed to reproduce, as nearly as possible, the results of a mosquito resting on an insecticide-treated surface (wall) inside a house, either before or after a blood meal. The goal reflects the original development of the method as an assessment tool in malaria control (WHO, 1981c). Although, limitations and criticism have been lodged in disfavor of this testing procedure (Davidson & Zahar, 1973; Shidrawi, 1990; Brogdon & McAllister, 1998), particularly with the use of wild-caught mosquitoes, this test remains the most widely used and comparable method today.

Vector behavior in relation to insecticides and disease control

The behavior and habits of disease vectors are important components of disease transmission. The behavior of mosquitoes in general and the effects of insecticides on altered behavior are critical in the understanding and control of vector-borne diseases (Kennedy, 1946; Mattingly, 1962; de Zulueta & Cullen, 1963; de Zulueta, 1964, Hamon,

et al. 1970; Elliott, 1972; Elliott & de Zulueta, 1975; Gillies, 1988; Klowden, 1996). The modified behavior of mosquitoes in response to various chemicals has been known for decades (Muirhead-Thomson, 1950; Downs & Bordas, 1951; Muirhead-Thomson, 1960; Pampana, 1963). The phenomenon of excito-repellency, especially irritancy, has generated both interest and controversy in terms of measurement, mechanisms of genetic expression, interpretation and significance in control of vectors and/or disease (Gabaldon, 1953; Muirhead-Thomson, 1960; de Zulueta, 1962; Coluzzi, 1963; Busvine, 1964; Elliott, 1969; Roberts, 1993; Roberts & Andre, 1994). Many specialists have considered the strong excito-repellency of DDT and various other chemicals used as residual sprays to be an important obstacle to vector and malaria control (Muirhead-Thomson, 1951; Bruce-Chwatt, 1970). However, others have advocated a serious reexamination of the issue in light of the facts (Roberts & Andre, 1994; Roberts, et al. 1997a). Contrary to conventional thought, in areas that have documented resistance and/or excito-repellency behavior in the local vectors, effective control is still being achieved by the regular use of DDT. A recently developed stochastic model of vector behavior, supported by analyses of selected field data, indicates that irritancy and repellency (avoidance behavior) predominate, quantitatively, over the toxic properties of DDT as the primary means of reducing indoor human-vector contact (Roberts, et al. unpub. doc.). The impact of DDT residues on the indoor activities of vectors helps explain the continued effectiveness of some spraying programs despite the presence of physiological resistance in mosquito populations. The development of resistance to pyrethroids in the Americas has raised public health concern (Beach, et al, 1989a; Cordon-Rosales, et al. 1992). However, physiological resistance to pyrethroids may have limited operational impact on their

continued effectiveness either as residual insecticides applied inside homes or when impregnated on bednets/curtains provided a vector-human barrier persists in the form of behavioral avoidance (Beach et al. 1989a; Miller, et al. 1991; Lindsey, et al. 1992; Evans, 1993; Arredondo-Jimenez, et al. 1997; Chareonviriyaphap, et al. 1997).

As with physiological/biochemical resistance patterns, mosquito behavioral patterns must be evaluated for discrete and separate populations to determine their specific role in the transmission of disease pathogens and the assessment of control methods. The epidemiological relationship and response of each mosquito varies with physiological condition, innate response to stimuli and environmental conditions (Hamon, et al. 1970, Roberts, et al. 1984). Problems in the interpretation of behavioral observations still exist because of the difficulty in detecting and analyzing behavioral alterations in populations and the paucity of quality field studies. Unresolved controversies still surround issues of development of so-called behavioral 'resistance' or adaptations (i.e., a population-based change in a species genetics) in response to insecticides as opposed to behavioral avoidance as innate natural responses present in a population and not necessarily 'selected' for or against during time of exposure. (Muirhead-Thomson, 1960; Gerold & Laarman, 1964; de Zulueta, 1964; Hooper & Brown, 1965; Gould, 1984; Lockwood, et al. 1984). Although behavioral resistance has been described or mentioned as having occurred in insects as far back as 1945 (Sparks, et al. 1989), there is, as yet, no convincing example of behavioral 'resistance' to insecticides in mosquitoes (Hamon, et al. 1970). In other words, it appears reasonable that a vector's host-seeking activities are altered more as a result of natural behavioral responses to a chemical, and not necessarily due to fixed genetic changes.

Roberts (1993) has reviewed much of the background and issues surrounding behavioral avoidance of mosquitoes to insecticides. Many terms have been applied, some with conflicting definitions, describing chemically-induced behaviors while insects are in motion or at rest (van Thiel, 1951; Dethier, et al. 1960; Georghiou, 1972a; Kennedy, 1978; Lockwood, et al. 1984). The terms 'repellent', 'irritant' and 'excito-repellent' as defined by Roberts (1993) have been adopted in this study. Herein, 'behavioral avoidance' (excito-repellency) is the ability of an insect to detect and avoid an insecticide-treated surface by contact irritancy (i.e., with physical tarsal contact) and/or non-contact repellency (i.e., without tarsal or physical contact). Irritability is a general property of most insects and represents one of the possible responses to disagreeable external stimuli detected by chemoreception (Chapman, 1982). It is well known that DDT deposits exert a direct irritant effect on adult mosquitoes (Kennedy, 1946). DDT contact has been shown to elicit specific effects on insect chemoreceptors and sensory hairs (Smyth & Roys, 1955; Soliman & Cutkomp, 1963).

Compared to irritancy, the repellent effect of insecticides has been far more difficult to measure objectively. Shortly after DDT and BHC (benzene hexachloride) were introduced for house spraying, the observed 'fumigant' effect and repellent properties of both were being advanced (Gabaldon, 1949a, 1952; Field, 1950; van Thiel, 1951; Ludvik, et al. 1951; Muirhead-Thomson, 1951). Smith and Webley (1969) were the first to demonstrate an outflow of DDT from the house interior after spraying which, in their estimation, resulted in strong repellency ("deterrency") on the part of the local anophelines. DDT residues have been detected in indoor air over many months post-spray (Singh, et al. 1992). Gebert (1948), in an apparent fit of mind, had suggested

mosquito (*An. funestus*) antennae were able to detect the "....disintegration of DDT accompanied by the emission of micro-waves, either electronic or sound....which would warn them of the impending danger." As far as known, Gebert was never again allowed to publish on this subject.

Field studies from Africa, Asia and the Americas have clearly demonstrated strong behavioral avoidance of sprayed walls by anophelines when using modified native houses or experimental huts (Muirhead-Thomson, 1960; Roberts, 1993). De Zulueta and Cullen (1963) described the repellent ("deterrency") effect of DDT and dieldrin that effectively prevented some anophelines from entering insecticide-treated houses. Similarly, others have found convincing evidence for a repellent effect on *An. gambiae* and /or *An. funestus* avoiding entering sprayed houses (Muirhead-Thomson, 1950; Wilkerson, 1951; Kuhlow, 1962; de Zulueta et al. 1961; Cullen & de Zulueta, 1962; Smith, 1963). In the Americas, Roberts and Alecrim (1991) demonstrated significant repellency in *An. darlingi* for up to two months post-spray in the Amazon Basin. Similar conclusions regarding reduced house entering by *An. darlingi* have been made in Guyana (=British Guiana) and Surinam (Symes & Hadaway, 1947; Rozendaal, et al. 1989). Repellency was considered a factor in the reduction of house entering and indoor human blood feeding by *An. pseudopunctipennis* in Mexico (Downs & Bordas, 1951; Loyola, et al. 1990; 1991a). In Asia, Colless (1953) described DDT as a contact repellent, with a suggestion of a "vapour effect" (true repellency), which altered or inhibited the normal host-seeking behavior of *Anopheles balabacensis* in northern Borneo. Repellency has been shown or strongly indicated for members of the *An. punctulatus* complex in New Guinea (Slooff, 1964), *An. minimus* and *An. dirus* in Thailand (Nutsathapana, et al. 1986;

Suwonkerd, et al. 1990), *An. maculatus* (with γ-isomer BHC) in Peninsular Malaysia (Wharton, 1951), and *An. culicifacies* in India (Shalaby, 1966). Roberts (1993) has provided further background evidence of insecticide excito-repellency in mosquitoes.

The two preferred methods for vector behavior studies have involved specially constructed experimental houses (huts), designed with entrance and exit traps to sample mosquito populations, and use of excito-repellency test boxes to quantify direct responses to contact (irritancy) and noncontact (repellency) aspects of avoidance behavior. Various test designs and apparatuses, amendable to laboratory and field settings, have been developed for evaluating behavioral responses of mosquitoes to residual insecticides (Duret, 1958; Muirhead-Thomson, 1960; Coluzzi, 1962, 1963; Elliott, 1964; Busvine, 1964; WHO, 1970; Elliott, 1972; Gheorghiu, et al. 1972; Roberts, 1993; Roberts et al. 1997b). Unfortunately, no single method or analysis for the study of behavior has been widely accepted, often resulting in difficulties interpreting and comparing excito-repellency data (Rachou, et al. 1965; Roberts et al. 1984). In response, Roberts et al. (1997b) have designed an excito-repellency test box as a standardized test procedure to measure graded behavioral avoidance to insecticides under differing conditions. The concept and box design is analogous to a single house where mosquitoes are inside and provided free behavioral options to rest, fly or exit the chamber. Chareonviriyaphap et al. (1997) has proven the utility of excito-repellency boxes in the evaluation of behavioral responses to insecticides in laboratory and field settings. The box is similar in principal to the design of Rachou et al. (1973). A same set of exposure chambers was used in this study. The improved design has allowed a highly reproducible means of objectively

measuring both irritancy and repellency responses (with and without physical contact with insecticides), among populations, insecticides and various concentrations of chemicals over time. Moreover, the interpretation of data has been greatly enhanced by use of survival analysis and the log-rank method as a means of comparing and estimating mosquito escape rates among and between populations, different species and dose levels.

The use of specially constructed experimental huts has held considerable importance in the evaluation of the efficacy of spraying houses with residual insecticides or using insecticide-impregnated bed nets (Muirhead-Thomson, 1968; Service, 1993b). As mentioned, experimental huts have also been instrumental in the study of normal and altered mosquito behavior concerning the movements of mosquitoes in and out of occupied houses. From the mid 1940s to the early 1970s, experimental huts played an important role in assessing new insecticides and vector behavior (Muirhead-Thomson, 1960, Smith, 1964). For reasons that are unclear, recent decades have seen a diminishing number of studies using experimental hut techniques. Considerable time must be spent designing huts that most favorably reflect the needs of the research objectives while maintaining, as close as possible, the similarity of the structure to houses used by local populations (WHO, 1975b; Rapley, 1961). Most often, these huts are fitted with a variety of entry and exit traps fitted to windows, doors or eaves (Worth, 1953; van Thiel & Metselaar, 1955). Other devices have been designed to augment captures such as verandah traps, louvre traps and the "Colombia curtain" (van den Assem, 1959; Smith, 1963, 1965; Elliott, 1972; Bown, et al. 1986). A review of the various hut designs, associated trapping methods and their use and interpretation is given by Service (1993b).

Anopheles: the target of study

There are approximately 14 species of *Anopheles* listed as present in the country of Belize (Wilkerson, et al. 1990). Fortunately, only a few represent important vectors of malaria in the country and the region as a whole (e.g., *An. albimanus*, *An. darlingi*, *An. pseudopunctipennis*) (Kumm & Ram, 1941; Bertram, 1971; Roberts, et al. 1993). During the course of my study in Caledonia, five species of *Anopheles* were collected inside and outside of experimental huts. The 2 most common were *An. albimanus* Wiedemann, 1820 and *An. vestitipennis* Dyar and Knab, 1906, followed in relative abundance by *An. crucians* Wiedemann, 1828. *An. gabaldoni* Vargas, 1941, and rarely *An. punctimacula* Dyar and Knab, 1906. The presence of these species in northern Belize has been documented (Kumm & Ram, 1941; Rejmankova, et al. 1993, 1995, 1998). *Anopheles pseudopunctipennis* was not encountered, presumably because of unacceptable riverine habitats (Rejmankova, et al. 1993). *Anopheles darlingi* was not detected in Caledonia, despite its presence along the New River north and south of the study site (Manguin et al. 1996; Andre, et al. unpub. data.). Species abundance is a critical factor in the study of mosquitoes and is among the greatest limiting factors in mosquito research. In this study, only *An. albimanus* and *An. vestitipenniis* were in sufficient numbers to merit detailed study and are reviewed herein. The other three species were relatively uncommon or very rare during the study periods and will be covered, as appropriate, in Chapter 4.

***Anopheles (Nyssorhynchus) albimanus* Wiedemann 1820**

Anopheles albimanus [Latin. *albus*, white; *manus*, hand] (Kitzmiller, 1982).

Relative to other anophelines in Central America, an impressive amount of published research has been generated on *An. albimanus* (Breeland, 1980; Elliott, 1969; Fredrickson, 1993; PAHO, 1996). Its malaria vector status, wide distribution and presence in high population numbers has made this species an easy target of study. The ecology and habits of this species has been described from many localities, including Haiti (Taylor, 1966; Hobbs, et al. 1986), Dominican Republic (Mekuria, et al. 1990b, 1991), Nicaragua (Mendoza, et al. 1991), Costa Rica (Kumm, et al. 1940), El Salvador (Breeland, 1972; Rachou, et al. 1973; Breeland, et al. 1974), Colombia (Elliott, 1968; Marten, et al. 1996), Venezuela (Gabaldon, 1949a), Jamaica (Boyd & Aris, 1929; Muirhead-Thomson & Mercier, 1952a,b; Belkin, et al. 1970), Cuba (Carr & Hill, 1942), Mexico (Savage, et al. 1990; Rodriguez, et al. 1992a, Rodriguez, et al. 1993, 1996), Panama (Simmons, et al. 1939; Trapido, 1946), Guatemala (Zamora & Calderon, 1991) and Belize (Rejmankova, et al. 1992, 1993, 1996; Roberts, et al. 1993).

Anopheles albimanus is distributed widely throughout the tropics and subtropics of the Americas. Its distribution extends from the southern United States (Texas and Florida), most of the Caribbean Islands, Mexico and Central America, the Atlantic coastal plains of northern South America and along the Pacific coast to northern Peru (Fig. 2) (Charles & Senevet, 1953; Faran, 1980; Darsie & Ward, 1981). It is predominately a tropical lowland species, most abundant at elevations less than 500 meters, and occurring

in greatest abundance along coastal lowlands and waterways, generally at distances less than 100 miles from ocean coasts (Rubio-Palis & Zimmerman, 1997).

Anopheles albimanus has long been recognized as a major vector of malaria throughout most of its range (Zetek, 1915, Rozeboom, 1941; Komp, 1942; Horsfall, 1955; Carpenter & LaCasse, 1955; Foote & Cook, 1959). This species has been found naturally infected with *Plasmodium falciparum* and *P. vivax* in nearly every country in which it is encountered (Fredrickson, 1993). It is regarded the most important coastal vector of malaria in Mexico and Central America. It is considered a primary malaria vector in Belize (Kumm & Ram, 1941; Foote & Cook, 1959; Russell, 1963) and is generally the more abundant vector encountered on the northern coastal plain of Belize (Faran, 1980; Rejmankova, et al., 1993; Rodriguez, et al., 1993).

Taxonomically, the species has been reviewed morphologically in the adult, larval and egg stages (Faran, 1980; Rodriguez, et al. 1992b), cytogenetically by analysis of polytene chromosomes (Hobbs, 1962; Keppler, et al. 1973; Narang, et al. 1991), by reciprocal hybridization studies (Keppler & Kitzmiller, 1969; Narang, et al. 1991), by use of allozyme frequencies (Narang, et al. 1991), and by ribosomal DNA (Beach, et al. 1989b; De Merida, et al. 1995). Despite its apparent morphological and chromosomal uniformity, the species is an ecologically and behaviorally polymorphic taxon (Beach et al. 1989). Although, it has yet to be definitively proven, the existence of cryptic (morphologically identical) species within *An. albimanus* remains a possibility because of its extensive geographic range, phenotypic variation and diversity of habitats (Breeland, 1972, 1974; Faran, 1980; Elliott, 1969; Zimmerman, 1992).

This species often occurs in high densities, with periodic, seasonal fluctuations. Periods of maximum abundance generally coincide or immediately follow periods of high rainfall (Kumm & Zuniga, 1944). Throughout its range it has feeding habits considered primarily exophagic and zoophagic (Elliott, 1969; Elliott, 1972; Breeland, 1972; Garrett-Jones, 1964; Garrett-Jones, et al. 1980). In general, this species has shown exceptionally low natural sporozoite rates, rarely reaching 2.0%, and generally below 1.0% (Horsfall, 1955; Rachou, et al. 1973; Warren, et al. 1975; Ramsey, et al. 1986; Mekuria, et al. 1991; Beach, et al. 1992; Fredrickson, 1993). It has likewise shown low relative experimental vector competence compared to other species (Eyles and Young, 1950; Chan, et al. 1994; Chege & Brier, 1998). This species is considered to have a low vectorial capacity and generally requires large population numbers for effective malaria transmission (Elliott, 1972; Rodriguez, et al. 1992a; Loyola, et al. 1993). However, even high population densities of this species do not necessarily translate to increased transmission rates to the human population (Bown, et al. 1991).

Dispersion (flight range) and survival of a vector is an important consideration when conducting studies on biology (mark-release techniques), transmission potential and planning control operations. *Anopheles albimanus* is recognized as a strong flier and periodic (non-appetial, migratory) flights up to 12 miles from breeding places have been recorded (Curry, 1934). However, maximum dispersal appears to be far shorter, being in the range of 1-1.5 miles (Zetek, 1915; Eyles, 1944). Studies in El Salvador found appetitive (goal-oriented) flights averaged 500 m in the dry season and <1 km in the wet, with survival extending to 14 days post-release (Lowe, et al. 1975). Hobbs et al. (1974)

recorded maximum distances of 3 km (maximum range 1-3 km), with averages of only 500 m from the release site and survival up to 11 days post-release during the dry season.

A wide variety of suitable larval habitats and chemical/temperature characteristics has been described over its geographic range (Rozeboom, 1941; Breeland, 1972; Frederickson, 1993). The most extensive surveys of larval habitats in Central America were conducted by Heinemann and Belkin (1977). In general, this species prefers sun-exposed sites, ranging from fresh water pools to brackish water swamps. On occasion, it has been found in crab holes. tree holes and large artificial containers (Faran, 1980). Depending on the regional ecology (e.g., Pacific verses Caribbean coasts), it can be found commonly in open canopy swamp forests, flooded pastures, ponds, lakes, backwashes of streams, lagoons and open marshes (Rejmankova, et al. 1993). Other sites can include permanent and semi-permanent pools, seepages, and irrigation ditches. Larval habitats are generally rich in microorganisms and floating or emergent vegetation (Savage, et al. 1990; Rejmankova, et al. 1992, 1993; Marten, et al. 1996). In Belize, environmental determinants associated with larval distribution and adult abundance have been described (Rejmankova, et al. 1993, 1995). In particular, marshes, sparsely populated with emergent macrophytes and dense cyanobacterial (blue-green algae) mats have been identified as very productive *An. albimanus* larval habitats in northern Belize (Rejmankova, et al. 1996). However, aquatic vegetation was not shown to be a reliable indictor of *An. albimanus* production in Colombia (Marten, et al. 1996). River margins consisting of aquatic grasses and water hyacinth have also been found productive. During dry seasons, the permanently flooded marshes and river margins seem to account for high abundance (Rejmankova, et al. 1995). On the other hand, seemingly ideal wet

season flooding of nutrient poor savannas in northern Belize does not create favorable larval habitats (Rejmankova, et al. 1993). Extensive investigations have revealed characteristic vegetation types and aquatic habitats detected by satellite data can provide high predictive value for identifying relative wet and dry seasonal distribution and densities of *An. albimanus* (Rejmankova, et al. 1995).

Resistance to insecticides has been prolifically documented in *An. albimanus* (Davidson, 1963; Brown & Pal, 1971; Georghiou, et al. 1972; WHO, 1986, Brown, 1986). Fredrickson (1993) has reviewed the importance and historical development of resistance in this species. By the middle 1960s, DDT and dieldrin resistance in Latin America populations of *An. albimanus* was widely distributed (Fig. 3). *Anopheles albimanus* was the first species to show multiple resistance to compounds other than organochlorines (DDT, dieldrin) (Davidson & Sawyer, 1975). However, it was soon realized that the extent and degree of resistance is not uniform over its geographical range (Brogdon, et al. 1988). The wide scale use of insecticides in agriculture, in particular rice, cotton and irrigated sugarcane, has greatly influenced the selection of high levels of multiple resistance seen wherever vectors and agriculture co-exist (Georghiou, et al. 1973; Hobbs, 1973; Bown, 1987; Lines, 1988). In Central America and Mexico, resistance patterns have been dramatically higher along the Pacific coastal regions, in association with the more extensive use of land for pesticide-intensive crops compared to the eastern seaboard. The correlation of high agricultural insecticide coverage and resistance patterns in *An. albimanus* extended to include other organophosphates and carbamates (Georghiou, 1972b). Reduced susceptibility to other carbamates and pyrethroids also occurred, in part, because of parallel cross-resistance mechanisms

(Ariaratnam & Georghiou, 1974; Malcolm, 1988; Beach, et al. 1989a; Brogdon & Barber, 1990). Elevated nonspecific esterases and insensitive acetylcholinesterase are regarded as the principal resistance mechanisms involved. The development of resistance to insecticides has often been cited as an important reason for the apparent or predicted failures to control malaria in Central America (Busvine & Pal, 1969; Brown & Pal, 1971; Beach, et al. 1989a; Frederickson, 1993).

With the exception of *Anopheles gambiae*, *An. albimanus* has been the most commonly evaluated anopheline regarding behavior and the impact of insecticides (Roberts, 1993). The behavior of *An. albimanus* in response to insecticides, particularly DDT, was noted shortly after the introduction of residual house spraying in Panama (Trapido, 1952). The DDT-induced excito-repellent response (irritability) to DDT in *An. albimanus* has been reported over most of its geographic range, from Mexico to Colombia (Brown, 1958; de Zulueta, 1964; Frederickson, 1993). Recently, tests carried out in Belize using excito-repellency boxes, have shown a strong excito-repellency escape response by 2 field populations to DDT, permethrin, and deltamethrin (Chareonviriyaphap, et al. 1997). Similarly, in coastal Chiapas (southern Mexico) and northern Guatemala, the pyrethroids, lambdacyhalothrin and permethrin, respectively, when impregnated on bednets produced noticeable excito-repellency against *An. albimanus* (Richards, et al. 1994; Arredondo-Jimenez et al. 1997).

***Anopheles (Anopheles) vestitipennis* Dyar and Knab 1906**

Anopheles vestitipennis [Latin, *vestitius*, clothing, mourning garmets; *penna*, feather, wing; *-is* adjectival suffix] (Kitzmiller, 1982).

Relative to *An. albimanus*, very little is known about the taxonomy, genetics, bionomics and malaria vectorial status of *An. vestitipennis*. It would be fair to say that this species deserves much more attention given its frequent feeding behavior on humans and suspected importance as a vector. This large, predominately dark-scaled beast, is found from southern Mexico through Central America (Wilkerson, et al. 1990) where distribution appears restricted to lower elevation coastal zones. Gabaldon (1949b) mentioned it occurring in Central America and the Greater Antilles. It has been identified from Jamaica, Hispaniola, Cuba, Puerto Rico and the Lesser Antilles (Belkin, et al. 1970; Belkin & Heinemann, 1972). It appears more commonly in the northern extent of its distribution (Mexico, Guatemala, Belize). It is also reportedly found along northern South America (Columbia, Venezuela) (Rozeboom, 1941; Lane, 1953; Arredondo-Jimenez et al., 1996a) (Fig. 4). Komp (1942) considered the species restricted to the coastal plains along the Atlantic and Gulf coasts only. Vargas and Martinez-Palacios (1956) described it occurring in the coastal zones of the Gulf of Mexico in southern Mexico and the Yucatan; however, it is also found commonly along the central Mexico-Guatemala border (Kumm, et al. 1943; Loyola, et al. 1991; Arredondo-Jimenez, et al. 1996a).

Evidence that *Anopheles vestitipennis* is an important malaria vector is epidemiologically strong. This species has been incriminated as a "potential" vector in northern Guatemala (Haworth, 1988; Padilla et al. 1992; Richards et al. 1994), as "probable" in southern Mexico (Rodriguez & Loyola, 1990; Loyola, et al. 1991b; Arredondo-Jimenez, et al. 1996a; Arredondo-Jimenez, et al. 1998), as "secondary" in the Dominican Republic (Mekuria et al. 1991) and "potentially important" in Belize (Kumm & Ram, 1941; Roberts, et al. 1993). It is also a suspected vector in the Greater Antilles island group (Boyd & Aris, 1929; Carley, 1931; Haworth, 1988). A few unsuccessful attempts with experimental infections have been conducted (Simmons, 1941; Horsfall, 1955). Carr and Hill (1942) were able to obtain oocysts from 1 of 12 specimens fed on a gametocytemic patient in Cuba. To date, only Kumm and Ram (1941) in Belize have detected a (1/41) salivary gland infection by dissection. All other natural infections have been based on the use of a monoclonal antibody based ELISA for sporozoite detection. In most cases, where *An. vestitipennis* has been 'incriminated', it has been a predominant species biting humans and is present in association with *An. albimanus*. In Belize, it is thought that the vector efficiency (e.g., indoor human-biting rates) of *An. vestitipennis* for malaria transmission may be a greater factor than numerical abundance alone (Roberts, et al. 1993; Rejmankova, et al. 1998). In southern Mexico, studies have indicated that relatively high longevity and multiple blood-feeding habits (Arredondo-Jimenez, et al. 1998) enhance the vectorial role of this species in the Lacandon Forest area (southern Mexico).

Anopheles vestitipennis is one of 26 species/subspecies of the subgenus *Anopheles* found in Mexico and Central America (Knight & Stone, 1977; Wilkerson, et al. 1990;

Harbach, 1994). Taxonomic keys and reviews (Vargas, 1963; Clark-Gil & Darsie, 1983; Wilkerson, et al.1990; Wilkerson & Peyton, 1990) clearly identify both adult and larval stages from closely allied forms in the Arribalzagia Series of mosquitoes (e.g., *An. gabaldoni, An. punctimacula*). The fourth stage larvae have shown high phenotypic variability from the same locality in southern Mexico (Bonilla et al. 1996), and variations in adult body color are seen in parts of its geographical range (Komp, 1942). Arredondo-Jimenez et al. (1996a) has recently suggested the existence of 2 genetically different subpopulations in southern Mexico based on behaviorally distinct host preferences.

Adult *An. vestitipennis* has been described as comparatively uncommon throughout its range, yet focally abundant. It generally is seen at highest biting densities during or immediately following rainy periods (Rozeboom, 1941; Komp, 1942; Boyd & Aris, 1929; Arredondo-Jimenez, et al. 1996b, Rejmankova, et al. 1998). Although exceptions exist, this species has been found to be anthropophilic (human-biting) and equally exo- and endophagic (outdoor/indoor feeding) (Komp, 1942; Foote & Cook, 1959). It is generally regarded as exophilic (outside resting) (Elliott & de Zulueta, 1975); however, Navarro et al. (1986) has reported the species as sometimes endophilic in Cuba.

This species has been reported commonly entering houses and readily feeding on humans in Mexico (Hoffmann, 1929; Vargas & Martinez-Palacios, 1956; Loyola, et al. 1991), Cuba (Carr & Hill, 1942; Marquetti, et al. 1990, 1992), Costa Rica (Kumm, et al. 1940), Puerto Rico (Pritchard & Pratt, 1944), Jamaica (Boyd & Aris, 1929), Guatemala (Richards, et al. 1994), and Belize (Foote & Cook, 1959; Roberts, et al. 1993). At one site in southern Mexico, it has not shown a particular predilection for either indoor or

outdoor feeding (Loyola, et al. 1991). However, in the Dominican Republic it has been found to be mostly exophagic, feeding on humans in high numbers with a higher human-biting index than *An. albimanus* in the same area (Mekuria, et al. 1990b, 1991). Evening biting activity has been reported as unimodal (early evening) in the Dominican Republic (Mekuria, et al. 1990b) and bimodal in Cuba (Marquetti, 1990). In Belize, Heinemann and Belkin (1977) recorded information on landing-biting collections from surveys in 1967 in the Cayo District by Bertram (1971). In that work, *An. vestitipennis* was found to be a frequent human biter during late afternoon and early evening collections within deciduous broadleaf forests around Roaring Creek and the Caves Branch area. Information regarding natural longevity (survivorship), dispersal and gonotrophic cycle has only recently been reported (Mekuria, et al. 1991; Arredondo-Jimenez, 1995; Arredondo-Jimenez, et al. 1998).

The larval habitats of *An. vestitipennis* have been described. Most anecdotal accounts summarize preferred habitats as permanent, fresh, cool water, predominately or permanently shaded, in association with floating and/or heavy emergent vegetation (Boyd & Aris, 1929; Komp, 1942; Horsfall, 1955; Vargas & Martinez-Palacios, 1956; Foote & Cook, 1959; Belkin, et al. 1965, Loyola, et al. 1991; Rejmankova, et al. 1998). In Costa Rica, Kumm, et al. (1940) described finding larvae in "sluggish" streams, with vegetation and algal mats or in overgrown shaded pools. In Cuba, extensive swamps, with partially shaded spaces of open water among dense emergent vegetation were found productive (Carr & Hill, 1942). Mekuria, et al. (1990b) found many types of habitats apparently suitable for this species. Most had emergent vegetation and/or algal mats at peripheral sites of water bodies, including permanent ponds and rice fields. It was

frequently found in the same habitats with *An. albimanus* and *An. crucians*. Foote and Cook (1959) described habitats as cool, fresh water ponds, seepages and streams with abundant shade. They went further to mention, that in Puerto Rico this species was associated with sugar cane fields with stagnant ditches filled with dense vegetation, while in Belize, large numbers of larvae were found associated with "small clumps of coarse grass in flooded forest rain pools." Along the Pacific Ocean Coastal Plain of southern Mexico, this species has been associated with cattail (*Typha* spp.) marshes in environmentally disturbed transitional zones between mangrove-land ecosystems (Arredondo-Jimenez, 1995). Rejmankova et al. (1998), using larval habitat surveys found preferred locations included tree-dominated and tall dense macrophyte-dominated environments, such as marshes and flooded swamp forests. It was found with equal frequency in both swamp-forest and marshes associated with greater shade cover. Interestingly, many sites could not be readily identified by spectral analysis, presumably because of their small size. It is also possible that other suitable habitats for this species have yet to be identified.

Resistance to DDT in *An. vestitipennis* has been reported from Mexico, Guatemala (Brown, 1986; WHO, 1992) and the Dominican Republic (Mekuria et al. 1990a). Nothing definitive is known about susceptibility to other insecticides. The paucity of information on insecticide susceptibility is most likely a reflection of poor sampling efforts. Likewise, little has been reported on its behavioral responses to insecticides. Richards, et al. (1994) found a marked reduction in the indoor resting population in houses that had permethrin-impregnated bed nets compared to control households, suggesting to the authors both a repellency and toxic effect.

Malaria in Belize

The malaria endemic countries of the Americas, comprising 21 countries and territories, now report more than 1 million cases of malaria per year, with an estimated malaria mortality rate of 156 per 100,000 population (WHO, 1995). This represents a dramatic increase in disease incidence from the previous decades. Over 40% of the Latin American population are at risk for contracting malaria. Malaria is a highly significant health problem in Belize (PAHO, 1994c; Vanzie, 1995). In 1939, (then British Honduras), an estimated 40 % of all hospital patients and 50% of the population outside of the urban center of Belize City had malaria (Faust, 1949). In 1930, over 10% of all certified hospital/dispensary deaths were due to malaria (Scott, 1932). Decades later, in 1994, malaria ranked fifth among the ten leading causes of hospital admissions in Belize City (Vanzie, 1995).

In 1994, the entire population of Belize (est. 205,000) was considered at 'high risk' for malaria transmission, the only country in Latin America to have such a distinction (PAHO, 1994a). The country leads on all standard epidemiological indicators for malaria in the Central American region. Between 1993 and 1994, Belize had an incidence of 42 and 47.4 per 1000 inhabitants, respectively, the highest in Central America (WHO, 1996b, 1997b). Microscopically confirmed cases dramatically rose from 3,033 in 1990 to nearly 8,600 in 1993. In 1994, Belize reported 9,957 cases, an all time high for the country, with most of the cases reported from the Cayo, Toledo, and Corozal Districts. A substantial increase in *Plasmodium falciparum* malaria also has been seen, increasing from none in 1972 (Haworth, 1988) to 40 cases in 1990 to over 475 cases in

1995 (VCP, unpub.data). Beginning in 1989, the Belize government temporarily suspended nearly all use of DDT for malaria control, thus effectively ending most organized vector control activities. Excluding those years when spraying was stopped (the years 1963-66, and 1990-91), 1993-96 represented the lowest house coverage rates since the program began.

Malaria Control in Belize

Indoor residual insecticide spraying remains the primary method of malaria vector control in the Americas (WHO, 1995). In 1992, DDT accounted for 73% by weight of the insecticide used for indoor residual spraying in the Americas. In many instances, large-scale application of insecticides has not been sustainable because of financial and operational constraints, together with a shift in re-prioritization on how best to combat the disease (Roberts, et al. 1997a). The reputed impact of DDT resistance in *An. albimanus* continues to be cited as the most serious technical/operational problem in Central America and Mexico (WHO, 1995).

The important malaria vectors in Belize are still considered *Anopheles albimanus* in the lowlands and *An. pseudopunctipennis* in the forested and higher elevations. *Anopheles darlingi* and *An. vestitipennis* have also been implicated as important (Kumm & Ram, 1941; Roberts, et al. 1993, 1996). Physiological resistance to DDT and other insecticides by anophelines in Belize has not been seen as widespread or severe (Chareonviriyaphap, et al., unpub. data). This matches a general trend noted along the

eastern coasts of Central America and Mexico compared to the much lower susceptibility levels seen along the Pacific coast (WHO, 1992).

In Belize, the use of DDT as an indoor residual spray for malaria control began in 1957 (Brown et al., 1976). By 1963, as a result of an intensified national spray program, the disease had virtually disappeared (PAHO, 1986). The entire country was placed in a 'consolidation phase' of eradication that same year. From 1963 onwards, yearly spray coverage fluctuated from near complete to no coverage at all. Retrospectively, a strong correlation between decreased spray coverage and the increase in the number of malaria cases was seen (D.R. Roberts, per. comm.). In 1988, Belize banned the use of DDT for all agriculture, while greatly reducing its use in public health, presumably until existing in-country stocks were depleted. Belize abandoned the nationwide coverage of routine house spraying the following year, while continuing limited focal spraying operations in areas experiencing high rates of malaria. Apparently, little or no spraying occurred in 1990-1991 (PAHO, 1994a). Inadequate spray coverage, and possibly more effective surveillance, contributed to the dramatic (reported) increases in the incidence of malaria countrywide during this period. Despite the alarming disease trend, the Government of Belize (Belize Pest Control Board), temporarily suspended all use of DDT for malaria control in 1993, principally because of continuing objections from some developed countries and international funding agencies. Shortly before suspension (1992), spray coverage was less than 24 per 1000 inhabitants. This effectively ended vector control activities for most of the country and further aggravated a deteriorating condition (Vanzie, 1995). The Government of Mexico, fearing a wave of malaria along their southern border, provided selective DDT spraying in the northern districts from 1993-

1995. In late 1996, DDT was reinstated for indoor residual spraying, but was restricted for use in only the most malarious villages per district. Although the renewed spraying program has shown a significant impact on transmission, the risk of malaria still exists throughout the year in all rural parts of the country.

In 1993, less than 5 per cent of the total Health Sector budget was devoted to malaria control programs (PAHO, 1994a). Vector Control Progamme (VCP) units are set up and minimally staffed in each of the 6 administrative districts. During the time of this study (1995-1996), an inter-country agreement between Mexico and Belize was re-initiated to coordinate anti-malaria spraying activities especially along the shared border. Belize continues both passive and limited active case detection, in addition to maintaining a village-level voluntary collaborator network (MOH, 1993). As of 1995, chloroquine remained effective for the treatment of all malaria species present (*P. falciparum*, *P. vivax* and *P. malariae*), augmented with primaquine.

Study site: Belize and Caledonia Village

The country of Belize is located on the southeastern part of the Yucatan peninsula of Central America (Fig. 5). Belize has a total land area of 21,400 square kilometers and a population density of less than 10 per km^2. It shares borders with southern Mexico and eastern Guatemala. Nearly half of the country is low-lying coastal plain with numerous waterways and lagoons, the remainder is hilly or mountainous (Fig. 6). The coastal plain regions consist of typically flat savanna and swampy lowlands, at elevations of generally less than 20 meters asl. The interior region has been uplifted over 1,000 m to form the

forested montane region of the Maya Mountains (peak elevation 1,122 m). Climate in Belize is primarily subtropical, with smaller tropical wet transition zones extending southward (Hartshorn, et al. 1984). Maximum and minimum temperatures are relatively constant throughout the year. Average diel temperatures in the coastal region range from 24°C in January to 27°C in July. There is a distinct dry/cooler season of around 3 months (December-April), but it can vary from year to year. The rainy season typically lasts from May/June through November. Annually, the southern part of the country receives on average of three times more rain than the north (385 vs. 128 cm/yr). Only a small portion of the country is under regular cultivation. The principal commercial crops consist of sugarcane (in the north), and tracts of banana and citrus fruit plantations elsewhere. Irrigated rice is commonly grown in the southern district of Toledo. For additional information on Belize, see Merrill (1993) and PAHO (1994b). For detailed information on land resources and the diverse environmental profiles of the country, consult Wright, et al. (1959), Hartshorn, et al. (1984) and King, et al. (1992).

A proposed 'ecoregional' classification for malaria vectors in the Neotropics is based on environmental determinants, including rainfall, vegetation type, mean temperatures, elevation and geomorphology places northern Belize in the 'coastal ecoregion' (Rubio-Palis & Zimmerman, 1997). Similarly, the worldwide malaria epidemiological classification developed by Macdonald (1957) and adopted by Russell et al. (1963), places Belize plainly in the 'Central American' zone. These 12 worldwide zones are based primarily on *Anopheles* species distribution in relation to general landscape epidemiology (geography, seasonality, altitude and aquatic habitats). Unfortunately, such classifications are far too general for characterizing specific habitat

diversity as needed to predict local vector distribution and potential risk of malaria transmission.

Malaria occurrence in the rural areas of the northern coastal plain is influenced by a mosaic sylvan epidemiology involving extensive herbaceous wetlands, including swamp forests, permanently flooded marshes, and river/riparian ecosystems. Hartshorn, et al. (1984) has categorized the northern lowlands of Belize as part of a larger "subtropical moist forest life zone", with characteristic broadleaf forests, rich in lime-loving species (Wright, et al. 1959), and with average annual rainfall between 130 and 200 cm. This life zone encompasses a large area surrounding Caledonia and all of the Corozal District. I am grateful to colleagues who have provided detailed vector ecology information for northern Belize, including an independent assessment around the site chosen for this study. Detailed descriptions of the northern coastal plain and the wetland ecosystems have been provided by Rejmankova et al. (1993, 1995, 1996, 1998). A substantial part of the lowlands of northern Belize are characterized by relatively undisturbed herbaceous wetlands providing a variety of important anopheline larval habitats (Rejmankova, et al. 1995). Two principal river systems, the Rio Hondo and the New River drain the Northern Coastal Region. Water quality in this region is typically high in calcium carbonate (limestone) and calcium sulfate (gypsum). Many undeveloped areas in northern Belize are waterlogged much of the year, supporting diverse freshwater and mangrove swamp forests. Epidemiologically, important areas include freshwater marshes dominated by tall macrophytes (e.g., sawgrass, rushes, and cattails) (Rejmankova, et al. 1996). The influence of plants on anopheline larval habitats has been recognized for a long time (Hall, 1972). The so-called 'intersection line' where

plant structures or flotage intersect the water surface is an important determinate of site selection by gravid female mosquitoes and successful larval development (Hess & Hall, 1943). The relative density of these emergent plants greatly influence production and distribution of particular species of mosquitoes. The difference in seasonal larval habitat availability has been found more pronounced in northern Belize, reflecting the 3-fold lower annual rainfall compared to southern Belize (King et al. 1992; Rejmankova, 1998). Average annual rainfall for this region does not normally exceed 139 cm (54 in).

Rejmankova, et al. (1993, 1995, 1996) have described larval habitats of *Anopheles albimanus* and associated species (e.g., *An. crucians*) in northern Belize. *Anopheles vestitipennis* and *An. punctimacula* larval habitats have also been described (Rejmankova, et al. 1998). Although, *An. albimanus* and *An. vestitipennis* can commonly occur together as adult populations, the partiality for their respective larval habitats differs considerably. *Anopheles albimanus* prefers wetlands dominated by sparse emergent macrophytes (*Eleocharis* spp. and sawgrass), areas often developing extensive floating mats of cyanobacteria (blue-green algae) promoted by higher (limestone) water pH values. On the other hand, *An. vestitipennis* has a strong predilection for areas dominated by larger trees or tall dense macrophytes (e.g., swamp forest/marsh habitats) that provide the necessary shade and cool fresh waters this species prefers.

Caledonia is considered an ancient settlement area of pre-Columbian Mayan culture that emerged in the lowland areas of the Yucatan Peninsula from 4500 to 1050 BP (before present) (Merrill, 1993; Thompson, 1972). A former minor Mayan ceremonial center, a large abandoned mound masonry site (85% limestone) still exists in Caledonia. [During my study, there was plenty of evidence of ancient pottery, stone and obsidian

arrowheads, shell objects, jadeite jewelry and other artifacts found by the local community, attesting to the antiquity of the location. Very close to the study site were several 'trash' (midden) mounds with many examples of exposed pottery shards and worked flint from a civilization long past]. The general environment of the village proper is one of trees, shrubs and garden plots. Soil type, with the exception of areas subject to periodic river flooding, is high in limestone. At the time of the study (1995-96), Caledonia Village had an official population of approximately 1,227, comprising 226 families and 395 houses (VCP, unpub. data). The native population is predominantly Spanish-speaking Mestizo (Honduran/Salvadoran extraction) and income is derived mostly from seasonal sugarcane production. Housing quality is generally good, walls of wooden planks or palm, cement block, metal roofs and screened windows. Access to the village is available year-round by an all-weather road.

Malaria incidence in Caledonia has been among the highest in the northern health sector of Belize since 1988 (VCP, unpub. report). In 1994, 100 cases (incidence 91/1,000) were recorded for the village. In this area, the vast majority of the infections are *Plasmodium vivax*, only rarely is *P. falciparum* reported. Regular indoor residual spraying (2-cycle/yr) with DDT (75% wettable powder suspension or technical grade product in kerosene) had been conducted in Caledonia on a near continuous basis for nearly 40 years at the time of this investigation.

Caledonia Village (18°13'78"N, 88°28'38"W) is located in this coastal zone along the New River floodplain, in the Corozal District, of northern Belize (Fig. 7). King et al. 1992 (Table 1, Fig. 8) has provided a land resource assessment of the general Caledonia

area. A SPOT (Systeme Pour l'Observation de la Terre) XS (multispectral) satellite digital image (Fig. 9) taken during the dry season in February, 1992 includes the Caledonia area and a breakdown of the land cover classification (Rejmankova, et al. 1998). In general, the area is characterized by a diverse combination of different ecological systems including forests, swamp forests, savanna, marshes, pasture, and cropland dominated by large tracts of sugarcane fields under various stages of growth. Some of these areas have a significant impact on mosquito production, both seasonal and perennial (Rejmankova, et al. 1993). The specific study locality within Caledonia was marked by a small depression along the New River floodplain. Experimental huts were less than 20 meters from the river's edge. A general habitat assessment was made in October 1995 (E. Rejmankova). The habitat was characterized as principally composed of 'graminoids' (aquatic grasses and sedges), with sparse trees. The total cover of emergent vegetation, approximating 20%, included Gramineae spp. (10%), *Sagittaria lancifolia* –arrowleaf (5%), *Eichhornia crassipes*- water hyacinth (1%), *Crinum* sp. (1%), *Mimosa priga* (1%), *Ludwigia octovalis* (1%), *Cypers* cf. *odoratus* (1%), and *Typha domingensis*- cattail (1%). Species composition in an extensive marsh immediately across the river was characterized as 'sparse *Cladium jamaicense* with cyanobacterial mats. Emergent vegetation represented 10-25%, including *Cladium jamaicense*- sawgrass (5-15%), *Conocarpus erectus* (1-5%), *Eleocharis cellulosa* –spike-rush (1-5%), *Dichromea* sp. (1%), and *Acoelorraphe wrighti* (1-5%).

Only recently have detailed studies of specific habitats and aquatic ecosystem associations been identified in northern Belize (Rejmankova, et al. 1995, 1998). Identification of vegetation types that indicate particular larval habitats has led to the

development of accurate predictions of vector distribution in relation to disease risk (Rejmankova, et al. 1995; Roberts, et al. 1996). Based on these predictions, Caledonia was rated "high risk" for malaria transmission as distances from identified larval habitats were less than 1.5 km from the village (i.e., within average *An. albimanus* flight range) (Rejmankova, et al. 1995).

The introduction presented was provided for background on general study design, relevance of research questions and general information on the study site and mosquito vectors that will not necessarily be presented in detail in the following chapters. Appendices I and II are simplified dichotomous keys that were developed for the identification of the common larval and adult *Anopheles* species found in Caledonia. Appendix III is a copy of the Human-Use Agreement form that was required for all mosquito collectors participating in the study. Appendix VI summarizes mosquito collection data that occurred in the Cayo, Belize and Corozal districts during the site selection phase. Appendix V provides some ancillary biological information for general interest on some of the mosquitoes collected in Caledonia during this study.

REFERENCES CITED

Abedi, Z.H. and A.W.A. Brown. 1960. Development and reversion of DDT-resistance in *Aedes aegypti*. Can. J. Genet. Cytol. 2:252-261.

Arata, A.A. 1994. Difficulties facing vector control in the 1990's. Am. J. Trop. Med. Hyg. 50(Suppl.):6-10.

Ariaratnam, V. and A.W.A. Brown. 1969. Exposure time versus concentration in the WHO standard test for mosquito resistance to chlorohydrocarbon insecticides. Bull. Wld. Hlth. Organ. 40:561-567.

Ariaratnam, V. and G.P. Georghiou. 1974. Carbamate resistance in *Anopheles albimanus* cross-resistance spectrum and stability of resistance. Bull. Wld. Hlth. Organ. 51:655-659.

Arredondo-Jimenez, J.I. 1995. Comparative ecology of allopatric populations of *Anopheles* (*Anopheles*) *vestitipennis* (Diptera: Culicidae). Ph.D. Dissertation, Department of Entomology, University of California, Davis, 172 pp.

Arredondo-Jimenez, J.I., J. Gimnig, M.H. Rodriguez and R.K. Washino. 1996a. Genetic differences among *Anopheles vestitipennis* subpopulations collected using different methods in Chiapas state, southern Mexico. J. Amer. Mosq. Control Assoc. 12:396-401.

Arredondo-Jimenez, J.I., M.H. Rodriguez and R.K. Washino. 1996b. Temporal and spatial dynamics of *Anopheles* vectors in Chiapas, Mexico. VI Symposium. Mosquito Vector Control and Biology in Latin America. 62nd Ann. Mtg. Am. Mosq. Control Assoc. Norfolk, VA. (Abstract).

Arredondo-Jimenez. J.I., M.H. Rodriguez, E.G. Loyola and D.N. Bown. 1997. Behavior of *Anopheles albimanus* in relation to pyrethroid-treated bednets. Med. Vet. Entomol. 11:87-94.

Arredondo-Jimenez. J.I., M.H. Rodriguez and R.K. Washino. 1998. Gonotrophic cycle and survivorship of *Anopheles vestitipennis* (Diptera: Culicidae) in two different ecological areas of southern Mexico. J. Med. Entomol. 35:937-942.

Assem, J. van den. 1959. A window-trap experiment to test the effects of dieldrin under local conditions in the Merauke area (Netherlands New Guinea). Trop. Geog. Med. 11:32-43.

Bang, Y.H. 1985. Implications in the control of malaria vectors with insecticides in tropical countries of South East Asia. J. Com. Dis. 17:199-218.

Beach, R.F., C. Cordon-Rosales and W.G. Brogdon. 1989a. Detoxifying esterases may limit the use of pyrethroids for malaria vector control in the Americas. Parasitol. Today. 5:326-327.

Beach, R.F., D. Mills and F.H. Collins. 1989b. Structure of ribosomal DNA in *Anopheles albimanus* (Diptera: Culicidae). J. Med. Entomol. 82:641-648.

Beach, R.F., C. Cordon-Rosales, E. Molina and A. Wirtz. 1992. Field evaluation of an enzyme-linked immunosorbent assay for estimating the sporozoite rate in *Anopheles albimanus*. Am. J. Trop. Med. Hyg. 47:478-483.

Belkin, J.N., R.X. Schick and S.J. Heinemann. 1965. Mosquito studies (Diptera: Culicidae). V. Mosquitoes originally described from Middle America. Contr. Am. Entomol. Inst. 1(5):1-95.

Belkin J.N., S.J. Heinemann and W.A. Page. 1970. The Culicidae of Jamaica. Cont. Am. Entomol. Inst. 6:1-458.

Belkin, J.N. and S.J. Heinemann. 1972. A tentative annotated list of the Culicidae of the island of Hispaniola. Mosq. Syst. 4:63-72.

Bertram, D.S. 1971. Mosquitoes of British Honduras, with some comments on malaria, and on arbovirus antibodies in man and equines. Trans. Roy. Soc. Trop. Med. Hyg. 65:742-762.

Bidlingmayer, W.L. 1985. The measurement of adult mosquito population changes some considerations. J. Am. Mosq. Control Assoc. 1:328-348.

Bonilla, A.O., J.I. Arredondo-Jimenez and M.H. Rodriguez. 1996. Classic morphology of fourth instar larvae, pupae and adults of *Anopheles vestitipennis*. VI Symposium. Mosquito Vector Control and Biology in Latin America. 62nd Ann. Mtg. Am. Mosq. Control Assoc. Norfolk, VA. (Abstract).

Bown, D.N., J.R. Rios, C. Frederickson, G. Del Angel Cabanas and J.F. Mendez. 1986. Use of an exterior curtain-net to evaluate insecticide/mosquito behavior in houses. J. Am. Mosq. Control Assoc. 2:99-101.

Bown, D.N. 1987. Agricultural use of pesticides and their effect on vector borne disease transmission in the WHO region of the Americas. In: *Effects of Agricultural Development on Vector-borne Diseases*. Food Agric. Organ. AGL/MISC/12/87. pp. 53-56.

Bown, D.N., M.H. Rodriguez, J. I. Arredondo-Jimenez, E.G. Loyola and M. del Carmen Rodriguez. 1991. Age structure and abundance levels in the entomological evaluation of an insecticide used in the control of *Anopheles albimanus* in southern Mexico. J. Am. Mosq. Control Assoc. 7:180-187.

Boyd, M.F. and F.W. Aris. 1929. A malaria survey of the island of Jamaica, B.W.I. Am. J. Trop. Med. 9:309-399.

Breeland, S. 1972. Studies on the ecology of *Anopheles albimanus*. Am J. Trop. Med. Hyg. 21:751-754.

Breeland, S.G., G.M. Jeffery, C.S. Lofgren and D.E. Weidhaas. 1974. Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. I. Characteristics of the test site and the natural population. Am. J. Trop. Med. Hyg. 23:274-281.

Breeland, S. 1980. A bibliography to the literature of *Anopheles albimanus* (Diptera: Culicidae). Mosq. Syst. 12:50-150.

Brent, K.J. 1986. Detection and monitoring of resistant forms: an overview. In: *Pesticide Resistance: Strategies and Tactics for Management*. National Academy Press, Washington, D.C. pp. 298-312.

Brogdon, W.G., R.F. Beach, J.M. Stewart and L. Castanaza. 1988. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. Bull. Wld. Hlth. Organ. 66:339-346.

Brogdon, W.G. 1989. Biochemical resistance detection: an alternative to bioassay. Parasitol. Today 5:56-60.

Brogdon, W.G. and A. M. Barber. 1990. Fenitrothion-deltamethrin cross-resistance conferred by esterases in Guatemalan *Anopheles albimanus*. Pest. Biochem. Physiol. 37:130-139.

Brogdon, W.G. and J.C. McAllister. 1998. Simplification of adult mosquito bioassays through the use of time-mortality determinations in glass bottles. J. Am. Mosq. Control Assoc. 14:159-164.

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

Brown, A.W.A. and R. Pal. 1971. *Insecticide Resistance in Arthropods*. Wld. Hlth Organ. Monogr. Series No. 38. WHO, Geneva. 491 pp.

Brown, A.W.A. 1972. The ecological implications of insecticidal usage in malaria programs. Am. J. Trop. Med. Hyg. 21:829-834.

Brown, A.W.A., J. Haworth and A.R. Zahar. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13:1-25.

Brown, A.W.A. 1983. Insecticide resistance as a factor in the integrated control of Culicidae. In: *Integrated mosquito control methodologies, Vol. 1: Experience and components from conventional chemical control.* M. Laird and J.W. Miles (Eds.). Academic, London. pp. 61-235.

Brown, A.W.A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2:123-140.

Brown, T.M. 1981. Countermeasures for insecticide resistance. Bull. Entomol. Soc. Am. 27:198-201.

Brown, T.M. and W. Brogdon. 1987. Improved detection of insecticide resistance through conventional and molecular techniques. Annu. Rev. Entomol. 32:145-162.

Bruce-Chwatt, L.J. 1970. Global review of malaria control and eradication by attack on the vector. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Amer. 7(1):7-27.

Bruce-Chwatt, L.J. 1979. Man against malaria: conquest or defeat. Trans. Roy. Soc. Trop. Med. Hyg. 73:605-617.

Busvine, J.R. 1956. The significance of insecticide-resistant strains, with special reference to pests of medical importance. Bull. Wld. Hlth Organ. 15:389-401.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitoes. Bull. Wld. Hlth. Organ. 31:645-656.

Busvine, J.R. and R. Pal. 1969. The impact of insecticide-resistance on control of vectors and vector-borne diseases. Bull. Wld. Hlth. Organ. 40:731-744.

Campbell C.C. 1997. Malaria: an emerging and re-emerging global plague. Imm. Med. Microbio. 18:325-331.

Carley, P.S. 1931. Results of the dissection of 1,017 wild-caught anophelines in Jamaica. Am J. Trop. Med. 11:293-296.

Carr, H.P. and R.B. Hill. 1942. A malaria survey of Cuba. Am. J. Trop. Med. 22:587 607.

Carpenter, S.J. and W.J. LaCasse. 1955. *Mosquitoes of North America.* U.C. Press, Berkeley. 360 pp. + plates.

Chan, A.S.T., M.H. Rodriguez, J. A. Torres, M. del Carmen Rodriguez and C. Villarreal. 1994. Susceptibility of three laboratory strains of *Anopheles albimanus* (Diptera: Culicidae) to coindigenous *Plasmodium vivax* in southern Mexico. J. Med. Entomol. 31:400-403.

Chapin, G. and R. Wasserstrom. 1983. Pesticide use and malaria resurgence in Central America and India. Soc. Sci. Med. 17:273-290.

Chapman, R.F. 1982. *The Insects: Structure and Function*. 3ed. Harvard Univ. Press, Cambridge. Chapter 30: Chemoreception. pp. 736-755.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.H. Harlan, S. Manguin and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus* (Diptera: Culicidae), a malaria vector in the Americas. J. Am. Mosq. Control Assoc. 13:171-183.

Chege, G.M. and J.C. Beier. 1998. Blood acquisition and processing by three *Anopheles* (Diptera: Culicidae) species with different innate susceptibilities to *Plasmodium falciparum*. J. Med. Entomol. 35:319-323.

Charles, L.J. and G. Senevet. 1953. The distribution of *Anopheles albimanus* in the Caribbean Islands. Am. J. Trop. Med. Hyg. 2:1109-1115.

Chow, E., R.A. Wirtz and T.W. Scott. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich of enzyme-linked immunosorbent assay. J. Am. Mosq. Control Assoc. 9:196-205.

Clark-Gil, S. and R.F. Darsie. 1983. The mosquitoes of Guatemala. Their identification, distribution and bionomics with keys to adult females and larvae. Mosq. Syst. 15:151-284.

Colless, D.H. 1953. The effect of DDT and kerosene upon adult behaviour in *Anopheles balabacensis* Baisas (=*A. leucosphyrus* Auct.). Ann. Trop. Med. Parasitol. 47:261-271.

Collins, F.H. and S. M. Paskewitz. 1995. Malaria: current and future prospects for control. Annu. Rev. Entomol. 40:195-219.

Coluzzi, M. 1962. An experimental method for determining the irritability of adult mosquitoes to insecticides. WHO/MAL/329: 15 pp.

Coluzzi, M. 1963. Studies on irritability of DDT to anopheline mosquitoes. WHO/VBC 6/25/63, 33: 22 pp.

Cordon-Rosales, C., N. Padilla and R. Zeissig. 1992. Impact of partial deltamethrin resistance in *Anopheles albimanus* on the efficacy of intradomiciliary spraying for malaria control. J. Am. Mosq. Control Assoc. 8:308-309.

Cullen, J.R. and J. De Zulueta. 1962. Observations on the irritability of mosquitos to DDT in Uganda. Bull. Wld. Hlth. Organ. 27:239-250.

Curry, D.P. 1934. Breeding of anopheline mosquitoes among aquatic vegetation of Gatun Lake, accompanied by periodic long flights of *A. albimanus* Wied. Southern Med. J. 27:644-651.

Curtis, C.F. 1994. Should DDT continue to be recommended for malaria vector control? Med. Veter. Entomol. 8:107-112.

Darsie, R.F. and R.A. Ward. 1981. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. Mosq. Syst. Suppl. 1:1-313.

Davidson, G. 1958. Studies on resistance in anopheline mosquitoes. Bull. Wld. Hlth. Organ. 18:579-621.

Davidson, G. 1963. DDT-resistance and dieldrin-resistance in *Anopheles albimanus*. Bull. Wld. Hlth Organ. 28:25-33

Davidson, G. and A.R. Zahar. 1973. The practical implications of resistance of malaria vectors to insecticides. Bull. Wld. Hlth. Organ. 49:475-483.

Davidson, G. and B. Sawyer. 1975. Carbamate and organophosphate resistance in *Anopheles albimanus*. Trans. Roy. Soc. Trop. Med. Hyg. 64:431

Davidson, G. 1989. Insecticide usage: an anti-alarmist view of its advantages and disadvantages. Trop. Dis. Bull. 86:R1-6.

De Merida, A.M., De Mata, M.P., E. Molina, C.H. Porter and W.C. Black IV. 1995. Variation in ribosomal DNA intergenic spacers among populations of *Anopheles albimanus* in South and Central America. Am. J. Trop. Med. Hyg. 53:469-477.

De Zulueta, J. 1959. Insecticide resistance in *Anopheles sacharovi*. Bull. Wld. Hlth Organ. 20:797-822.

De Zulueta, J., G.W. Kafuko, J.R. Cullen and C.K. Pedersen. 1961. The results of the first year of a malaria eradication pilot project in northern Kigezi (Uganda). E. Afr. Med. J. 38:1-26.

De Zulueta, J. 1962. The irritability of mosquitos to DDT and its importance in malaria eradication. Riv. Malariol. 41:169-178.

De Zulueta, J. and J.R. Cullen. 1963. Deterrent effect of insecticides on malaria vectors. Nature, Lond. 200(4909):860-861.

De Zulueta, J. 1964. Ethological changes in malaria vectors. A review of the situation in the light of recent findings. Riv. Malariol. 43:29-36.

Dethier, V. G., L.B. Browne and C.N. Smith, C. N. 1960. The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Entomol. 53:134-36.

Devonshire, A.L., L.M. Field and M.S. Williamson. 1992. Molecular biology of insecticide resistance. In: *Insect Molecular Science*. J.M. Crampton & P. Eggleston, (Eds.). Academic Press, London. pp. 173-183.

Downs, W.G. and E. Bordas. 1951. Control of *Anopheles pseudopunctipennis* in Mexico with DDT residual sprays applied in buildings. Part V: Effectiveness of residual applications of DDT and gammexane up to one year after application under controlled conditions. Am. J. Hyg. 54: 150-156.

Duret, J.P. 1958. Quantitative measurements toward an understanding of behavioristic resistance in Panama (in the field). WHO/PAHO seminar on the susceptibility of insects to insecticides. pp. 318-322.

El-Mallakh, R.S., H. Rubin and D.L. Baumgartner. 1998. Trends in funding for medical entomological research. J. Med. Entomol. 35:1-2.

Elliott, R. 1964. Studies on the kinetic response of mosquitos to chemicals. Bull. Wld. Hlth. Organ. 31:657-667.

Elliott, R. 1968. Studies on man-vector contact in some malarious areas in Colombia. Bull Wld. Hlth. Organ. 38:239-253.

Elliott, R. 1969. Ecology and behaviour of malaria vectors in the American region. Cah. ORSTOM., ser. Ent. Med. et Parasitol. 7:29-33.

Elliott, R. 1972. The influence of mosquito behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21:755-763.

Elliott, R. and J. de Zulueta. 1975. Ethological resistance in malaria vectors: behavioural response to intradomestic residual insecticides. WHO/VBC/75.569. 15 pp.

Eyles, D.E. 1944. A critical review of the literature related to the flight and dispersion habits of anopheline mosquitoes. Pub. Hlth. Rep., No. 287. 39 pp.

Eyles, D.E. and M.D. Young. 1950. The comparative susceptibility of *Anopheles albimanus* and *An. quadrimaculatus* to a South Carolina strain of *Plasmodium falciparum*. J. Infect. Dis. 87:189-193.

Faran, M. E. 1980. Mosquito studies (Diptera: Culicidae) 34. A revision of the Albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. 15:1-215.

Farid, M.A. 1991. Views and reflections on anti-malaria programmes in the world. Kaohsiung J. Med. Sci. 7:243-255.

Faust, E.C. 1949. Malaria incidence in North America. In: *Malariology. A Comprehensive Survey of All Aspects of this Group of Diseases*. M.F. Boyd, (Ed.). Saunders, Philadelphia. pp. 749-763.

Field, J.W. 1950. Fumigant and repellent effects of BHC (Gammexane) and DDT upon *Anopheles*. Trans. Roy. Soc. Trop. Med. Hyg. 43:547-548.

Foote, R. and D. Cook. 1959. *Mosquitoes of Medical Importance*. Agriculture Handbook No. 152. U.S. Dept. of Agriculture. Washington, D.C. 158 pp.

Frederickson, E.C. 1993. Bionomics and control of *Anopheles albimanus*. Tech. Paper 34. Pan Am. Hlth. Organ. Washington, D.C. 76 pp.

Gabaldon, A. 1949a. The nation-wide campaign against malaria in Venezuela. Trans. Roy. Soc. Trop. Med. Hyg. 43:113-160.

Gabaldon, A. 1949b. Malaria incidence in the West Indies and South America. In: *Malariology. A Comprehensive Survey of All Aspects of this Group of Diseases.* M.F. Boyd, (Ed.). Saunders, Philadelphia. pp. 764-787.

Gabaldon, A. 1952. The effect of DDT on the population of anopheline vectors in Venezuela. Riv. Parassitol. 13:29-41.

Gabaldon, A. 1953. Possible effects of residual insecticides on the interruption of malaria transmission. Riv. Malariol. 32:155-171.

Gabaldon, A. 1978. What can and cannot be achieved with conventional anti-malaria measures. Am. J. Trop. Med. Hyg. 27:653-658.

Garrett-Jones, C. 1964. The human blood index of malaria vectors in relation to epidemiological assessment. Bull. Wld. Hlth. Organ. 30:241-260.

Garrett-Jones, C. 1970. Problems of epidemiological entomology as applied to malariology. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):168-180.

Garrett-Jones, C., P.F.L. Boreham and C.P. Pant. 1980. Feeding habits of anophelines (Diptera: Culicidae) in 1971-78, with references to the human blood index: a review. Bull. Entomol. Res. 70:165-185.

Gebert, S. 1948. Notes on certain aspects of the action of DDT residual sprays, and on the partial treatment of dwellings as a means of anti-anopheline protection. Trans. Roy. Soc. Trop. Med. Hyg. 42:295-297.

Georghiou G.P. 1972a. The evolution of resistance to pesticides. Ann. Rev. Ecol. Syst. 3:133-168.

Georghiou, G.P. 1972b. Studies on resistance to carbamate and organophosphorus insecticides in *Anopheles albimanus*. Am. J. Trop. Med. Hyg. 21: 797-806.

Georghiou, G.P., V. Ariaratnam and S.G. Breeland. 1972. Development of resistance to carbamates and organophosphorus compounds in *Anopheles albimanus* in nature. Bull. Wld. Hlth. Organ. 46:551-554.

Georghiou, G.P., S. Breeland and V. Ariaratnam. 1973. Seasonal escalation of organophosphorus and carbamate resistance in *Anopheles albimanus* by agricultural sprays. Environ. Entomol. 2:369-374.

Georghiou G.P. and T. Saito (Eds.). 1983. *Pest Resistance to Pesticides*. Plenum, New York. 809 pp.

Georghiou, G.P. and R.B. Mellon. 1983. Pesticide resistance in time and space. In: *Pest Resistance to Pesticides*. G.P. Georghiou & T. Saito (Eds.) Plenum, New York. pp. 1-46.

Georghiou G.P. 1986. The magnitude of the resistance problem. In: *Pesticide Resistance: Strategies and Tactics for Management*. National Academy Press, Washington, D.C. pp. 14-43.

Gerold, J.L. and J.J. Laarman. 1964. Selection of some strains of *Anopheles atroparvus* with different behavioural responses to contacts with DDT. Nature. 204(4957):500-501.

Gheorghiu, T., E.M. Ungureanu and C. Garrett-Jones. 1972. An apparatus for the study of the behavior patterns of mosquitoes under the influence of insecticides. Bull. Wld. Hlth. Organ. 46:122-126.

Gillies, M.T. 1956. The problems of exophily in *Anopheles gambiae*. Bull. Wld. Hlth. Organ. 15:437-449.

Gillies, M.T. 1970. Assessment of control, some problems in the measurement of anopheline populations. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):156-167.

Gillies, M.T. 1988. Anopheline mosquitoes: vector behaviour and bionomics. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer and I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 453-485.

Gould, F. 1984. Role of behavior in the evolution of insect adaptation to insecticides and resistant host plants. Bull. Entomol. Soc. Am. 30(4):34-40.

Hall, T.F. 1972. The influence of plants on anopheline breeding. Am. J. Trop. Med. Hyg. 21: 787-794.

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. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Amer. 7(1):28-41.

Harbach, R.E. 1994. Review of the internal classification of the genus *Anopheles* (Diptera: Culicidae): the foundation for comparative systematics and phylogenetic research. Bull. Entomol. Res. 84:331-342.

Harrison, G. 1978. *Mosquitoes, Malaria and Man: A History of the Hostilities Since 1880.* John Murrey, London. 314 pp.

Hartshorn, G., L. Nicolait, L. Hartshorn, G. Bevier, R. Brightman, J. Cal, A. Cawich, W. Davidson, R. DuBois, C. Dyar, J. Gibson, W. Hawley, J. Leonard, R. Nicolait, D. Weyer. H. White and C. Wright. 1984. Belize Country Environmental Profile A Field Study. R. Nicolait & Associates, Belize City, Belize. 151 pp.

Haworth, J. 1988. The global distribution of malaria and the present control effort. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer and I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 1379-1420.

Heinemann, S.J. and J.N. Belkin. 1977. Collection records of the project "Mosquitoes of Middle America", 8. Central America: Belize (BH), Guatemala (GUA), El Salvador (SAL), Honduras (HON), Nicaragua (NI, NIC). Mosq. Syst. 9:403-454.

Hess, A.D. and T.F. Hall. 1943. The intersection line as a factor in anopheline ecology. J. Nat. Mal. Soc. 3:93-98.

Higley, L.G., M.R. Zeiss, W.K. Wintersteen and L.P. Pedigo. 1992. National pesticide policy: a call for action. Am. Entomologist. Fall Issue. pp. 139-146.

Hobbs, J.H. 1962. Cytogenetics of *Anopheles albimanus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 55:245-251.

Hobbs, J.H. 1973. Effect of agricultural spraying on *Anopheles albimanus* densities in a coastal area of El Salvador. Mosq. News 33:420-423.

Hobbs, J., R. Lowe and C. Schreck. 1974. Studies of the flight range and survival of *Anopheles albimanus* Wiedemann in El Salvador. I. Dispersal and survival during the dry season. Mosq. News 34:389-393.

Hobbs, J.H., J.D. Sexton, Y. St. Jean and J.R. Jacques. 1986. The biting and resting behavior of *Anopheles albimanus* in northern Haiti. J. Am. Mosq. Control Assoc. 2:150-153.

Hoffmann, C.C. 1929. La Distribucion Geografica de los Mosquitoes *Anopheles* en al Estado de Veracruz. [The Geographic Distribution of the *Anopheles* Mosquitoes in the State of Veracruz.] Dept. Sal. Publ. Mex. 76 pp.

Hooper, G.H.S. and A.W.A. Brown. 1965. A case of developed irritability to insecticides. Bull. Wld. Hlth. Organ. 32:131-32.

Horsfall, W. 1955. *Mosquitoes: their Bionomics and Relation to Disease.* Ronald, New York. 723 pp.

Hoskins, W.M. and Gordon, H.T. 1956. Arthropod resistance to chemicals. Annu. Rev. Entomol. 1:89-122.

Kennedy, J.S. 1946. The excitant and repellent effects on mosquitos of sublethal contacts with DDT. Bull. Entomol. Res. 37:593-607.

Kennedy, J.S. 1978. The concepts of olfactory 'arrestment' and 'attraction'. Physiol. Entomol. 3:91-98.

Keppler, W.J. and J.B. Kitzmiller. 1969. Reciprocal fertility among five populations of *An. albimanus*. Genetics 61:31.

Keppler, W.J., J.B. Kitzmiller and M.G. Rabbani. 1973. The salivary gland chromosomes of *Anopheles albimanus*. Mosq. News 33:42-49.

Kerkut, G.A. and L.I. Gilbert. (Eds.). 1985. *Comparative Insect Physiology Biochemistry and Pharmacology.* Vol. 12: Insect Control. Pergamon Press, Oxford. 849 pp.

King, R.B., I.C. Baillie, T.M.B. Abell, J.R. Dunsmore, D.A. Gray, J.H. Pratt, H.R. Versey, A.C.S. Wright and S.A. Zisman. 1992. Land resource assessment of Northern Belize. Natural Resources Institute Bulletin 43. Kent, United Kingdom. 513 pp.

Kitzmiller, J.B. 1982. *Anopheline Names, Their Derivations and Histories*. Thomas Say Foundation. Volume 8. 639 pp.

Klowdon, M.J. 1996. Vector Behavior. In: *The Biology of Disease Vectors*. B.J. Beaty and W.C. Marquardt (Eds.). University Press, Colorado. pp. 34-50.

Knight, K.L and A. Stone. 1977. *A Catalog of the Mosquitoes of the World.* Thomas Say Foundation. Volume 6. 611 pp.

Komp, W.H.W. 1942. The anopheline mosquitoes of the Caribbean region. U.S. Public Health Service, Nat. Inst. Hlth. Bull. No.179. 195 pp.

Krogstad, D.J. 1996. Malaria as a reemerging disease. Epidemiol. Rev. 18:77-89.

Kuhlow, F. 1962. Field experiments on the behaviour of malaria vectors in an unsprayed hut and in a hut sprayed with DDT in northern Nigeria. Bull. Wld. Hlth Organ. 26:93-102.

Kumm, H.W., W.H.W. Komp and H. Ruiz. 1940. The mosquitoes of Costa Rica. Am. J. Trop. Med. 20: 385-422.

Kumm, H.W. and L.M. Ram. 1941. Observations on the *Anopheles* of British Honduras. Am. J. Trop. Med. 21:559-566.

Kumm, H.W., M.E. Bustamante and J.R. Herrera. 1943. Report concerning certain anophelines found near the Mexican-Guatemalan frontier. Am J. Trop. Med. 23:373-376.

Kumm, H.W. and H. Zuniga. 1944. Seasonal variations in the numbers of *Anopheles albimanus* and *A. pseudopunctipennis* caught in stable traps in Central America. Am. J. Hyg. 39:8-15.

Lane, J. 1953. *Neotropical Culicidae*. Vol I/II. Univ. Sao Paulo, Brazil. 1,112 pp.

Lines, J.D. 1988. Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. Parasitol. Today 4:S17-20.

Lindsay, S.W., J.H. Adiamah and J.R.M. Armstrong. 1992. The effect of permethrin impregnated bednets on house entry by mosquitoes (Diptera: Culicidae) in The Gambia. Bull. Entomol. Res. 82:49-55.

Lockwood, J.A., T.C. Sparks and R.N. Story. 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30:41-51.

Lopez-Carrillo, L., L. Torres-Arreola, L. Torres-Sanchez, F. Espinosa-Torres, C. Jimenez, M. Cebrian, S. Waliszewski and O. Saldate. 1996. Is DDT use a public health problem in Mexico? Environ. Hlth. Persp. 104:584-588.

Lowe, R.E., C.E, Schreck, J.H. Hobbs, D.A. Dame and C.S. Lofgren. 1975. Studies of flight range and survival of *Anopheles albimanus* Wiedemann in El Salvador. II. Comparisons of release methods with sterile and normal adults in wet and dry seasons. Mosq. News 35:160-168.

Loyola, E.G., M.H. Rodriquez, L. Gonzalez, J.L. Arredondo, D.N. Bown and M.A. Vaca. 1990. Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of *Anopheles pseudopunctipennis* in Mexico. J. Am. Mosq. Control Assoc. 6:635-640.

Loyola, E.G., M.A. Vaca, D.N. Bown, E. Perez and M.H. Rodriguez. 1991a. Comparative use of bendiocarb and DDT to control *Anopheles pseudopunctipennis* in a malarious area of Mexico. Med. Veter. Entomol. 5:233-242.

Loyola, E.G., J.I. Arredondo-Jimenez, M.H. Rodriguez, D.N. Bown and M. A. Vaca Marin. 1991b. *Anopheles vestitipennis*, the probable vector of *Plasmodium vivax* in the Lacandon forest of Chiapas, Mexico. Trans. Roy. Soc. Trop. Med. Hyg. 85:171-174.

Loyola, E.G., L. Gonzalez-Ceron, M.H. Rodriquez, J.I. Arredondo-Jimenez, S. Bennett and D.N. Bown. 1993. *Anopheles albimanus* (Diptera: Culicidae) host selection patterns in three ecological areas of the coastal plains of Chiapas, southern Mexico. J. Med. Entomol. 30:518-523.

Ludvik, G.F., W.E. Snow and W.B. Hawkins. 1951. The susceptibility of *Anopheles quadrimaculatus* to DDT after five years of routine treatment in the Tennessee River Valley. J. Nat. Malar. Soc. 10:35-43.

Macdonald, G. 1957. *The Epidemiology and Control of Malaria*. Oxford Univ. Press.

Malcolm, C.A. 1988. Current status of pyrethroid resistance in anophelines. Parasitol. Today 4(7): S13-15.

Manguin, S., D.R. Roberts, R.A. Andre, E. Rejmankova and S. Hakre. 1996. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. J. Med. Entomol. 33:205-211.

Marquetti, M.C., A. Navarro and J.A. Bisset. 1990. Estudio de la actividad hematofagica de *Anopheles vestitipennis* Dyar & Knab, 1906 (Diptera: Culicidae). [Hematophagous activity of *Anopheles vestitipennis* Dyar & Knab, 1906 (Diptera: Culicidae)]. Rev. Cub. Med. Trop. 42:101-108.

Marquetti del Carmen, M., A. Navarro, J. Bisset and F.A. Garcia. 1992. Comparison of three catching methods for collecting anopheline mosquitoes. Mem. Inst. Oswaldo Cruz. 87:457-458.

Marten, G.G., M.F. Suarez and R. Astaeza. 1996. An ecological survey of *Anopheles albimanus* larval habitats in Colombia. J. Vect. Ecol. 21:122-131.

Mattingly, P.F. 1962. Mosquito behaviour in relation to disease eradication programmes. Annu. Rev. Entomol. 7:419-436.

Mekuria, Y., D.C. Williams, M.A. Tidwell and T.A. Santana. 1990a. Studies of the susceptibility of *Anopheles albimanus* and *Anopheles vestitipennis* from Dajabon, Dominican Republic, to insecticides. J. Am. Mosq. Control Assoc. 6:645-650.

Mekuria, Y., M.A. Tidwell, D.C. Williams and J.D. Mandeville. 1990b. Bionomic studies of the *Anopheles* of Dajabon, Dominican Republic. J. Am. Mosq. Control Assoc. 6:651-657.

Mekuria, Y., R. Granados, M.A. Tidwell, D.C. Williams, R.A. Wirtz and D.R. Roberts. 1991. Malaria transmission potential by *Anopheles* mosquitoes of Dajabon, Dominican Republic. J. Amer. Mosq. Control Assoc. 7:456-461.

Mendoza, P.R. and M.M. Lopez. 1991. Ecology and biology of *Anopheles albimanus* in a locality of the Pacific Coast of Nicaragua. J. Am. Mosq. Control Assoc. 7:635.

Merrill, T.L. ed. 1993. *Guyana and Belize: Country Studies.* 2ed. Area Handbook Series. U.S. Lib. Congress, DA Pam 550-82. 369 pp.

Metcalf, R.L. 1983. Implications and prognosis of resistance to insecticides. In: *Pest Resistance to Pesticides*. G.P. Georghiou & T. Saito (Eds.). Plenum, New York. pp. 703-733.

Miller, J.E., S.W. Lindsay and J.R.M. Armstrong. 1991. Experimental hut trials of bednets impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia. Med. Vet. Entomol. 5:465-476.

Miller, T.A. 1988. Mechanisms of resistance to pyrethroid insecticides. Parasitol. Today 4(7): S8-12.

[MOH] Ministry of Health (Belize). 1993. Evaluation of program activities. Vector Control Programme, Belize.

Molineaux, L., G.R. Shidrawi, J.L. Clarke, J.R. Boulzaguet and T.S. Ashkar. 1979. Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition. Bull. Wld, Hlth. Organ. 57:265-274.

Mouchet, J. S. Manguin, J. Sircoulon, S. Laventure, O. Faye, A.W. Onapa, P. Carnevale, J. Julvez and D. Fontenille. 1998. Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors. J. Am. Mosq. Control Assoc. 14:121-130.

Muirhead-Thomson, R.C. 1950. DDT and Gammexane as residual insecticides against *Anopheles gambiae* in African houses. Trans. Roy. Soc. Trop. Med. Hyg. 43:401-412.

Muirhead-Thomson, R.C. and E.C. Mercier. 1952a. Factors in malaria transmission by *Anopheles albimanus* in Jamaica. Part I. Ann. Trop. Med. Parasitol. 46:103-116.

Muirhead-Thomson, R.C. and E.C. Mercier. 1952b. Factors in malaria transmission by *Anopheles albimanus* in Jamaica. Part II. Ann. Trop. Med. Parasitol. 46:201-213

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. Wld. Hlth. Organ. 22:721-734.

Muirhead-Thomson, R.C. 1968. *Ecology of Insect Vector Populations*. Academic Press, London. 174 pp.

Najera, J.A. 1989. Malaria and the work of WHO. Bull. Wld. Hlth. Organ. 67:229-243.

Narahashi, T. 1983. Resistance to insecticides due to reduced sensitivity of the nervous system. In: *Pest Resistance to Pesticides*. G.P. Georghiou & T. Saito (Eds.). Plenum, New York. pp. 333-352.

Narang, S.K., J.A. Seawright and M.F. Suarez. 1991. Genetic structure of natural populations of *Anopheles albimanus* in Colombia. J. Am. Mosq. Control Assoc. 7:337-345.

Navarro O., A., M. del Carmen Marquetti, J.A. Bisset Lazcano and F.A. Garcia. 1986. Estudio estacional de sitios de reposo de anophelinos (Diptera: Culicidae). [Studies of the resting places of anophelines (Diptera: Culicidae).] Informe preliminar. Rev. Cub. Med. Trop. 38:21-26.

Nutsathapana, S., P. Sawasdiwongphom, U. Chitprarop and J.R. Cullen. 1986. The behaviour of *Anopheles minimus* Theobald (Diptera: Culicidae) subjected to differing levels of DDT selection pressure in northern Thailand. Bull. Entomol. Res. 76:303-312.

O'Brien, R.D. 1978. The biochemistry of toxic action of insecticides. In: *Biochemistry of Insects*. M. Rockstein, (Ed.). Academic Press, New York. 515-539.

Oaks, S.C., V.S. Mitchell, G.W. Pearson and C.C.J. Carpenter, (Eds.). 1991. *Malaria. Obstacles and Opportunities*. Institute of Medicine, National Academy of Sciences. National Academy Press, Washington, D.C. 309 pp.

Olliaro, P., J. Cattani and D. Wirth. 1996. Malaria, the submerged disease. J. Am. Med. Assoc. 275(3):230-233.

Oppenoorth, F.J. 1984. Biochemistry of insecticide resistance. Pest. Biochem. Physiol. 22:187-193.

Oppenoorth, F.J. 1985. Biochemistry and genetics of insecticide resistance. In: *Comprehensive Insect Physiology Biochemistry and Pharmacology*. G.A. Kurkut and L.I. Gilbert (Eds.). Vol. 12: Insect Control. pp. 731-767. Pergamon, Oxford.

Padilla, N., P. Molina, J. Juarez, D. Bown and C. Cordon-Rosales. 1992. Potential malaria vectors in northern Guatemala. J. Am. Mosq. Control Assoc. 8:307-308.

Pampana, E. 1963. *A Textbook of Malaria Eradication*. Oxford, London. 508 pp.

[PAHO] Pan American Health Organization. 1986. Malaria control in the Americas: a critical analysis. Bull. Pan Am. Hlth. Organ. 20:1-17.

[PAHO] Pan American Health Organization. 1994a. Status of malaria programs in the Americas,XLII report. CSP24/INF/2 (Eng.) XXIV Pan American Sanitary Conference, Washington, D.C. 116 pp.

[PAHO] Pan American Health Organization 1994b. Prohibition of DDT. Med. Veter. Entomol. 8:164

[PAHO] Pan American Health Organization. 1994c. Health Conditions in the Americas. Vol. II. Sci. Pub. No. 549. Pan Am. Hlth. Organ. Washington, D.C. pp. 50-57.

[PAHO] Pan American Health Organization. 1996. Biology and ecology of *Anopheles albimanus* Weidmann in Central America. Tech. Rep. Ser. No. 43. Washington, D.C. 44 pp.

Pant, C.P. 1988. Malaria vector control: imagociding. In: *Malaria, Principals and Practice of Malariology.* W.H. Wernsdorfer and I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 1173-1212.

Plapp, Jr. F.W. 1986. Genetics and biochemistry of insecticide resistance in arthropods: prospects for the future. In: *Pesticide Resistance: Strategies and Tactics for Management.* National Academy Press, Washington, D.C. pp. 75-86.

Prasittisuk, C. and J.R. Busvine. 1977. DDT-resistant mosquito strains with cross resistance to pyrethroids. Pestic. Sci. 8:527-533.

Pritchard, A.E. and H.D. Pratt. 1944. I. A comparison of light trap and animal bait trap anopheline mosquito collections in Puerto Rico II. A list of the mosquitoes of Puerto Rico. Pub. Hlth. Rep. 59:221-233.

Rachou, R.G., G. Lyons, M. Moura-Lima and J.A. Kerr. 1965. Synoptic epidemiological studies of malaria in El Salvador. Am. J. Trop. Med. Hyg. 14:1-62.

Rachou, R.G. and M. Moura-Lima. 1966. Preliminary note on the epidemiological studies made in El Salvador to determine the causes of the failure of residual spraying to interrupt the transmission of malaria. Rev. Brasil. Malariol. Doencas Trop. 18(3/4):763-779.

Rachou, R.G., L.A. Schinazi and M.M. Lima. 1973. An extensive epidemiological study of the causes for the failure of residual DDT-spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages on the coastal plain of El Salvador, Central America. Rev. Bras. Malariol. Doencas Trop. 25:3-293.

Ramsey, J.M., D.N. Bown, J.L. Aron, R.L. Beaudoin and J.F. Mendez. 1986. Field trial of a rapid detection method for malaria in anopheline vectors with low infection rates in Mexico. Am. J. Trop. Med. Hyg. 35:234-238.

Rapley, R.E. 1961. Notes on the construction of experimental huts. Bull Wld. Hlth. Organ. 24:659-663.

Read, R.G., A.J. Adames and P. Galindo. 1978. A model of microenvironment and man biting tropical insects. Environ. Entomol. 7:547-552.

Rejmankova, E., H.M. Savage, H.M. Rodriquez and D.R. Roberts. 1992. Aquatic vegetation as a basis for classification of *Anopheles albimanus* Wiedemann (Diptera: Culicidae) larval habitats. Environ. Entomol. 21:598-603.

Rejmankova, E., D.R. Roberts, R.E. Harbach, J. Pecor, E.L. Peyton, S. Manguin, R. Krieg, J. Polanco and L. Legters. 1993. Environmental and regional determinants of *Anopheles* (Diptera: Culicidae) larval distribution in Belize, Central America. Environ. Entomol. 22:978-992.

Rejmankova, E., D.R. Roberts, A. Pawley, S. Manguin and J. Polanco. 1995. Predictions of adult *Anopheles albimanus* densities in villages based on distances to remotely sensed larval habitats. Am. J. Trop. Med. Hyg. 53:482-488.

Rejmankova, E., D.R. Roberts, S. Manguin, K.O. Pope, J. Komarek and R.A. Post. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. Environ Entomol. 25:1058-1067.

Rejmankova, E., K.O. Pope, D.R. Roberts, M.G. Lege, R. Andre, J. Greico and Y. Alonzo. 1998. Characterization and detection of *Anopheles vestitipennis* and *Anopheles punctimacula* (Diptera: Culicidae) larval habitats in Belize with field survey and SPOT satellite imagery. J. Vect. Ecol. 23:74-88.

Richards, F.O., Jr., R.Z. Flores, J.D. Sexton, R.F. Beach, D.L. Mount, C. Cordon Rosales, M. Gatica and R.E. Klein. 1994. Effects of permethrin-impregnated bed nets on malaria vectors in northern Guatemala. Bull. Pan Am. Hlth. Organ. 28:112-121.

Roberts, D.R., W.D. Alecrim, A.M. Tavares and K.M. McNeill. 1984. Influence of physiological condition on the behavioral response of *Anopheles darlingi* to DDT. Mosq. News 44:357-362.

Roberts, D.R. and W.D. Alecrim. 1991. Behavioral response of *Anopheles darlingi* to DDT-sprayed house walls in Amazonia. Bull. Pan Am. Hlth. Organ. 25:210-217.

Roberts, D.R. 1993. Insecticide repellency in malaria vector control: a position paper. VBC Report no. 81131. USAID, Arlington, VA. 72 pp.

Roberts, D.R., O. Chan, J. Pecor, E Rejmankova, S. Manguin, J. Polanco and L.J. Legters. 1993. Preliminary observations on the changing roles of malaria vectors in southern Belize. J. Am. Mosq. Control Assoc. 9:456-459.

Roberts, D.R. and R.G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. Am. J. Trop. Med. Hyg. 50(Suppl):21-34.

Roberts, D.R., J.F. Paris, S. Manguin, R.E. Harbach, R. Woodruff, E. Rejmankova, J. Polanco, B. Wullschleger and L.J. Legters. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. Am. J. Trop. Med. Hyg. 54:304-308.

Roberts, D.R. 1997. Remote Sensing for Research and Control of Malaria in Belize: Progress Report 1996. 413 pp.

Roberts, D.R., L.L. Laughlin, P. Hsheih and L.J. Letgers. 1997a. DDT: global strategies, and a malaria control crisis in South America. Emerg. Inf. Dis. 3:295-302.

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

Roberts, D.R., W.D. Alecrim, R.G. Andre and P. Hsheih. A stochastic model of vector behavior: effects of DDT repellency, irritancy and toxicity in malaria control. (unpub. doc.).

Rodriguez, A.D., M.H. Rodriguez, R.A. Meza, J.E. Hernandez, E. Rejmankova, H.M. Savage, K.O. Pope, D.R. Roberts and L. Legters. 1993. Dynamics of population densities and vegetation associations of *Anopheles albimanus* larvae in a coastal area of southern Chiapas, Mexico. J. Am. Mosq. Control Assoc. 9:46-58.

Rodriguez, A.D., M.H. Rodriguez, J.E. Hernandez, S.W. Dister, L.R. Beck, E. Rejmankova and D.R. Roberts. 1996. Landscape surrounding human settlements and *Anopheles albimanus* (Diptera: Culicidae) abundance in southern Chiapas, Mexico. J. Med. Entomol. 33:39-48.

Rodriguez, M.H. and E.G. Loyola. 1990. Malaria in Mexico. Proc. 58th Ann. Conf. Calif. Mosq. Vect. Control Assoc. pp. 49-52.

Rodriguez, M.H., D.N. Bown, J.I. Arredondo-Jimenez, C. Villarreal, E.G. Loyola, and C.E. Frederickson. 1992a. Gonotrophic cycle and survivorship of *Anopheles albimanus* (Diptera: Culicidae) in southern Mexico. J. Med. Entomol. 29:395-399.

Rodriguez, M.H., B. Chavez, A. Orozco, E.G. Loyola and A. Martinez-Palomo. 1992b. Scanning election microscopic observations of *Anopheles albimanus* (Diptera; Culicidae) eggs. J. Med. Entomol. 29:400-406.

Rouch, R.T. and G.L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79:293-298.

Rozeboom, L.E. 1941. Distribution and ecology of the *Anopheles* mosquitoes of the Caribbean region. In: *A symposium on human malaria with special reference to North America and the Caribbean region.* F.R. Moulton, (Ed.). Am. Assoc. Adv. Sci., Pub. No. 15. Washington, D.C. pp. 98-107.

Rozendaal, J.A. 1989. Impregnated mosquito nets and curtains for self-protection and vector control. Trop. Dis. Bull. 86:R1-41.

Rozendaal, J.A., J.P.M. van Hoof, J. Voorham and B.F.J. Oostburg. 1989. Behavioral responses of *Anopheles darlingi* in Suriname to DDT residues on house walls. J. Am. Mosq. Control Assoc. 5:339-350.

Rubio-Palis, Y. and R.H. Zimmerman. 1997. Ecoregional classification of malaria vectors in the neotropics. J. Med. Entomol. 34:499-510.

Russell, P.F., L.S. West, R.D. Manwell and G. Macdonald. 1963. *Practical Malariology*. 2ed. Oxford University, London. 750 pp.

Savage, H.M., E. Rejmankova, J.I. Arredondo-Jimenez, D.R. Roberts and M.H. Rodriguez. 1990. Limnological and botanical characterization of larval habitats of two primary malaria vectors, *Anopheles albimanus* and *Anopheles pseudopunctipennis*, in coastal areas of Chiapas state, Mexico. J. Am. Mosq. Control Assoc. 6:612-620.

Scott, H.H. 1932. Medical and sanitary reports from British colonies, protectorates and dependencies for the year 1930. Trop. Dis. Bull. 29:127-129.

Service, M.W. 1989. The importance of ecological studies on malaria vectors. Bull. Soc. Vect. Ecol. 14:26-38.

Service, M.W. 1992. Vector Control. Where are we now? Bull. Soc. Vect. Ecol. 17:94-108.

Service, M.W. 1993a. The role of ecology and ecological modeling in vector control. Bull. Soc. Vect. Ecol. 18:85-98.

Service, M.W. 1993b. *Mosquito Ecology, Field Sampling Methods*. 2ed. Elsevier, London. 988 pp.

Shalaby, A.M. 1966. Observations on some responses of *Anopheles culicifacies* to DDT in experimental huts in Gujarat State, India. Ann. Entomol. Soc. Am. 59:938-944.

Shidrawi, G.R. 1990. A WHO global programme for monitoring vector resistance to pesticides. Bull. Wld. Hlth. Organ. 68:403-408.

Simmons, J.S., G.R. Callender, D.P. Curry, S.C. Schwartz and R. Randall. 1939. *Malaria in Panama*. Am. J. Hyg. Monogr. Ser. No. 13. 326 pp.

Simmons, J.S. 1941. The transmission of malaria by the *Anopheles* mosquitoes of North America. In: *A symposium on human malaria with special reference to North America and the Caribbean region*. F.R. Moulton, (Ed.). Am. Assoc. Adv. Sci. Pub. No. 15. Washington, D.C. pp. 113-130.

Singh, P.P., A.S. Udeaan and S. Battu. 1992. DDT and HCH residues in indoor air arising from their use in malaria control programmes. Sci. Total Environ. 116:83-92.

Slooff, R. 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *Anopheles punctulatus* group. Ph.D. Dissertation, Leyden: A.W. Sythoff. 144 pp.

Smith, A. 1963. Deterrent effect of insecticides on malaria vectors. Nature. 200:861 862.

Smith, A. 1964. A review of the origin and development of experimental hut techniques used in the study of insecticides in East Africa. E. Afr. Med. J. 41:361-374.

Smith, A. 1965. A verandah-type hut for studying the house-frequenting habits of mosquitos and for assessing insecticides. I. A description of the verandah-trap and of studies on the egress of *Anopheles gambiae* Giles and *Mansonia uniformis* (Theo.) from an untreated hut. Bull. Entomol. Res. 56:161-167.

Smith, A. and D.J. Webley. 1969. A verandah-type hut for studying the house frequenting habits of mosquitoes and for assessing insecticides. III. The effect of DDT on behaviour and mortality. Bull. Entomol. Res. 59:33-46.

Smits, A., P. Roelants, W. Van Bortel and M. Coosemans. 1996. Enzyme polymorphisms in the *Anopheles gambiae* (Diptera: Culicidae) complex related to feeding and resting behavior in the Imbo Valley, Burundi. J. Med. Entomol. 33:545-553.

Smyth, T., Jr. and C.C. Roys. 1955. Chemoreception in insects and the action of DDT. Biol. Bull. 108:66-76.

Soliman, S.A. and L.K. Cutkomp. 1963. A comparison of chemoreceptor and whole-fly response to DDT and parathion. J. Econ. Entomol. 56:492-494.

Sparks, T.C., J.A. Lockwood, R.L. Byford, J.B. Graves and B.R. Leonard. 1989. The role of behavior in insecticide resistance. Pestic. Sci. 26:383-399.

Suwonkerd, W. B. Amg-Ung, K. Rimwangtrakul, S. Wongkattiyakul, B. Kattiyamongkool, U. Chitraprop and M. Takagi. 1990. A field study on the response of *Anopheles dirus* to DDT and fenitrothion sprayed to huts in Petchabun Province, Thailand. Trop. Med. (Nagasaki). 32:1-5.

Symes, C.B. and A.B. Hadaway. 1947. Initial experiments in the use of DDT against mosquitoes in British Guiana. Bull. Entomol. Res. 37:399-430.

Taylor, R.T. 1966. The ecology of *Anopheles albimanus* (Wied.) in Haiti. Mosq. News 26:393-397.

Thiel, P.H. van. 1951. The repellent effect of insecticides, especially of contact insecticides. Doc. Neerl. Indones. Morb. Trop. 3:117-125.

Thiel, P.H. van and D. Metselaar. 1955. A pilot project of residual spraying as a mean of controlling malaria transmitted by anophelines of the punctulatus group in Netherlands New Guinea. Doc. Med. Geog. Trop. 7:164-181.

Thompson, J.E.S. 1972. The Maya of Belize historical chapters since Colombus, Government of Belize. Belize City.

Tonn, R.J. 1983. Recent developments in Latin America and the Caribbean. Bull. Soc. Vect. Ecol. 8:3-22.

Trapido, H. 1946. The residual spraying of dwellings with DDT in the control of malaria transmission in Panama, with special reference to *Anopheles albimanus.* Am. J. Trop. Med. 26:383-415.

Trapido, H. 1952. Modified response of *Anopheles albimanus* to DDT residual housing spraying in Panama. Am. J. Trop. Med. Hyg. 1:853-861.

Trigg, P.I. and A.V. Kondrachine. 1998. Commentary: Malaria control in the 1990s. Bull. Wld. Hlth. Organ. 76:11-16.

Vanzie, E. 1995. Situational analysis of the vector control program: Magnitude and trend of malaria transmission. Vector Control Programme, Ministry of Health, Belize. 17 pp.

Vargas, L. and A. Martinez-Palacios. 1956. Anofelinos mexicanos. Taxonomia y distribucion [Mexican anophelines. Taxonomy and distribution]. Mex., D.F., ecr. Salubr. Assist. CNEP/SSA, 181 pp.

Vargas, L. 1963. Keys to the *Anopheles* of the World. Neararctic and Neotropical Regions. In: *Practical Malariology*. 2ed. Russell, P.F., L.S. West, R.D. Manwell and G. Macdonald, (Eds.). Oxford University, London. pp. 641-654.

Ware, G.W. 1994. *The Pesticide Book*. 4th ed. Thomson, Fresno, CA. 386 pp.

Warren, McW., J. Mason and J. Hobbs. 1975. Natural infections of *Anopheles albimanus* with *Plasmodium* in a small malaria focus. Am J. Trop. Med. Hyg. 24:545-546.

Wharton, R.H. 1951. The behaviour and mortality of *Anopheles maculatus* and *Culex fatigans* in experimental huts treated with DDT and BHC. Bull. Entomol. Res. 42:1-20.

[WHO] World Health Organization. 1970. Insecticide resistance and vector control. Instructions for determining the irritability of adult mosquitoes to insecticide, Wld. Hlth. Organ. Tech. Rep. Ser. 433:158-163.

[WHO] World Health Organization. 1975a. Ecology and control of vectors in public health. Wld. Hlth. Organ. Tech. Rep. Ser. No. 561. 35 pp.

[WHO] World Health Organization. 1975b. Manual on practical entomology in malaria. Part II. Methods and techniques. WHO Offset Pub. No, 13. Geneva. 191 pp.

[WHO] World Health Organization. 1981a. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Establishment of the base-line. WHO/VBC/81.805. Geneva. 6 pp.

[WHO] World Health Organization. 1981b. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Diagnostic Test. WHO/VBC/81.806. Geneva. 6 pp.

[WHO] World Health Organization. 1981c. Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides. WHO/VBC/81.6. Geneva. 4 pp.

[WHO] World Health Organization. 1986. Resistance of vectors and reservoirs of disease to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 737. 87 pp.

[WHO] World Health Organization. 1992. Vector resistance to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 818. 62 pp.

[WHO] World Health Organization. 1994. Use of DDT in vector control. Med. Veter. Entomol. 8:113.

[WHO] World Health Organization. 1995. Vector control for malaria and other mosquito-borne diseases. Wld. Hlth. Organ. Tech. Rep. Ser. No. 857. 91 pp.

[WHO] World Health Organization. 1996. World malaria situation in 1993. Part II. Weekly Epidemiol. Rec. 71:25-29.

[WHO] World Health Organization. 1997a. World malaria situation in 1994. Part I. Weekly Epidemiol. Rec. 72:269-276.

[WHO] World Health Organization. 1997b. World malaria situation in 1994. Part II. Weekly Epidemiol. Rec. 72:277-284.

Wilkerson, P.R. 1951. Distribution and fate of *Anopheles gambiae* and *A. funestus* in two different types of huts treated with DDT and BHC in Uganda. Bull. Entomol. Res. 42:45-54.

Wilkerson, R.C., D. Strickman and T.R. Litwak. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. J. Am. Mosq. Control Assoc. 6:7-34.

Wilkerson, R.C. and E.L. Peyton. 1990. Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). J. Med. Entomol. 27:207-224.

Wolda, H. and P. Galindo. 1981. Population fluctuations of mosquitoes in the non-seasonal tropics. Ecol. Entomol. 6:99-106.

Worth, C.B. 1953. Construction and use of a simplified window trap for insects. Mosq. News 13:204-206.

Wright, A.C.S., D.H. Romney, R.H. Arbuckle and V.E. Vial. 1959. Land in British Honduras. Colonial Research Publications, No. 24. Her Majesty's Stationery Office. London. 327 pp.

Wright, J.W., R.F. Fritz and J. Haworth. 1972. Changing concepts of vector control in malaria eradication. Annu. Rev. Entomol. 17:75-102.

Zamora, F.V. and M.L. Calderon. 1991. Biology and vectorial capacity of *Anopheles albimanus* on the southern coast of Guatemala. J. Am. Mosq. Control Assoc. 7:635.

Zerba, E. 1988. Insecticidal activity of pyrethroids on insects of medical importance. Parasitol. Today 4(7): S3-7.

Zetek, J. 1915. Behavior of *Anopheles albimanus* Wiede. and *tarsimaculata* Goeldi. Ann. Entomol. Soc. Am. 8:221-271.

Zimmerman, R.H. 1992. Ecology of malaria vectors in the Americas and future direction. Mem. Inst. Oswaldo Cruz. 87 (suppl. III):371-383.

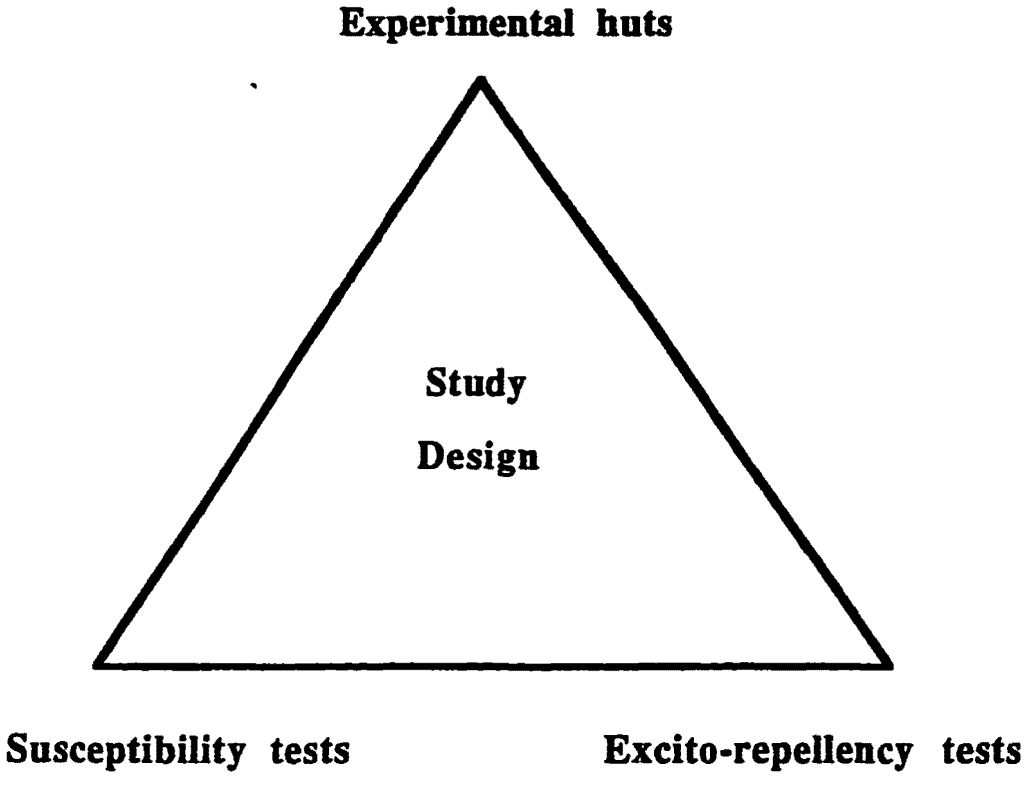


Figure 1. Experimental study design triangle.

Figure 2. Geographical distribution of *Anopheles albimanus* in the Americas (Faran, 1980).

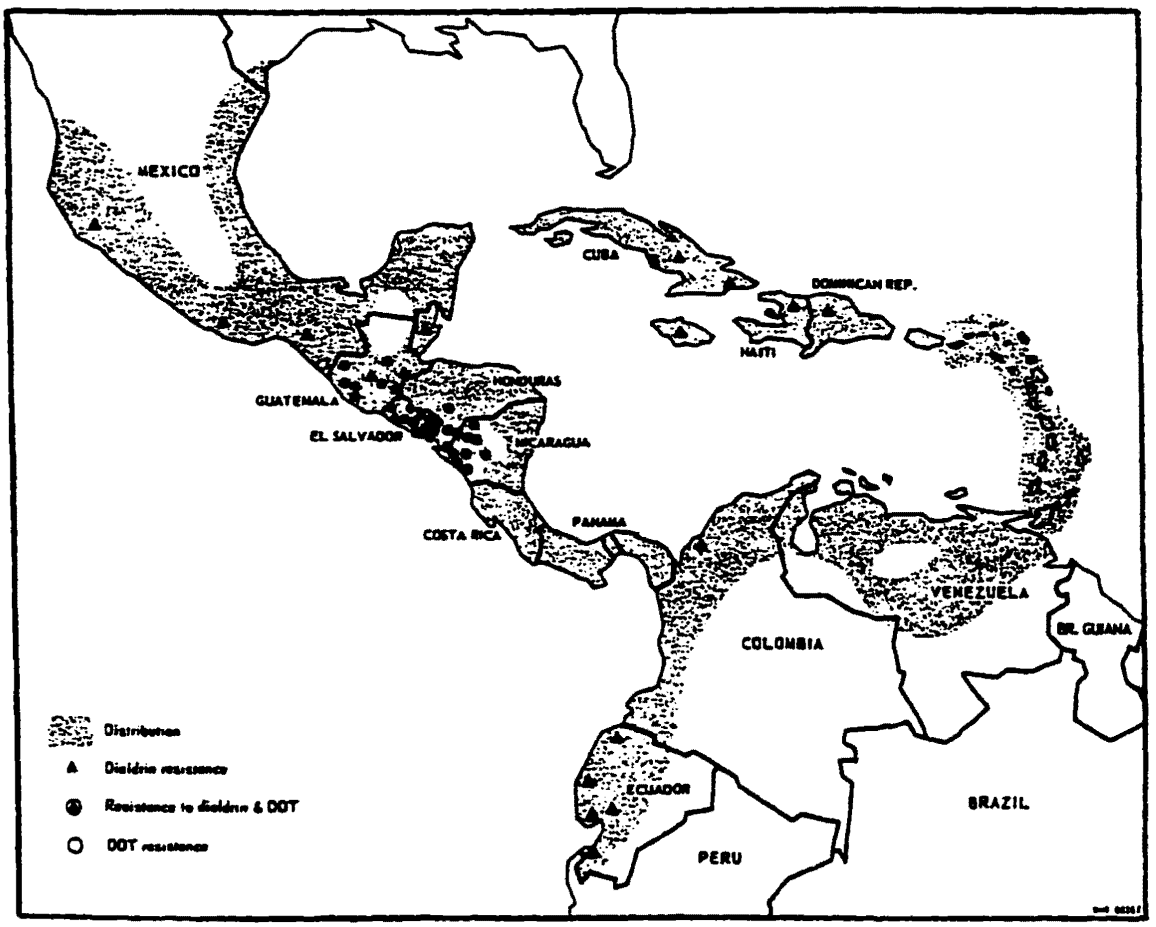


Figure 3. Distribution of insecticide resistance to DDT and dieldrin (circa 1965) over the geographic range of *Anopheles albimanus* in the Americas (Brown & Pal, 1971).

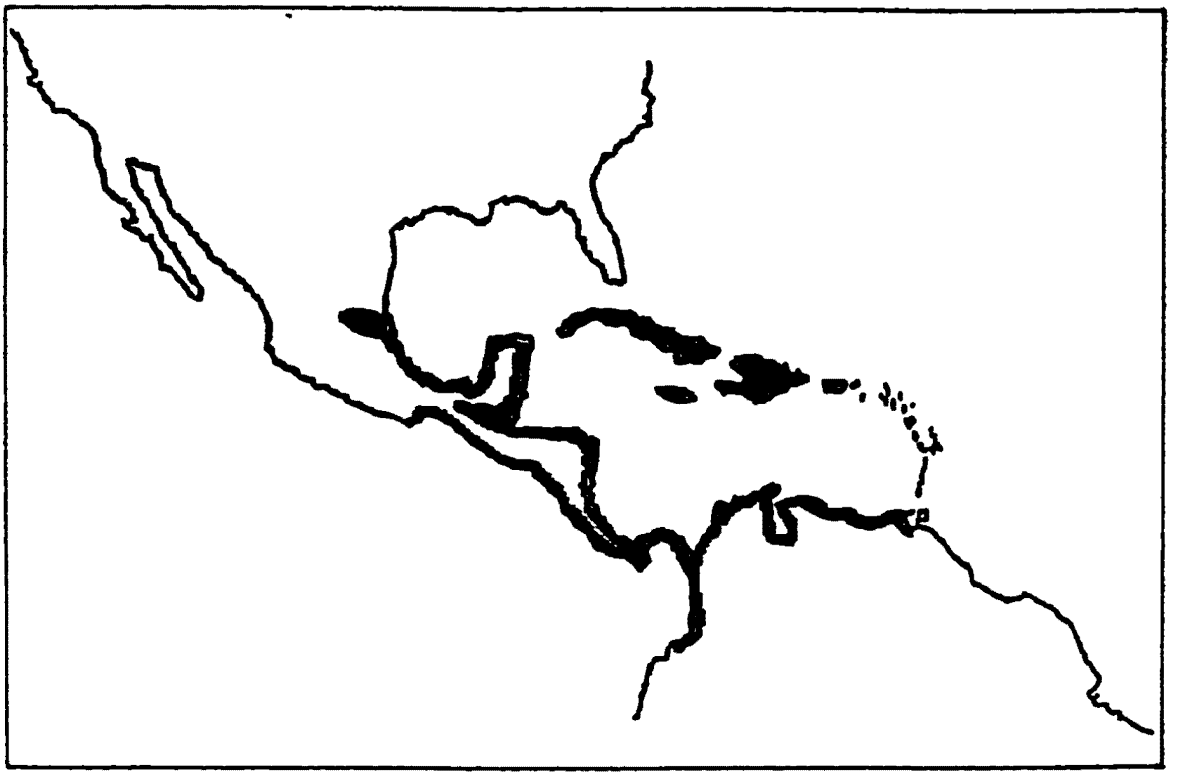


Figure 4. Geographical distribution of *Anopheles vestitipennis* in the Americas (Arredondo-Jimenez, et al. 1996).

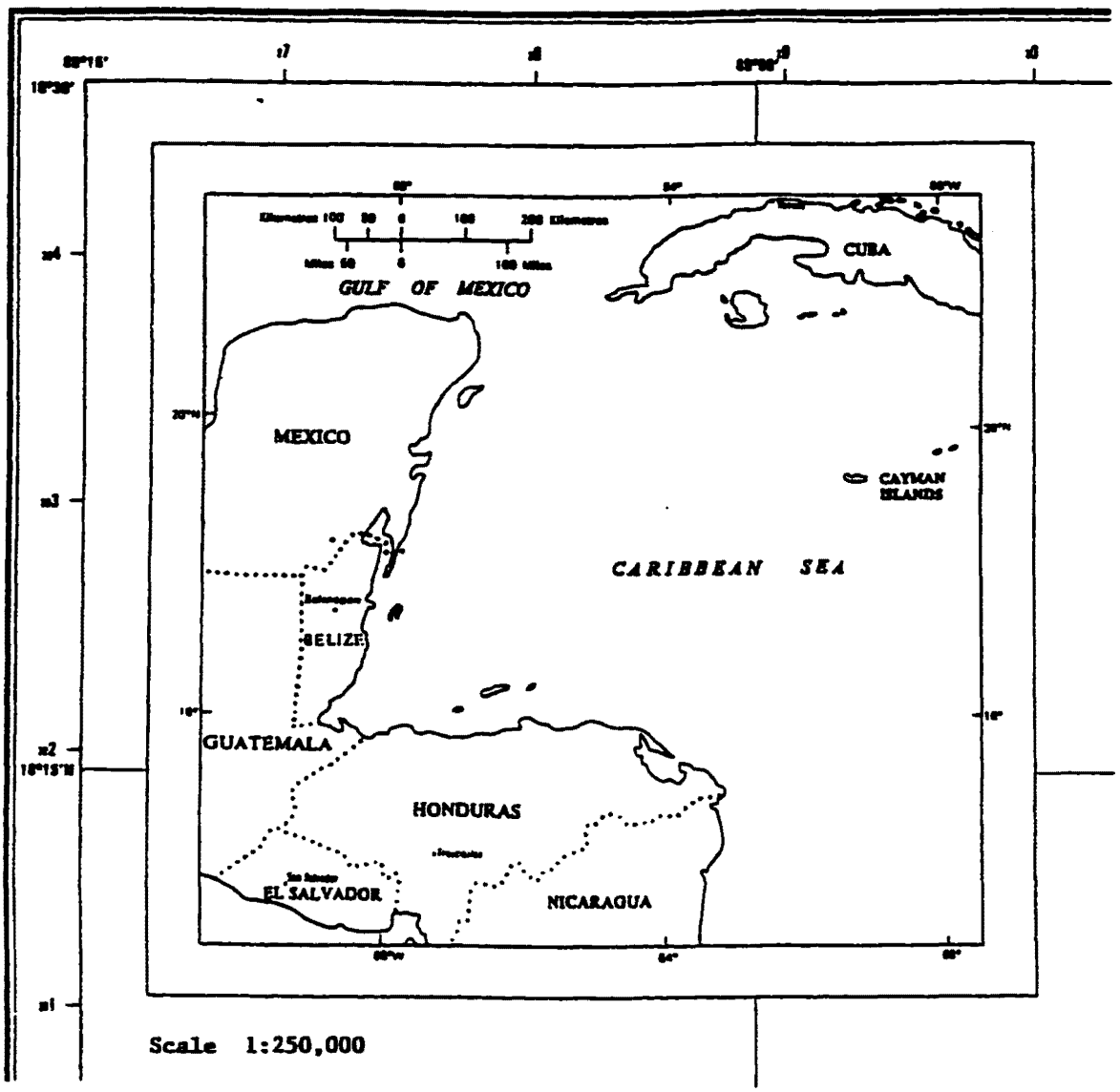


Figure 5. Map of Belize relative to other Central American nations (Merrill, 1993).

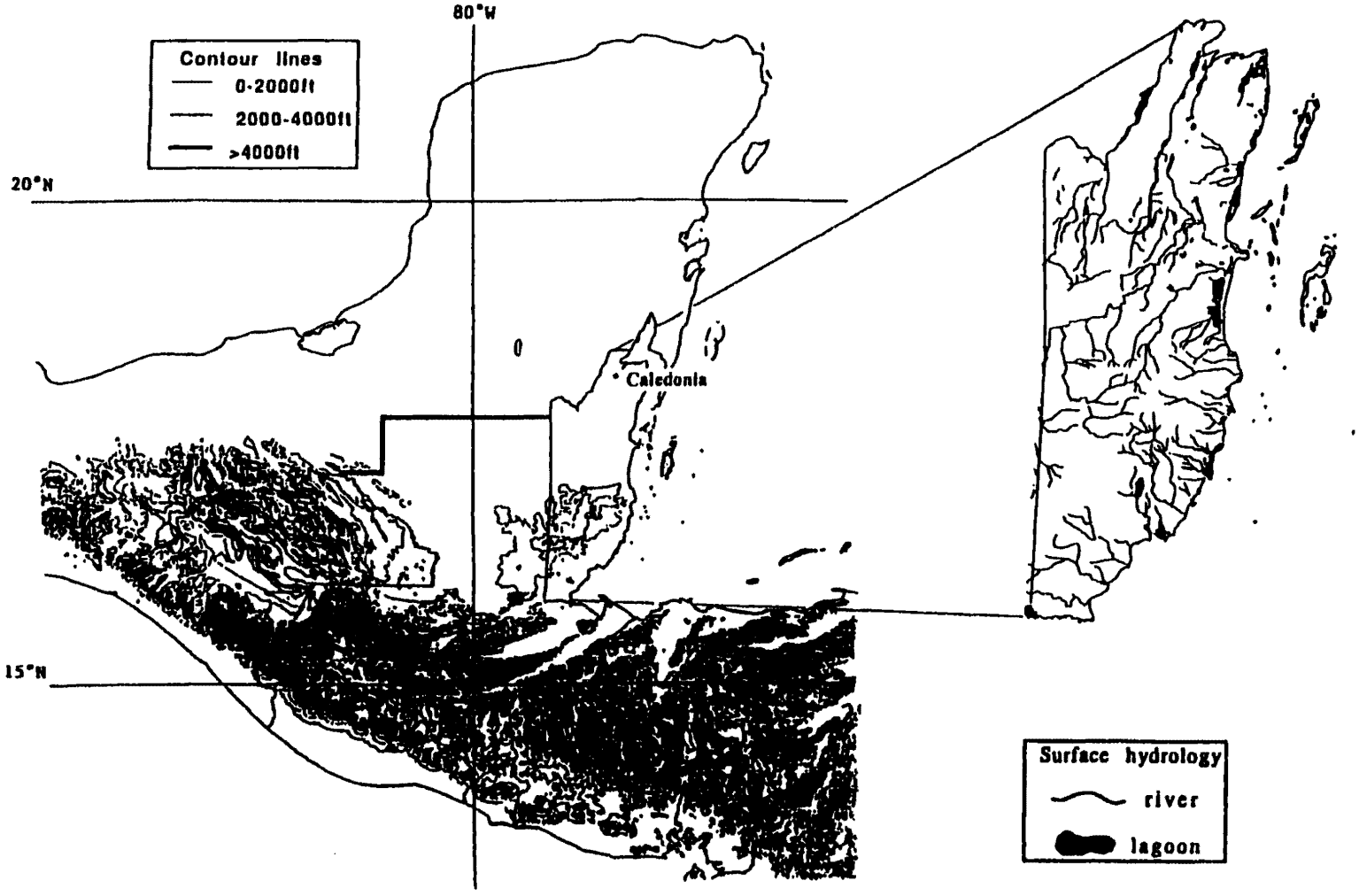


Figure 6. Map of Belize and surrounding landscape depicting general elevation and surface hydrology.

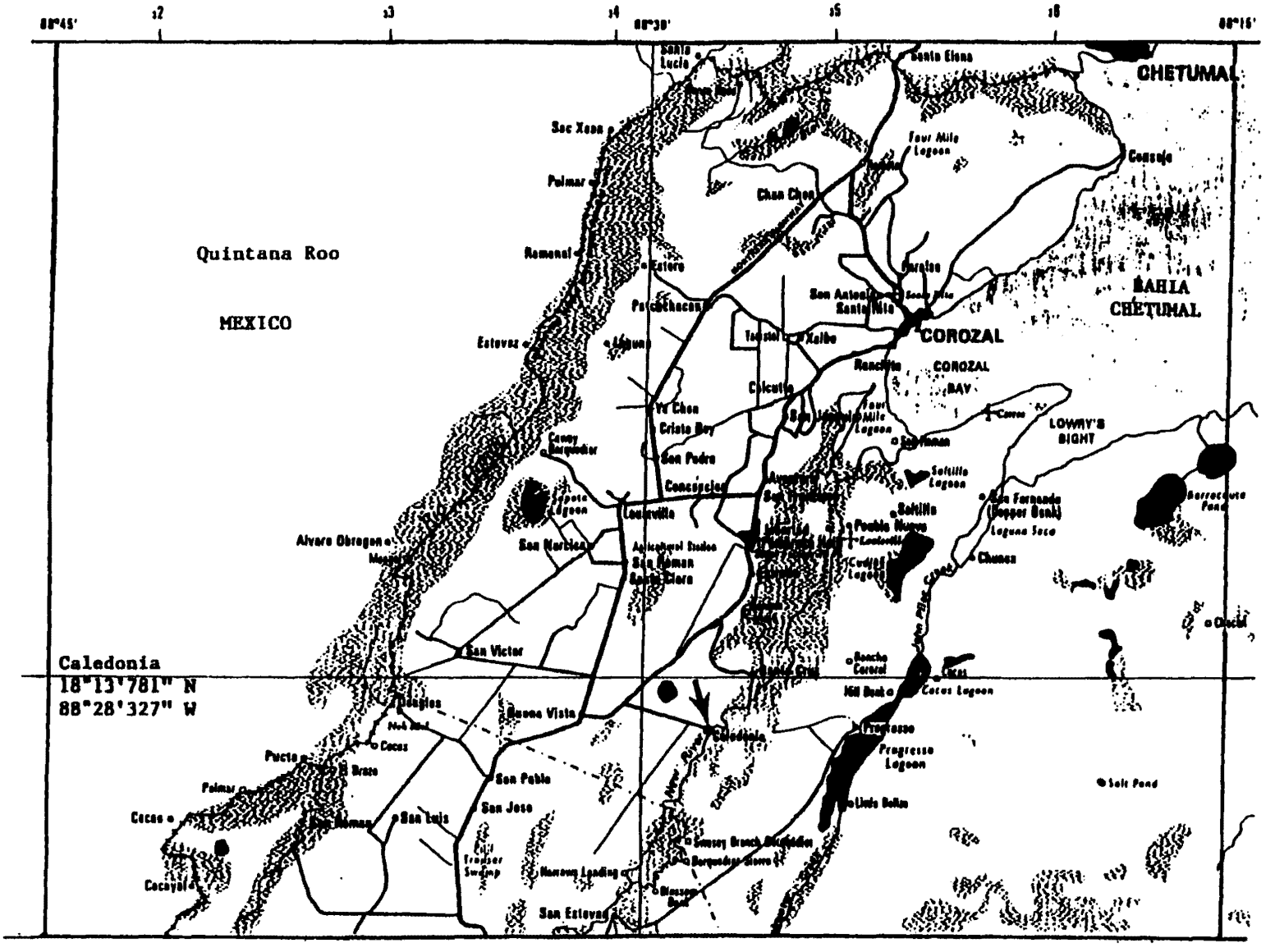


Figure 7. Map of Corozal District, northern Belize depicting position of study site, Caledonia Village, (arrow) along the New River.

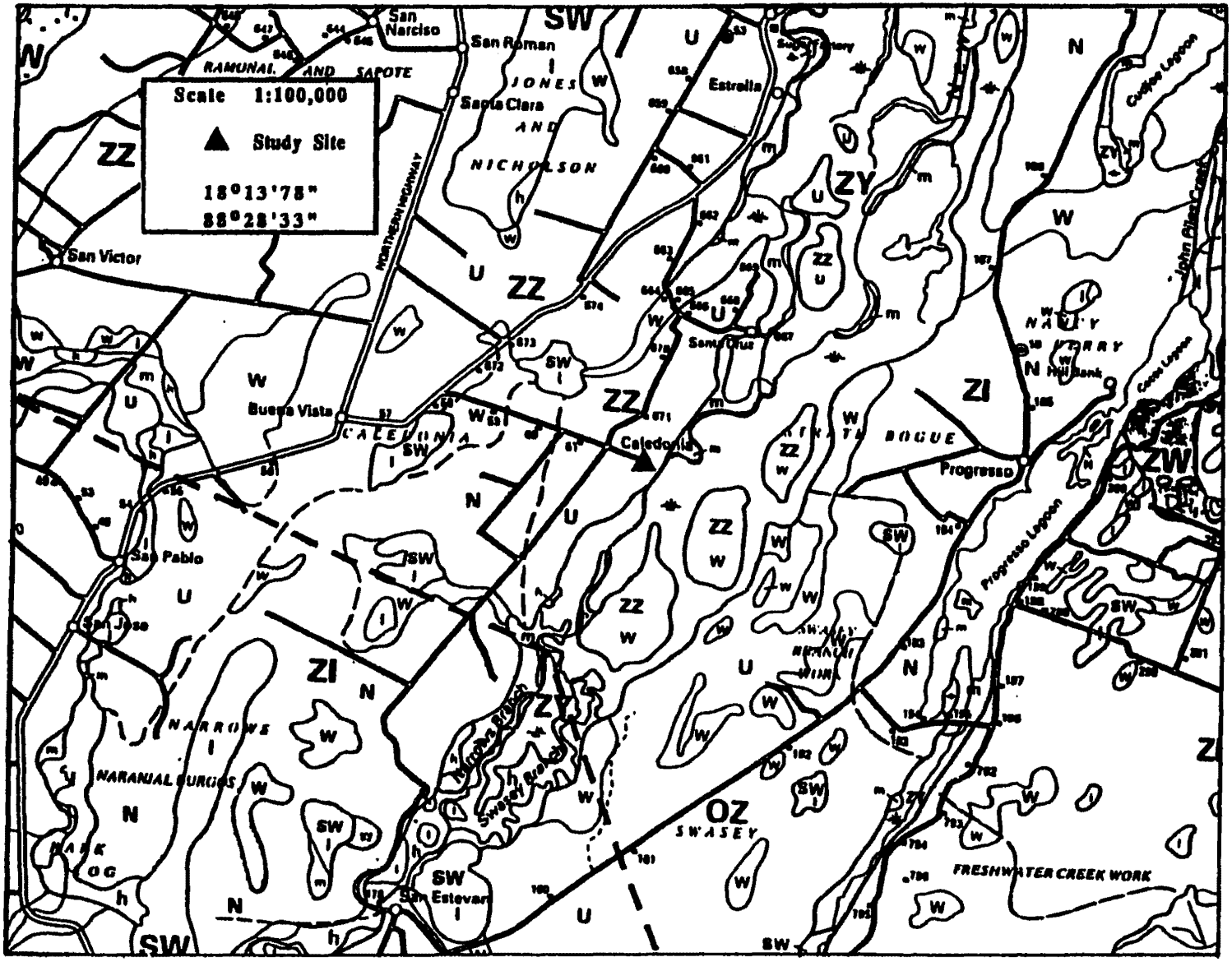


Figure 8. Land resource assessment map depicting land characteristics of surrounding study site, Caledonia Village (King et al. 1992).

Table. Legend remarks for land resource assessment map (Fig. 8) of northern Belize and Caledonia area (King, et al. 1992).

Land system	Dominant rock type	Drainage density	Altitude (m)	Dominant subunits	Characteristic vegetation	Water availability
ZZ	Sascab	None	<20	Undulating plain lower slope	High broadleaf semi-deciduous forest with many cohune palms	Low
OZ	Sascab sand	Very low	<50	Undulating plain lower slope	High broadleaf semi-deciduous forest with many cohune palms	Low
ZI	Limestone variable hardness	None	<20	Flat plain lower slope	Low broadleaf semi-deciduous forest with sapote, mahogany and cohune	Low
SW	Alluvium	Zero	<100	Herbaceous swamp, savanna plain	Herbaceous: rushes and sedges; marsh forest	High
ZW	Limestone	Zero	<5	Flat plain	Herbaceous-low broad-leaf semi-deciduous forest mosaic	High
ZY	Alluvium limestone	Very low	<5	Savanna Swamp	Mangrove, savanna Herbaceous swamp	High

Figure 9. Land cover classification of SPOT multispectral image, Caledonia area, northern Belize (approximate distance across 3.2 km, north at top).

Key: white (1) – urban/bare ground; yellow (2) – pasture/fallow; dark brown (3) – cropland; dark green (4) – forest; light green (5) – swamp forest; light brown (6) – savanna; pink (7) – tall dense marsh; purple (8) – tall sparse marsh; red (9) – low sparse marsh; and blue (10) – water.

Arrow ↑ indicates approximate location of study site along the New River.

CHAPTER 2

Susceptibility of *Anopheles* (Diptera: Culicidae) species to DDT and deltamethrin in Caledonia, northern Belize, Central America

ABSTRACT

Knowledge of insecticides' effectiveness and how insecticides function to reduce disease transmission is critical information for malaria control programs seeking reassurance of current control policies and availability of proven alternatives. Through timely surveillance mechanisms, frank or incipient resistance to insecticides can be detected and the impact on control assessed. In response to a deteriorating malaria situation in Belize, which was initiated by reduction and eventual cessation of indoor spraying, the present study assessed the insecticide susceptibility status of anophelines captured in a malarious village in northern Belize.

Dose-response, using the WHO conventional contact susceptibility test, was analyzed by probit statistics on *Anopheles albimanus*, *Anopheles crucians* and *Anopheles vestitipennis* captured in Caledonia Village from September 1995 to May 1996. Serial dilutions of the target (diagnostic) dosage of DDT (4%, 2 gm/m^2) and deltamethrin (0.025%, 0.00925 gm/m^2) were used. Mortality and knockdown rates after contact with different concentrations or varying exposure times to single concentrations are presented.

These findings indicate, that in northern Belize, all 3 *Anopheles* species remain susceptible to the diagnostic dosage of DDT despite decades of routine indoor residual spraying. This information is important for malaria control with regards to *An. albimanus*, a major vector in the region, and *An. vestitipennis* as a suspected secondary vector in Belize. The median lethal dosage of DDT varied from 0.435 (0.87%) to 0.277 gm/m^2 (0.55%). Deltamethrin, a pyrethroid never before used for malaria control in

Belize, was a potent contact toxin for *An. albimanus* and *An. vestitipennis*, producing high mortality (>84.5%) after 60 min contact as low as 0.0015% concentration of insecticide. The few *An. punctimacula* and *An. gabaldoni* tested also were completely susceptible to both compounds. Probit slopes and SE of the estimates indicate low heterogeneity in species population response to DDT and deltamethrin. Significant differences in response between species were seen at the lower concentrations of both chemicals. Proportion knockdown showed strong positive correlation with 24-hr mortality at the higher doses. It is most probable that DDT would remain effective in suppressing transmission by remaining either toxic to the majority of the vector population and/or continuing to lower human-vector contact by vector behavioral avoidance of sprayed structures.

INTRODUCTION

"*It is important not to assume that if resistance has been reported "in a given species* [it] *is at an operational level and that a rapid change to alternatives is necessary* [and that] *the species is universally resistant throughout the country in question.*" **WHO, 1986.**

MALARIA remains a serious health and economic problem in the Americas (WHO, 1997; PAHO, 1994a). In Belize (formerly British Honduras), the use of DDT as an indoor residual spray for malaria control began in 1957 (Brown et al., 1976; PAHO, 1986). By 1963, as a result of an intensified national spray program, the disease had virtually disappeared (PAHO, 1986). The entire country was placed in the consolidation phase of eradication that same year. From 1963 onwards, yearly spray coverage fluctuated from complete to no coverage. Retrospectively, a strong correlation between decreased spray coverage and the increase in the number of malaria cases was seen (D.R. Roberts, per. comm.). The country abandoned the nationwide coverage of routine house spraying in 1989, while continuing limited focal spraying operations during 1991-1992. Inadequate spray coverage, and possibly more effective surveillance, contributed to the dramatic increases in the reported incidence of malaria countrywide. Between 1993 and 1994, Belize had an incidence of 42 and 47.4 per 1000 inhabitants, respectively, the highest in Central America (WHO, 1996, 1997). Microscopically confirmed cases dramatically rose from 3,033 in 1990 to over 8,600 in 1993. In 1994, Belize reported 9,957 cases, an all time high for the country, with most of the cases reported from the districts Cayo, Toledo and Corozal. A substantial increase in *Plasmodium falciparum*

malaria also has been seen, increasing from only 40 cases in 1990 to over 400 cases in 1994. Despite this alarming trend, the Government of Belize (Belize Pest Control Board) again temporarily suspended the use of DDT for malaria control beginning in 1993, principally because of objections from some developed countries and international funding agencies. This effectively ended vector control activities and further aggravated a deteriorating condition (Vanzie, 1995). In late 1996, DDT was reinstated for vector control, but was restricted for use in only the most malarious villages. Although the renewed spraying program has shown an impact on transmission, the risk of malaria still exists throughout the year in the rural parts of the country.

Resistance to DDT by *An. albimanus*, a major vector of malaria in Belize and much of Central America, has been considered a serious operational problem in certain areas of the region (Davidson, 1963; WHO 1986, 1992, Brown & Pal, 1971). Frederickson (1993) has provided a recent review of the historical development of resistance in *An. albimanus*. Other potential vectors, also present in Belize, have been found with varying degrees of reduced susceptibility to DDT in other locations in the Americas (Brown 1986; Brown & Pal ,1971; WHO, 1992; Mekuria et al. 1990). These include *Anopheles darlingi* (Colombia, Venezuela), *Anopheles apicimacula* (Panama), *Anopheles punctimacula* (Colombia, Ecuador, Panama), *An. crucians* (Mexico), *Anopheles pseudopunctipennis* (Bolivia, Guatemala, Honduras, Mexico, Panama, Peru) and *An. vestitipennis* (Dominican Republic, Guatemala, Mexico). Moreover, *An. albimanus* and *An. pseudopunctipennis* have been found multi-resistant across a broad spectrum of compound classes (organochlorines, organophosphates, carbamates, and pyrethroids) commonly used in both agriculture and public health (Davidson & Sawyer,

1975; Loyola et al. 1991a; WHO, 1992). The profuse use of pesticides in agriculture has been cited as the principal reason behind the dramatic selection patterns of insecticide resistance (Hobbs, 1973; Bown, 1987; Breeland, et al. 1970; Lines, 1988). Intensive use of insecticides on crops has been pronounced along the Pacific coast of Central America where a far greater proportion of agro-industrial production takes place. In areas of intensive agricultural spraying, particularly in cotton producing regions, rapid development of cross resistance has been well documented (Georghiou, 1972; Georghiou et al. 1972, 1973; Frederickson, 1993).

In Belize, *An. albimanus* has been reported resistant to DDT (WHO, 1992) and dieldrin (Brown & Pal, 1971). Chareonviriyaphap (1995) detected DDT resistance in 35% of tested *An. albimanus* from southern Belize (Toledo District) using the diagnostic (4%) dosage, but found complete susceptibility to 0.025% deltamethrin. However, they reported that a population from northern Belize (Chan Chin, Corozal District) was found completely susceptible to both DDT and deltamethrin. As far as known, information on insecticide susceptibilities of other anopheline species has not been reported for Belize.

In response to the alarming rise in malaria, the present study assessed the current susceptibility status of anophelines captured in an endemic malarious village of northern Belize. Dose-response data using the WHO conventional contact susceptibility test was analyzed by probit statistics on *Anopheles albimanus*, *An. crucians* and *An. vestitipennis* captured in Caledonia Village from September 1995 to May 1996. Serial dilutions of the target (diagnostic) dosage of DDT (4%, 2 gm/m^2) and deltamethrin (0.025%, 0.00925 gm/m^2) were used.

MATERIALS AND METHODS

Study area: The country of Belize is located on the southeastern part of the Yucatan peninsula of Central America (Fig.). Nearly half of the country is low-lying coastal plain, the remainder is hilly or mountainous. Climate in Belize is subtropical with relatively constant maximum and minimum temperatures throughout the year. Average temperatures in the coastal regions range from 24°C in January to 27°C in July. The rainy season typically lasts from May/June through November. The coastal plain regions consist of lowlands with elevations generally less than 20 meters above sea level. A substantial part of the lowlands is characterized by relatively undisturbed herbaceous wetlands providing important anopheline larval habitats. A detailed description of the northern coastal plain and the wetland ecosystems has been provided by Rejmankova et al. (1995, 1996).

Caledonia Village is located in the northern coastal zone along the New River floodplain, Corozal District, northern Belize (18°13'78"N, 88°28'38"W). Average annual rainfall for this region does not normally exceed 1,350mm (54 in). Access is available year round by an all-weather road. The village consists of approximately 395 houses with an estimated population of 1,227 (Vector Control Programme [VCP] unpub. data). The native population is predominantly Spanish-speaking Mestizo (Honduran and Salvadoran extraction) and income is derived mostly from seasonal sugarcane production. Malaria incidence in Caledonia has been among the highest in the northern sector of Belize since 1988 (VCP unpub. data). In 1994, 100 cases (91/1000) were recorded for

the village. The vast majority of the infections are *Plasmodium vivax*, only rarely is *P. falciparum* encountered. Regular indoor residual spraying (2-cycles/yr) with DDT (75% wettable powder or technical grade product in kerosene) had been conducted in Caledonia on a near continuous basis for nearly 40 years at the time of this investigation. Before conducting the susceptibility assays, the last village-wide indoor application of DDT was in March 1995 in direct response to the high number of village malaria cases.

Insecticides: Only analytical grade chemicals were used in the impregnation of test and control papers. DDT (1,1,1,-trichloro-2,2-bis [*p*-chlorophenyl] ethane), 99% pure *p,p'*-isomer, was provided by the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine (USACHPPM), Aberdeen Proving Ground, Maryland, USA. Deltamethrin (K-Othrine®, Decis®) [(S)-α-cyano- 3-phenoxybenzyl (IR)-cis-trans-3-(2,2-dibromovinyl)-2, 2-dimethylcyclo-propane carboxylate], 99.7% pure, was kindly provided by AgroEvo Environmental Health (U.K.), United Kingdom in January 1994.

Test papers: Insecticide impregnated papers (MN261 Chromatography paper, Lot# SA231, Macherey-Nagel, Germany) were made to World Health Organization specification by the USACHPPM, Entomological Sciences Division (Zeichner, 1992). The following concentrations were used (all mg and gm amounts expressed as active ingredients [AI] per ml or m^2): **DDT**: 4% (13.09 mg/ml or 2 gm/m^2), 2% (6.546 mg/ml or 1 gm/m^2), 1% (3.273 mg/ml or 0.5 gm/m^2), 0.25% (0.818 mg/ml or 0.125 gm/m^2); and

Deltamethrin: 0.025% (0.0605 mg/ml or 0.0925 gm/m^2), 0.0125% (0.03025 mg/ml or 0.0046 gm/m^2), 0.00625% (0.015125 mg/ml or 0.0023 gm/m^2), 0.003125% (0.00756 mg/ml or 0.0011 gm/m^2) and 0.00156% (0.00378 mg/ml or 0.00057 gm/m^2). All 12 x 15 cm papers were prepared separately and treated at the rate of 2.75 ml of insecticide solution per 180 cm^2 of paper surface using the WHO recommended gm/m^2 diagnostic dosage, with dose serial dilutions expressed on a weight of insecticide per volume of carrier (WHO, 1981b). Trichloroethylene (TCE) was the solvent used to aid uniform dispersal of the solution onto the paper. The carrier for DDT was Semtol 85 (Witco) + TCE, and deltamethrin was dispersed using silicon fluid (Dow Corning #556) + TCE. Papers were separated based on chemical and concentration and hermetically sealed in airtight aluminum bags. Control papers were treated with Semtol or silicon fluid without insecticide as appropriate for each insecticide. All test papers were made and packaged in May 1995 and stored at ambient temperature (27 ± 5 °C).

Mosquito collections and handling: All mosquito collections were taken from the same study site location. Adult female anophelines were captured using mouth aspirators during early evening outdoor landing collections from exposed lower legs of human volunteers (WHO, 1975). Anophelines were placed into a clean, 3.8 L cardboard ice cream container and allowed to randomly mix. Mosquitoes were kept overnight and supplied water-saturated cotton pads only. Susceptibility tests took place the following morning. Mosquitoes were not held for more than 12 hours before testing. Because the tests required replicates and sufficient controls, a relatively high number of mosquitoes

were needed. To reduce potential handling damage, mosquitoes were not sorted to species before testing. Only *An. albimanus*, *An. crucians* and *An. vestitipennis* were present in sufficient numbers to allow appropriate analysis and presentation.

Test Conditions: All tests were conducted during the cooler morning hours under similar ambient conditions. Ambient temperature and relative humidity was recorded during the exposure period and maximum-minimum temperature was recorded over the 24-hr holding period. All tests took place in a well-ventilated, vacant house that had not been sprayed with DDT for over 6 years.

Test method by dose: A clean WHO standard test kit was used following the instructions set forth by WHO (1981a,b). Only female mosquitoes with no obvious evidence of blood in the abdomen were used in the tests. Unfed and non-gravid female mosquitoes (20-25) were selected randomly, placed in each holding tube by mouth aspirator, and allowed a 1-hr pre-test adjustment period. All pre-test damaged/dead specimens were excluded from the final analysis. Two control tubes containing representative populations of mosquitoes were run concurrently with each test series. A series consisted of either 4 DDT or 5 deltamethrin tubes, each tube containing one treated paper of a different concentration of chemical. Each test design was replicated at least 3 times. As recommended, individual papers were not used for more than 20 tests or after 3 weeks of removal from the package (Zeichner, 1992).

Mosquitoes were exposed to the particular concentration for 60 min, then carefully transferred into clean individual holding tubes, and provided a 10% sucrose

solution on a cotton pad as a carbohydrate source. Mosquitoes found dead or having experienced knockdown immediately after the contact period were recorded but not identified to species. Knockdown (KD) was defined as the condition when a specimen was unable to fly or effectively walk following exposure. Holding tubes were placed upright in a large plastic ice chest on plastic pans over a thin layer of water to thwart scavenging ants. At 24-hr post-exposure, mortality was recorded and each specimen identified to species according to Wilkerson et al. (1990). Only *An. albimanus* and *An. vestitipennis* were available in sufficient numbers for analyses of deltamethrin for 60 min exposure periods. Deltamethrin test series also were done using *An. albimanus* at 30 min exposures.

Tests with variable exposure periods: The same procedures as described by dose test method apply to tests using different exposure periods. Tests were conducted using only deltamethrin at the diagnostic (0.025%) and lowest (0.00156%) concentrations against *An. albimanus* and *An. vestitipennis*. Depending on availability of specimens, tests were conducted with 4 or 5 different exposure periods (60, 30, 15, 7.30, 3.15 min.). Mortality was recorded 24-hr post-exposure.

Analysis: Total mortality or knock-down is calculated and presented as a proportion. Abbott's formula [(% test mortality -% control mortality)/(100 -% control mortality)] was invoked to correct proportion mortality in any test series when control mortality over the same period was between 5-20% (Davidson & Zahar, 1973; WHO, 1981a). General interpretation of results is based on criteria and remarks established by

WHO, 1981c). Lethal dosage (LD_{50}, LD_{90}), lethal time (LT_{50}, LT_{90}), knockdown (KD_{50}, KD_{90}) and knockdown time (KDT_{50}, KDT_{90}) estimates reflecting 50 and 90% of a particular population's response were calculated using probit analysis from Abbott's adjusted dose-response data (Finney, 1971). SAS/STAT 6.04 (SAS® Institute, Inc. Cary, N.C., USA) 'Proc Probit' program (log-likelihood for normal distribution model) was used to derive log probit regression line probabilities and 95% fiducial limits. When appropriate, response variables were adjusted using Abbott's formula proportions before probit analysis. Comparisons between chemical doses, exposure times and species were completed using 2x2 contingency table chi-square (χ^2) statistics (Yates' corrected) for differences between tests (Remington & Schork, 1970). Significance was determined at level $p<0.05$.

RESULTS

Test results on field populations of *An. albimanus*, *An. crucians* and *An vestitipennis* are presented. Other anophelines collected and assayed in Caledonia, *Anopheles punctimacula* and *An. gabaldoni*, were found to be susceptible to the diagnostic dosage of DDT and deltamethrin, unfortunately too few in number were assayed for definitive analysis (Robertson, et al. 1984). Data derived from 0.5% DDT papers were consistent outliers in response outcomes across all tests and species and were excluded from analysis. Ambient temperature and relative humidity varied from 21-29.5°C (70-85°F) and 75-95% RH during the morning hours of test exposure. Maximum temperatures rarely exceeded 32°C (90°F) and midday relative humidity generally remained above 50% during the 24-hr holding periods. *Anopheles albimanus* was the most plentiful mosquito captured, and its greater sample size is reflected in the wider variety of tests performed. Overall control mortality in the DDT and many of the deltamethrin trials exceeded 5%. Accordingly, response data and proportion mortalities were adjusted using Abbott's formula.

Dose-response proportion mortality for DDT for the 3 species (Table 1) and the LD_{50} and LD_{90} values from probit analysis (Table 2) indicates all species were highly susceptible to the 4% diagnostic dosage (2 gm/m^2), while showing consistent reductions in mortality with decreasing concentrations of insecticide. Probit slopes and SE's of the estimates indicate low heterogeneity in species population response to DDT. Proportion mortality between doses within species were all significant by χ^2 analysis ($p<0.05$).

Anopheles albimanus had the highest LD_{50} and LD_{90} relative to *An. vestitipennis* and *An. crucians*. The LD_{50}/LD_{90} gm/m^2 (and % concentration) range limits for the 3 species was 0.4353 (0.87%) - 0.2777 (0.55%) and 0.9917 (1.98%) - 0.7731 (1.54%), respectively (Table 2). Mortality with 4% DDT for *An. albimanus* was 98.1% and within the established level of susceptibility (Davidson & Zahar, 1973). Successive trials at the diagnostic dosage generally resulted in 100% mortality. From Table 1, only at the lowest (0.25%) concentration were differences seen between species; *An. crucians* having a significantly higher proportion mortality (0.187) than either *An. vestitipennis* (0.011) or *An. albimanus* (0.019).

Deltamethrin was found to be extremely toxic for both *An. albimanus* and *An. vestitipennis* down to a 0.00156% concentration after 60 min exposure. Probit analysis was not possible at 60 min dose-response exposure to deltamethrin as mortality and knockdown exceeded 90% at all concentrations for both species. Complete mortality was noted from 0.025% (diagnostic dose) to 0.003125%, 8-fold lower than the recommended target dosage (Tables 3 & 4). *An. albimanus* showed complete susceptibility to 0.025 and 0.0125% after 30 min exposure and >83% kill to 0.00625 and 0.003125% (Table 5). The LD_{50} and LD_{90} values at 30 min exposure were 60 and 5-fold lower than the diagnostic dosage, respectively. (Table 6).

The median lethal time (LT_{50}) is a measure of mosquito response to a single dose in relation to differing time periods of exposure. Probabilities of LT were estimated from computed regression of probit mortality on log-time of exposure. For *An. albimanus* and *An. vestitipennis*, the highest and lowest concentrations were evaluated at 5 and 4 different exposure times, respectively (Tables 7 & 8). At 0.025%, complete mortality

was seen for both species down to 15 min exposure, while remaining high (> 77% mortality) after 7.30 min exposure. A significant χ^2 difference was seen between species at 3.15 min, with *An. vestitipennis* having > 88% survival (Table 7). At 0.00156%, significant differences were seen between species at 30, 15 and 7.30 time periods (Table 8). *An. vestitipennis* appeared far less susceptible at the lowest dose over the 3 time periods (0.328-0.069) than *An. albimanus* (0.686-0.389). This large difference is also reflected in the estimated LT_{50} values for 0.00156% shown in Table 9. Further analysis (Table 10) of dose-response LD_{50} and LD_{90} using high and low concentrations at the 7.30 min exposure period revealed a significantly higher tolerance at the lower concentration (0.00156%) in *An. vestitipennis* over *An. albimanus* (p=0.0001).

The use of mixed species in most trials precluded identification of individuals during the exposure period. Consequently, species-specific rates are not available for proportions knockdown in susceptibility tests. Proportion knockdown for all *Anopheles* species were analyzed using probit analysis. At 60 min exposures (Table 11 & 12), both compounds have dramatic knockdown properties at the higher doses. Deltamethrin showed overall stronger knockdown relative to DDT with all incrementally decreasing doses. The greater knockdown effect of deltamethrin also is illustrated by the KD_{50} and KD_{90} concentration values in 60 min exposures for both compounds (Table 13). At 30 min exposure, deltamethrin maintained strong knockdown capabilities at the 2 higher doses, dropping off precipitously with the 3 lower concentrations (Table 14). Differences in KD estimates as products of different exposure times are reflected in KD estimates at 30 min vs. 60 min (Tables 13 & 15). A 5-6 fold lower dosage was required for 50 and

90% KD after 60 min compared to 30 min. Although knockdown was strongly correlated with eventual mortality, overall, higher doses are required for 60 min KD than for 24-hr mortality. Comparing Tables 2 & 13, $LD_{50/90}$ vs. $KD_{50/90}$, it is apparent that few mosquitoes, if any, recovered within the 24-hr period after showing knockdown at 60 min.

Similarly, the proportion knockdown at the highest and lowest concentrations of deltamethrin, using time as the discriminating exposure variable (Tables 16 & 17) compared to mortality data (Tables 7 & 8), indicates that recovery after knockdown is an unlikely event (Tables 9 & 17). The KDT_{50} at 0.025% was 11.69 min compared to over 2- hr exposure with 0.00156%, the low dose (Table 18).

DISCUSSION

The WHO contact susceptibility test method was used to analyze 3 species of anophelines captured in Caledonia, northern Belize from September 1995 to May 1996. Four concentrations of DDT and five concentrations of deltamethrin were used. Probit analysis was used to derive log dose-response curves, 95% fiducial limits and respective LD, LT, KD and KDT estimates. Proportion knockdown after 1-hr exposure to different concentrations or varying exposure times to single concentrations also was amenable to probit statistics. Recovery from knockdown would suggest either tolerance or incipient resistance. The number of replicates and types of tests were based on availability of field-caught specimens and varied between the 3 species.

The standard WHO adult mosquito susceptibility test method (WHO, 1981a,b) was used because it is still the commonly recognized means of testing and comparability with results from other regions (Rouch & Miller, 1986; Shidrawi, 1990). This test uses standardized insecticide-treated papers of varying concentrations, placed in holding tubes, on which groups of mosquitoes are allowed contact over a set period of time. Knockdown response and mortality are measured during and after exposure for up to 24 hrs. Tests can be altered to reflect the response of mosquitoes to different chemical doses over a particular time period or use of a single dose (usually a designated diagnostic concentration) comparing varying exposure times (Ariaratnam & Brown, 1969). For example, doubling the exposure period has the same effect as doubling the DDT concentration, thus halving the LD_{50} obtained. As originally intended, this test was

designed to reproduce, as nearly as possible, the results of a mosquito resting on an insecticide-treated surface (wall) inside a house, either before or after a blood meal. The goal reflects the original development of the method as an assessment tool in malaria control (WHO, 1981c). Although, limitations and criticism have been given in disfavor of this testing procedure (Davidson & Zahar, 1973; Shidrawi, 1990; Brogdon & McAllister, 1998), it remains the most widely used and referenced method today.

The WHO base-line and diagnostic tests have been technical standards for comparability of intra- and interspecies variability in susceptibility to insecticides for many decades. The tests are useful for detecting the absence or presence of resistance, but unlike biochemical and molecular assays, provide no information on the underlying mechanisms of resistance (Brown & Brogdon, 1987). Likewise, behavioral responses of the insect determine the dose it receives once placed on insecticide-impregnated paper. Beyond crude observations of flight behavior relative to controls during test exposure, these self-dosing tests cannot discriminate between avoidance behavior and physiological resistance. Molineaux et al. (1979) has discussed the issue of the usual (implicit) assumption of uniform exposure of insecticides in vector populations, concluding that non-uniform exposure is more realistic when assessing the true insecticidal impact on vectorial capacity. Interpretation of bioassay results can be difficult because variation in response among individual insects from a susceptible population is common (Hoskins & Gordon, 1956). So-called 'vigor tolerance' also can impact test results, and appears influenced by age, physiological and nutritional states, overcrowding during larval development, and seasonality (Busvine, 1956; Raffaele et al. 1958; Gordon, 1961; Gilotra, 1966; Hadaway & Barlow, 1956). Tolerance, in this case, is defined as the

ability of an individual mosquito to withstand a dose of poison that would have been lethal at some point earlier in its lifetime. In general, there is a progressive increase in susceptibility with advancing age. The effects of seasonal climatic changes are assumed diminished in the subtropical climes; however, seasonality with respect to agricultural use of insecticides in Central America has been associated with changes in local vector susceptibility (Georghiou et al. 1973; Bown, 1987). Many of these factors are difficult to control or measure accurately in the field, and test interpretations must be made with these variables in mind.

Some tests had higher control mortalities (>5%) than would have been desired (WHO, 1981a). Increased control mortality may have resulted from the intentional withholding of sugar prior to testing. Sugar contact/feeding has been found to significantly reduce mortality compared to unfed (non-feeding) mosquitoes. (Elliott & Ochoa-Aguirre, 1974). Additionally, mosquitoes recently blood-fed are normally recommended for testing as they generally have lower mortality over the period of the observation than unfed mosquitoes (WHO, 1981b). Time and human-use issues precluded the use of blood-fed mosquitoes in this study. However, there is apparently little change in susceptibility to DDT between mosquitoes tested unfed compared to 3-hr post feeding, while a significant (2-fold) increase in LD_{50} has been noted for those mosquitoes exposed to DDT immediately after a blood meal (Hadaway & Barlow, 1956).

The probit statistic is a dose-response estimate representing the 'best-fit' straight line relationship to derive the maximum likelihood medium lethal dose. The interpretation and merit of susceptibility tests based on a calculated median lethal dosage

vary (Hoskins & Gordon, 1956; Brown & Brogdon, 1987; Miller, 1988; Brogdon & McAllister, 1998). Davidson & Zahar (1973) believed use of the log probit regression line may result in a failure to recognize early stages of resistance, and they advocated a more meaningful test would determine the concentration (diagnostic) that normally kills all individuals of a susceptible strain. They contend that probit mortality relationships of dose-response data can only apply when the tested population is homogeneous (Finney, 1971) in its linear response to the toxin and is of uniform age and general physiological condition. Seldom, if ever, are these conditions met when using field-caught specimens resulting in a heterogeneous test population. A variety of mathematical methods have been developed to help adjust for this apparent lack of homogeneity, but all still fall short in adequately detecting low levels or frequency of resistance (Rouch & Miller, 1986; Brogdon & McAllister, 1998). Admittedly, a limitation of all tests is often the wide variability of response between test subjects and an inherent inability to have precise reproducibility of results between all treatment trials, even under laboratory conditions. This can be overcome by stringent statistical treatment of binary categorical (quantel) data, to approximate a normal distribution (Muirhead-Thomson, 1960). Additionally, increasing the sample size, use of random sampling and adequate replication of tests will help reduce standard errors and increase the precision of the estimates (Finney, 1971).

The inherent biologic variability within a normally distributed field population actually increases the utility of probit analysis in summarizing natural (field) responses vice selected (unrealistic) homogeneous inbred laboratory strains (Hoskins & Gordon, 1956).. From the field, we assume genetic panmyxia (random mating) within the sampled population and near identical survival values of the genotypes. The interpretation of the

LD_{50} using the principal of maximum-likelihood remains valid despite stated limitations and untested assumptions. Probit methods provide a more complete picture of the type and amount of resistance in a population while still providing information on the response to the discriminating dose (Miller, 1988).

Insecticide susceptibility testing in *Anopheles* from Belize has been conspicuously lacking. Information on the susceptibility of *An. albimanus*, a major vector in the region (Frederickson, 1993), and *An. vestitipennis*, a strongly suspected vector in Belize (Roberts et al. 1993) and recently incriminated as an important vector in neighboring southern Mexico and northern Guatemala (Padilla et al. 1992; Loyola et al. 1991b) is needed for proper management of the national malaria control program. Only *Anopheles albimanus* has been documented to be resistant to insecticides (DDT and dieldrin) in the country (WHO, 1992; Brown & Pal, 1971; Chareonviriyaphap, 1995). As far as known, information on insecticide susceptibility status of other anopheline species has not been reported for Belize.

DDT resistance in *An. albimanus* has been reported from all countries in Central America (WHO, 1992). Deltamethrin resistance has been reported in *An. albimanus* from Guatemala and Mexico (Malcolm, 1988; Beach et al. 1989). In Caledonia, *An. albimanus* was found susceptible to the diagnostic dose of DDT and highly susceptible to deltamethrin at concentrations 16-fold below standard target dosage. *Anopheles vestitipennis* has been reported resistant to DDT in Mexico and Guatemala (WHO, 1968; Brown, 1986) and more recently in the Dominican Republic (Mekuria et al. 1990). In the Dominican Republic, they found only 74.4% (n=133) susceptible to 4% DDT. In

Caledonia, complete susceptibility at 2 gm/m^2 (4%) was seen. Deltamethrin was highly toxic below 0.003125% concentration, 8-fold below the recommended operational target dosage. Insecticide resistance in *An. crucians* has been reported for DDT in Mexico (Georghiou & Mellon, 1983; WHO, 1992) and dieldrin in the Dominican Republic and the USA (Brown, 1986). This species also was found highly susceptible to DDT and deltamethrin in Caledonia. Although a limited number of *An. gabaldoni* and *An. punctimacula* were examined, all were found susceptible to both compounds. Conclusions on susceptibility status for these 3 species must be tempered with regards deltamethrin and *An. crucians* and DDT and deltamethrin with *An. gabaldoni* and *An. punctimacula*, as sample sizes were below generally acceptable statistical levels of confidence (Robertson, et al. 1984; DeBanne & Haller, 1985). As a general rule, resistance to the diagnostic dose at levels exceeding 20% is considered an impediment to malaria suppression (Davidson & Zahar, 1973). From a control perspective, both DDT and deltamethrin would appear acceptable for malaria suppression in Caledonia, Belize.

In an earlier study, Chareonviriyaphap (1995) suggested that the difference in DDT susceptibility between the southern (resistant) and northern (susceptible) populations of *An. albimanus* owed to the greater use of agricultural chemicals in Toledo district. In particular, greater exposure to Gramoxone® (Paraquat dichloride), a common bipyridylium class, DDT-like herbicide used for weed control in rice fields, was cited as a possible difference in environmental exposure between the 2 locations. Rice fields, in particular, are considered favorable larval habitats for *An. albimanus* (Brown & Pal, 1971; Frederickson, 1993), thus affording greater contact with the herbicide and possibly

other extraneous pesticides. Gramoxone is also used extensively to clear weeds from commercial sugarcane in the north; however, these non-irrigated sites are dry most of the year and are not considered important larval habitats for mosquitoes. Runoff of chemicals into favorable larval habitats may also be minimized in the north which has less than half the annual rainfall compared to southern Belize. Because DDT resistance is a recessive trait, its appearance in the northern populations could be expected to be slower to develop than in the south where selection pressures may be greater (Macdonald, 1959; Davidson, 1963). Alternatively, resistance may not evolve in northern populations under present conditions.

Deltamethrin was highly potent at all 5 concentrations at 60 and 30 min exposures. In order to determine the insecticidal limits of this compound, the highest and lowest concentration were tested at differing exposure times. The diagnostic dosage (0.025%) produced complete mortality in *An. albimanus* and *An. vestitipennis* with 15 min. exposure and >75% mortality at 7.5 min. Most pyrethroids, like deltamethrin, share many characteristics with the insecticidal activity of DDT, including a negative temperature coefficient, and knockdown and neurotoxic killing activity targeting sodium channels and depolarizing motor nerve terminals in the peripheral and central nervous system (Zerba, 1988). Pyrethroids have been divided into a type 1 and 2 classification system based on chemistry and symptomology (Bloomquist, 1996). The more recent advanced development of type 2 compounds is associated with enhanced acute toxicity and hyperexcitatory effects than type 1 chemicals. DDT is a type 1 chemical. Deltamethrin is regarded as a fourth-generation (type 2) pyrethroid with an effective application rate one-tenth that of typical third generation (type 1) compounds, like

permethrin (Ware, 1994). Deltamethrin possesses one of the lowest LD_{50} levels (ng/insect) available for marketed pyrethroids (Zerba, 1988). For example, topically applied (ng/insect) deltamethrin activity against *Anopheles stephensi* was ~700 times more toxic than DDT (Pant, 1988).

Deltamethrin has been considered as a potential replacement of DDT in Belize, however, issues of cost (>3X that of DDT), increased number of required spray cycles and issues of spraymen/occupant health complaints (paresthesia) have delayed its possible routine use. In addition to greater insect toxicity, deltamethrin has shown an irritant or repellent effect on *An. albimanus* in treated houses in Mexico (Bown et al. 1987). In Guatemala, Beach et al. (1989) detected significant cross resistance between pyrethroids (deltamethrin) and other insecticides in widespread use, particularly organophosphates. They concluded that an extensive evaluation of the impact of deltamethrin on vector control was needed before making an operational switch.

DDT and pyrethroid associated cross-resistance has been extensively documented (Oppenoorth, 1985). It appears that 2 principal mechanisms are involved, either alone or in combination: 1) target-site (nerve) insensitivity, presumably expressed by the *kdr* (knock-down resistance) gene or a *kdr*-like factor (Narahashi, 1983; Oppenoorth, 1985) and 2) metabolic detoxification by increased esterase production, glutathione-*S*-transferase and/or mixed-function oxidases (Prasittisuk & Busvine, 1977; Amin & Hemingway, 1989). It remains unclear to what degree either mechanism influences cross-resistance, and it may be species and population specific (Chadwick et al. 1977; Vatan-Doost et al. 1996).

Malaria control in the Americas, before the wide-scale use of insecticides, had relied on reduction of larval habitats and exclusion of biting adult mosquitoes as the principal means of control (Gabaldon, 1949). Notable success in malaria abatement was achieved in a delimited area of the Canal Zone (CZ) in Panama as a result of intensive sanitation work begun in 1904 by William Gorgas, yet malaria persisted at high levels outside the CZ (Simmons et al. 1939). This prompted the authors to conclude: *"From this general survey of the malaria problem in Panama, it is obvious that, due to the brilliant work of various members of the local health agencies, it has been possible to partially control the disease among employees and troops in the field, and that more effective and less expensive procedures will be required for the prevention of malaria among the inhabitants of the Republic of Panama and similar regions in the American tropics"*. Despite years of considerable labor and expense during the pre-DDT era, most control areas in the Americas experienced only modest protection (Gabaldon, 1949).

The use of residual pesticides have been the cornerstone for the control of vectors of tropical diseases for over 50 years (WHO, 1992). The use of residual insecticides for the interception or exclusion of vectors, in particular, indoor spraying with DDT, had been the major reason for success in eliminating malaria from many countries and regions during the 1955-1969 global eradication effort (Haworth, 1988). Organized spraying activities have been primarily responsible for protecting over one billion people from the renewed risk of autochthonous malaria (WHO, 1990; Farid, 1991). Despite the apparent reduced efficacy of DDT to suppress malaria incidence in areas of high vector resistance, it continues to be used in many endemic countries, partly because of a lack of affordable alternatives.

The conventional notion of how an insecticide controls transmission is by reduction of the mean expectation of survival of the vector population that comes in contact with indoor sprayed surfaces (Russell et al. 1963; Pampana, 1963; Gilles & Warrell, 1993). Commonly, when only moderate levels of resistance have been reported, there is an urgent switch to a different insecticide without adequate investigation of possible alternative reasons for real or apparent control failure, such as interrupted spray schedules, incomplete coverage and lack of sprayable wall surfaces. Decisions have generally been based on the reported compromised insecticidal effects with little consideration on the continued impact of DDT or other compounds influencing behavioral responses (avoidance) that serve to prevent indoor transmission of malaria via irritancy and/or repellency (Gabaldon, 1953; de Zulueta, 1962; Roberts & Andre, 1994). It would seem premature to replace one insecticide for another as soon as resistance is confirmed, especially when the degree of resistance is not high and the vector is not considered highly efficient (Davidson & Zahar, 1973). A switch in chemical or strategy should be withheld until surveillance indicates control failure is directly due to resistance (Brown, 1983) or some behavioral phenomenon (e.g., exophagy) on the part of the vector that significantly promotes transmission.

Recently, many countries in the Americas have been encouraged to implement the WHO Global Malaria Control Strategy, changing the emphasis from vector control to promoting prompt diagnosis and treatment of cases (PAHO, 1994a; Trigg & Kondrachine, 1998). In contrast, Roberts et al. (1997) questioned the rationale behind the proposed wholesale abandonment of DDT for public health use advocated by others (Curtis, 1994; PAHO, 1994b; WHO, 1994; Lopez-Carrillo et al. 1996). A strong and

convincing argument to retain DDT for use in malaria control is based on solid historical evidence and a cost verses benefit analysis of increased health risk that would result from the proposed global ban on the insecticide (Roberts et al. 1997). Moreover, WHO (1995) recently stated that there was no justification on either toxicological or epidemiological evidence involving the alleged human risk associated with chronic insecticide exposure for changing the current policy of using DDT indoor spraying for vector-borne disease control. Residual insecticide spraying still remains the most commonly used method of vector control in the region and a valuable tool when applied in the correct circumstances (Pant, 1988; Brown et al. 1976; WHO, 1984). Moreover, insecticides continue to be used with success by malaria control programs in the Americas despite the patterns and degree of insecticide resistance (USAID, 1985).

The present findings clearly indicate, that for northern Belize, *An. albimanus*, *An. crucians* and *An. vestitipennis* remain susceptible to the diagnostic dosage of DDT despite decades of near routine public health use. Deltamethrin, a pyrethroid never before used for malaria control in Belize, also was shown to be a highly potent contact toxin for all 3 species. Based on the estimated slopes and standard errors, there appears to be low levels of response heterogeneity in the Caledonia *Anopheles* population with regards to both chemicals. Likewise, there was no indication of knockdown resistance in the populations. Significant recovery from knockdown would have suggested possible tolerance or incipient resistance. Because cross-resistance is a common phenomenon among DDT and pyrethroids, the high susceptibility seen for deltamethrin make it probable that other untested pyrethroids would be effective in Caledonia.

There are possible limitations in extrapolating these findings to other areas of northern Belize, provided ecological and agricultural conditions vary. In areas outside of Belize where intensive pesticide application occurs (e.g., Pacific Coast of C.A.), resistance in *An. albimanus* has appeared frequently but has had a heterogeneous and localized distribution (Frederickson, 1993). For example, in northern Guatemala (Pateń Department), areas of little or no intensive agriculture, *An. albimanus* had the greatest susceptibility to OP and carbamate insecticides compared to areas under heavy agriculture and associated pesticide use (Brogdon, et al. 1988). Given the dramatic increase in malaria in Belize, it was prudent to resume a regular (focal) spraying campaign using DDT (or some other affordable public health insecticide) for indoor residual spraying to achieve immediate control. This may not be the case in southern Belize where some degree of reduced sensitivity has been detected in a single *An. albimanus* population. It remains to be seen whether the resistance detected is operationally significant to warrant a possible change in chemicals or control strategy. Periodic susceptibility monitoring of the anopheline populations, together with disease surveillance, is needed in these 'suspect' areas under observed spray coverage before concluding operational failure. Based on the results reported herein, and recent work on pesticide avoidance behavior in *An. albimanus* (Chareonviriyaphap et al. 1997), it is believed that DDT will continue to be effective in suppressing malaria transmission in Belize, either by remaining toxic to the majority of the vector population and/or continuing to function as an excito-repellent by lowering the proportion of mosquitoes biting humans by behavioral avoidance of sprayed structures.

REFERENCES CITED

Amin, A.M. and J. Hemingway. 1989. Preliminary investigation of the mechanisms of DDT and pyrethroid resistance in *Culex quinquefasciatus* Say (Diptera: Culicidae) from Saudi Arabia. Bull. Entomol. Res. 79:361-366.

Ariaratnam, V. and A.W.A. Brown. 1969. Exposure time versus concentration in the WHO standard test for mosquito resistance to chlorohydrocarbon insecticides. Bull. Wld. Hlth. Organ. 40:561-567.

Beach, R.F., C. Cordon-Rosales and W.G. Brogdon. 1989. Detoxifying esterases may limit the use of pyrethroids for malaria vector control in the Americas. Parasitol. Today. 5:326-327.

Bloomquist, J.R. 1996. Ion channels as targets for insecticides. Annu. Rev. Entomol. 41:163-190.

Bown, D.N. 1987. Agricultural use of pesticides and their effect on vector borne disease transmission in the WHO region of the Americas. In: *Effects of Agricultural Development on Vector-borne Diseases*. Food & Agric. Organ. AGL/MISC/12/87. pp. 53-56.

Bown, D.N., E.C. Frederickson, G. del Angel Cabanas and J.F. Mendez. 1987. An evaluation of bendiocarb and deltamethrin applications in the same Mexican village and their impact on populations of *Anopheles albimanus*. Bull. Pan Am. Hlth. Organ. 21:121-135.

Breeland, S., J. Kliewer, J. Austin and C. Miller. 1970. Observations on malathion-resistant adults of *An. albimanus* Wiedemann in coastal El Salvador. Bull. Wld. Hlth. Organ. 43:627-631.

Brogdon, W.G., R.F. Beach, J.M. Stewart and L. Castanaza. 1988. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. Bull. Wld. Hlth. Organ. 66:339-346.

Brogdon, W.G. and J.C. McAllister. 1998. Simplification of adult mosquito bioassays through the use of time-mortality determinations in glass bottles. J. Am. Mosq. Control Assoc. 14:159-164.

Brown, A.W.A. and R. Pal. 1971. *Insecticide Resistance in Arthropods*. Wld. Hlth. Organ. Monogr. Series No. 38. 491 pp.

Brown, A.W.A., J. Haworth and A.R. Zahar. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13:1-25.

Brown, A.W.A. 1983. Insecticide resistance as a factor in the integrated control of Culicidae. In: *Integrated Mosquito Control Methodologies, Vol. 1. Experience and Components from Conventional Chemical Control.* Laird, M. & J.W. Miles (Eds.). Academic, London. pp. 61-235.

Brown, A.W.A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2:123-140.

Brown T. M. and W.G. Brogdon. 1987. Improved detection of insecticide resistance through conventional and molecular techniques. Annu. Rev. Entomol. 32:145-162.

Busvine, J.R. 1956. The significance of insecticide-resistant strains, with special reference to pests of medical importance. Bull. Wld. Hlth. Organ. 15:389-401.

Chadwick, P.R., J.F. Invest and M.J. Bowron. 1977. An example of cross-resistance to pyrethroids in DDT-resistance *Aedes aegypti*. Pestic. Sci. 8:618-624.

Chareonviriyaphap, T. 1995. Pesticide avoidance behavior in *Anopheles albimanus*, a malaria vector in Central and South America. Ph.D. Dissertation. Uniformed Services University of the Health Sciences, Bethesda, Md. 217pp.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.H. Harlan, S. Manguin and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus*, a malaria vector in the Americas. J. Am. Mosq. Control Assoc. 13:171-183.

Curtis, C.F. 1994. Should DDT continue to be recommended for malaria vector control? Med. Veter. Entomol. 8:107-112.

Davidson, G. 1963. DDT resistance and dieldrin resistance in *Anopheles albimanus*. Bull. Wld. Hlth. Organ. 28:25-33.

Davidson, G. and A.R. Zahar. 1973. The practical implications of resistance of malaria vectors to insecticides. Bull. Wld. Hlth. Organ. 49:475-483.

Davidson, G. and B. Sawyer. 1975. Carbamate and organophosphate resistance in *Anopheles albimanus*. Trans. Roy. Soc. Trop. Med. Hyg. 69:431.

DeBanne, S.M. and H.S Haller. 1985. Evaluation of statistical methodologies for estimation of median effective dose. Toxocol. Appl. Pharmacol. 79:274-282.

De Zulueta, J. 1962. The irritability of mosquitos to DDT and its importance in malaria eradication. Riv. Malariol. 41:169-178.

Elliott, R. and O. Ochoa-Aguirre. 1974. Overnight access to sugar and response to DDT in *Anopheles albimanus* Wied. Bull. Wld. Hlth. Organ. 51:311-313.

Farid, M.A. 1991. Views and reflections on anti-malaria programmes in the world. Kaohsiung J. Med. Sci. 7:243-255.

Finney, D.J. 1971. *Probit Analysis*. 3rd ed. Cambridge, London. 333 pp.

Frederickson, E.C. 1993. Bionomics and control of *Anopheles albimanus*. Tech. paper No. 34. Pan Am. Hlth. Organ., Washington, D.C. 76 pp.

Gabaldon, A. 1949. Malaria control in the neotropic region. In: *Malariology. A Comprehensive Survey of all Aspects of this Group of Diseases*. M.F. Boyd. (Ed.). Saunders, Philadelphia. pp. 1400-1415.

Gabaldon, A. 1953. Possible effects of residual insecticides on the interruption of malaria transmission. Riv. Malariol. 31:155-171.

Georghiou, G.P. 1972. Studies on resistance to carbamate and organophosphorus insecticides in *Anopheles albimanus*. Am. J. Trop. Med. Hyg. 21:797-806.

Georghiou, G.P., V. Ariaratnam and S.G. Breeland. 1972. Development of resistance to carbamates and organophosphorus compounds in *Anopheles albimanus* in nature. Bull. Wld. Hlth. Organ. 46:551-554.

Georghiou, G.P., S. Breeland and V. Ariaratnam. 1973. Seasonal escalation of organophosphorus and carbamate resistance in *Anopheles albimanus* by agricultural sprays. Environ. Entomol. 2:369-374.

Georghiou, G.P. and R.B. Mellon. 1983. Pesticide resistance in time and space. In: *Pest Resistance to Pesticides*. G.P. Georghiou & T. Saito (Eds.) Plenum, New York. pp. 1-46.

Gilotra, S. 1966. Effects of larval environments on adult resistance in *Anopheles albimanus* Wiedemann. Mosq. News 26:26-30.

Gilles, H.M. and D.A. Warrell. 1993. *Bruce-Chwatt's Essential Malariology*. 3ed. Edward Arnold, London. 340 pp.

Gordon, H. 1961. Nutritional factors in insect resistance to chemicals. Annu. Rev. Entomol. 6:27-54.

Hadaway, A.B. and F. Barlow. 1956. Effects of age, sex and feeding on the susceptibility of mosquitoes to insecticides. Ann. Trop. Med. Parasitol. 50:438-443.

Haworth J. 1988. The global distribution of malaria and the present control effort. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer & I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 1379-1420.

Hobbs, J.H. 1973. Effect of agricultural spraying on *Anopheles albimanus* densities in a coastal area of El Salvador. Mosq. News 33:420-423.

Hoskins, W.M. and Gordon, H.T. 1956. Arthropod resistance to chemicals. Annu. Rev. Entomol. 1:89-122.

Lines J.D. 1988. Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. Parasitol. Today 4: S17-20.

Lopez-Carrillo, L., L. Torres-Arreola, L. Torres-Sanchez, F. Espinosa-Torres, C. Jimenez, M. Cebrian, S. Waliszewski and O. Saldate. 1996. Is DDT use a public health problem in Mexico? Environ. Hlth. Persp. 104:584-588.

Loyola, E.G., M.A. Vaca, D.N. Bown, E. Perez and M.H. Rodriguez. 1991a. Comparative use of bendiocarb and DDT to control *Anopheles pseudopunctipennis* in a malarious area of Mexico. Med. Veter. Entomol. 5:233-242.

Loyola, E.G., J.I. Arredondo. M.H. Rodriguez, D.N. Bown and M. A. Vaca-Marin. 1991b. *Anopheles vestitipennis*, the probable vector of *Plasmodium vivax* in the Lacandon forest of Chiapas, Mexico. Trans. Roy. Soc. Trop. Med. Hyg. 85:171-174.

Macdonald, G. 1959. The dynamics of resistance to insecticides by anophelines. Riv. Parassitol. 20:305-315.

Malcolm, C.A. 1988. Current status of pyrethroid resistance in anophelines. Parasitol. Today. 4(7):S13-15.

Mekuria, Y., D.C. Williams, M.A. Tidwell and T.A. Santana. 1990. Studies of the susceptibility of *Anopheles albimanus* and *Anopheles vestitipennis* from Dajabon, Dominican Republic, to insecticides. J. Am. Mosq. Control Assoc. 6:645-650.

Miller, T.A. 1988. Mechanisms of resistance to pyrethroid insecticides. Parasitol. Today 4(7):S8-12.

[MOH] Ministry of Health. 1993. Evaluation of program activities. Vector Control Programme, Public Health Bureau, M.O.H., Belize C.A.

Molineaux, L., G.R. Shidrawi, J.L. Clarke, J.R. Boulzaguet and T.S. Ashkar. 1979. Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition. Bull. Wld. Hlth. Organ.. 57:265-274.

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. Wld. Hlth. Organ. 22:721-734.

Narahashi, T. 1983. Resistance to insecticides due to reduced sensitivity of the nervous system. In: *Pest Resistance to Pesticides*. G.P. Georghiou & T. Saito (Eds.) Plenum, New York. pp. 333-352.

Oppenoorth, F.J. 1985. Biochemistry and genetics of insecticide resistance. In: *Comprehensive Insect Physiology Biochemistry and Pharmacology*. G.A. Kurkut & L.I. Gilbert (Eds.). Vol. 12: Insect Control. Chapter 19, pp. 731-767. Pergamon, Oxford.

Padilla, N., P. Molina, J. Juarez, D. Bown and C. Cordon-Rosales. 1992. Potential malaria vectors in northern Guatemala. J. Am. Mosq. Control Assoc. 8:307-308.

Pampana, E. 1963. *A Textbook of Malaria Eradication*. Oxford, London. 508 pp.

[PAHO] Pan American Health Organization. 1986. Malaria control in the Americas: a critical analysis. Bull. Pan Am. Hlth. Organ. 20:1-17.

[PAHO] Pan American Health Organization. 1994a. Status of malaria programs in the Americas, XLII Report. CSP24/INF/2 (Eng.), XXIV Pan American Sanitary Conference, Washington, D.C. 116 pp.

[PAHO] Pan American Health Organization 1994b. Prohibition of DDT. Med. Veter. Entomol. 8:164.

Pant, C.P. 1988. Malaria vector control: imagociding. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer and I. McGregor (Eds.). Churchill Livingstone, New York. pp. 1173-1212.

Prasittisuk, C. and J.R. Busvine. 1977. DDT-resistant mosquito strains with cross-resistance to pyrethroids. Pestic. Sci. 8:527-533.

Raffaele. G., A. Coluzzi and J. de Zulueta. 1958. Observations on the effect of numbers and age on the susceptibility of mosquitoes to DDT. Bull. Wld. Hlth. Organ. 18:464-468.

Rejmankova, E., D.R. Roberts, A. Pawley, S. Manguin and J. Polanco. 1995. Predictions of adult *Anopheles albimanus* densities in villages based on distances to remotely sensed larval habitats. Am. J. Trop. Med. Hyg. 53:482-488.

Rejmankova, E., D.R. Roberts, S. Manguin, K.O. Pope, J. Komarek and R. A. Post. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. Environ. Entomol. 25:1058-1067.

Remington, R.D. and M.A. Schork. 1970. *Statistics with Applications to the Biological and Health Sciences*. 2ed. Prentice-Hall, N.J. 415 pp.

Roberts, D.R., O. Chan, J. Pecor, E .Rejmankova, S. Manguin, J. Polanco and L.J. Legters. 1993. Preliminary observations on the changing roles of malaria vectors in southern Belize. J. Am. Mosq. Control Assoc. 9:456-459.

Roberts, D.R. and R.G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. J. Am. Trop. Med. Hyg. 50(6) Suppl:21-34.

Roberts, D.R., L.L. Laughlin, P. Hsheih and L.J. Legters. 1997. DDT: global strategies, and a malaria control crisis in South America. Emerg. Inf. Dis. 3:295-302.

Robertson, J.L., K.C. Smith, N.E. Savin and R.J. Lavigne. 1984. Effects of dose selection and sample size on precision of lethal dose estimates in dose-mortality regression. J. Econ. Entomol. 77:833-837.

Rouch, R.T. and G.L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79:293-298.

Russell, P.F., L.S. West, R.D. Manwell and G. Macdonald. 1963. *Practical Malariology*. 2ed. Oxford University, London. 750 pp.

Shidrawi, G.R. 1990. A WHO global programme for monitoring vector resistance to pesticides. Bull. Wld. Hlth. Organ. 68:403-408.

Simmons, J.S., G.R. Callender, D.P. Curry, S.C. Schwartz and R. Randall. 1939. *Malaria in Panama*. Am. J. Hyg. Monogr. Ser. No. 13. 326 pp.

Trigg, P.I. and A.V. Kondrachine. 1998. Commentary: Malaria control in the 1990s. Bull. Wld. Hlth. Organ. 76:11-16.

[USAID] U.S. Agency for International Development. 1985. *Malaria: Meeting the Global Challenge*. Agency for International Development. 110 pp.

Ware, G.W. 1994. *The Pesticide Book*. 4ed. Thomson, Fresno, CA. 386 pp.

Wilkerson, R.C., D. Strickman and T.R. Litwak. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. J. Am. Mosq. Control Assoc. 6:7-34.

[WHO] World Health Organization. 1968. Information circular on insecticide resistance, insect behavior and vector genetics. VBC/IGR/68.1 43 pp.

[WHO] World Health Organization. 1975. Manual on Practical Entomology in Malaria. Part II. Methods and techniques. WHO Offset Pub. No. 13. Geneva. 160 pp.

[WHO] World Health Organization. 1981a. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Establishment of the base-line. WHO/VBC/81.805. Geneva. 6 pp.

[WHO] World Health Organization. 1981b. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Diagnostic Test. WHO/VBC/81.806. Geneva. 6 pp.

[WHO] World Health Organization. 1981c. Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides. WHO/VBC/81.6. Geneva. 4 pp.

[WHO] World Health Organization. 1984. *Chemical Methods for the Control of Arthropod Vectors and Pests of Public Health Importance.* Wld. Hlth. Organ. Geneva. 108 pp.

[WHO] World Health Organization. 1986. Resistance of vectors and reservoirs of disease to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 737. 87 pp.

[WHO] World Health Organization. 1990. World malaria situation, 1988. Wld. Hlth. Statist. Quart. 43:68-78.

[WHO] World Health Organization. 1992. Vector resistance to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 818. 62 pp.

[WHO] World Health Organization. 1994. Use of DDT in vector control. Med. Veter. Entomol. 8:113.

[WHO] World Health Organization. 1995. Vector control for malaria and other mosquito-borne diseases. Wld. Hlth. Organ. Tech. Rep. Ser. No. 857. 91 pp.

[WHO] World Health Organization. 1996. World malaria situation in 1993. Part II. Weekly Epidemiol. Rec. 71:25-29.

[WHO] World Health Organization. 1997. World malaria situation in 1994. Part II. Weekly Epidemiol. Rec. 72:277-284.

Vanzie, E. 1995. Situational analysis of the vector control programme. Magnitude and trend of malaria transmission. Vector Control Programme, Ministry of Health, Belize. (unpub. doc.) 17 pp.

Vatan-Doost, H., H. Townson and S. Ward. 1996. Cross-resistance spectrum in permethrin-resistant *Anopheles stephensi*. Ann. Trop. Med. Parasitol. 90:435.

Zeichner, B. 1992. Procedures for the diagnostic dose resistance test kits for mosquitoes, body lice, and beetle pests of stored products. U.S. Army Environ. Hyg. Agency Tech. Guide No. 189. 39 pp.

Zerba, E. 1988. Insecticidal activity of pyrethroids on insects of medical importance. Parasitol. Today 4:S3-7.

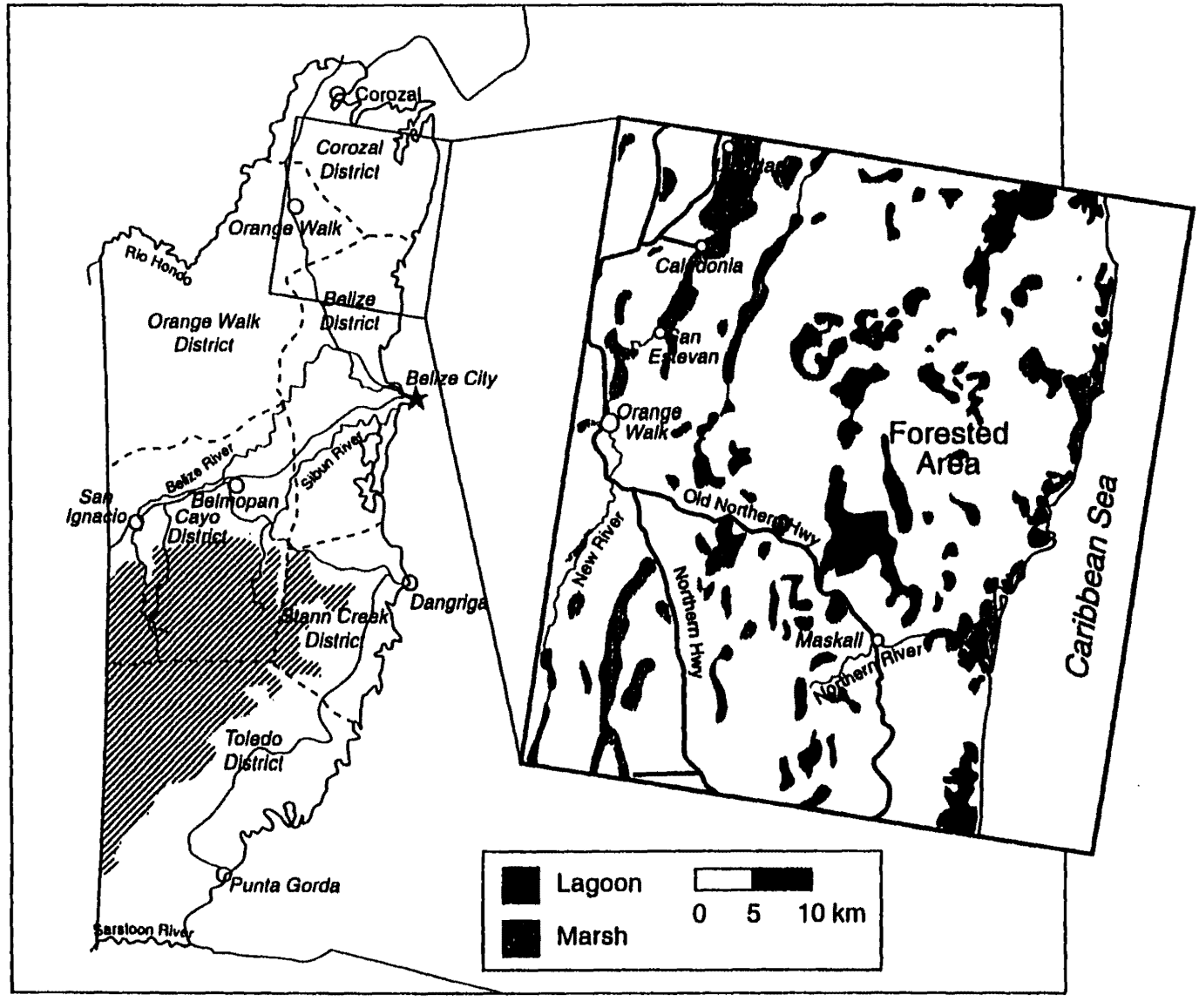


Figure. Map of Belize, depicting location of study site, Caledonia Village, along the New River in the northern District of Corozal.

Table 1. Proportion mortality of *Anopheles* species after 24-hr post-exposure with 4 serial concentrations of DDT for 60-min using the standard WHO susceptibility test method.

***Anopheles albimanus*/DDT**

Concentration	N	Response*	*P* mortality*
4.0 % (2 gm/m^2)	262	257	0.981
2.0 % (1 gm/m^2)	264	242	0.917
1.0 % (0.5 gm/m^2)	232	138	0.595
0.25 % (0.125 gm/m^2)	250	5	0.019[a]
Control	548	105	0.192

***Anopheles vestitipennis*/DDT**

Concentration	N	Response*	*P* mortality*
4.0 % (2 gm/m^2)	120	120	1.0
2.0 % (1 gm/m^2)	116	107	0.924
1.0 % (0.5 gm/m^2)	168	106	0.634
0.25 % (0.125 gm/m^2)	176	2	0.011[b]
Control	272	24	0.088

TABLE 1. (Continued)

***Anopheles crucians*/DDT**

Concentration	N	Response*	*P* mortality*
4.0 % (2 gm/m^2)	136	136	1.0
2.0 % (1 gm/m^2)	214	207	0.968
1.0 % (0.5 gm/m^2)	148	103	0.697
0.25 % (0.125 gm/m^2)	116	22	0.187[ab]
Control	368	40	0.109

* Dose-response data and proportion- Abbott's formula adjusted.

[ab] Significant difference between *An. crucians* and respective species (χ^2 corrected), $p<0.05$ **a**= χ^2 30.94 (p=0.0001) **b**= χ^2 27.15 (p=0.0001)

Table 2. Probit analysis of adjusted* dose-response data from DDT susceptibility tests, 60-min exposure with 4 serial concentrations, presented as lethal dosage (LD) in gm/m^2 for 50 and 90% mortality, with 95% fiducial limits (p<0.05) and the slope and standard error (SE) of the estimate.

Species	LD_{50} gm/m^2 (% dose) (fiducial limits)	LD_{90} gm/m^2 (% dose) (fiducial limits)	Slope (SE)
Anopheles albimanus (n=1008)	0.4353 (0.87%) (0.4004-0.4703)	0.9917 (1.98%) (0.9073-1.098)	3.583 (0.1939)
Anopheles vestitipennis (n=580)	0.4267 (0.85%) (0.3867-0.4668)	0.8777 (1.75%) (0.7864-1.005)	4.092 (0.3178)
Anopheles crucians (n=614)	0.2777 (0.55%) (0.1224-0.4388)	0.7731 (1.55%) (0.4865-2.042)	2.882 (0.3658)

* Abbott's formula

Table 3. Proportion mortality of *An. albimanus* after 24-hr post-exposure with 5 serial concentrations of deltamethrin for 60-min using the standard WHO susceptibility test method.

	***Anopheles albimanus*/deltamethrin**		
Concentration	N	Response*	*P* mort.*
0.025 % (0.00925 gm/m^2)	105	105	1.0
0.0125 % (0.0046 gm/m^2)	109	109	1.0
0.00625% (0.0023 gm/m^2)	89	89	1.0
0.003125% (0.00115 gm/m^2)	112	112	1.0
0.00156% (0.00057 gm/m^2)	89	83	0.935
Control	187	25	0.134

* Dose-response data and proportion mortality- Abbott's formula adjusted.

Table 4. Proportion mortality of *An. vestitipennis* after 24-hr post-exposure with 5 serial concentrations of deltamethrin for 60-min using the standard WHO susceptibility test method.

	***Anopheles vestitipennis*/deltamethrin**		
Concentration	N	Response*	*P* mort.*
0.025 % (0.00925 gm/m^2)	72	72	1.0
0.0125 % (0.0046 gm/m^2)	81	81	1.0
0.00625% (0.0023 gm/m^2)	93	93	1.0
0.003125% (0.00115 gm/m^2)	91	91	1.0
0.00156% (0.00057 gm/m^2)	97	82	0.845
Control	119	8	0.067

* Dose-response data and proportion mortality- Abbott's formula adjusted.

Table 5. Proportion mortality of *An. albimanus* after 24-hr post-exposure with 5 serial concentrations of deltamethrin for 30-min using the standard WHO susceptibility test method.

***Anopheles albimanus*/deltamethrin**

Concentration	N	Response*	*P* mort.*
0.025 % (0.00925 gm/m^2)	94	94	1.0
0.0125 % (0.0046 gm/m^2)	88	88	1.0
0.00625% (0.0023 gm/m^2)	94	83	0.883
0.003125% (0.00115 gm/m^2)	97	81	0.835
0.00156% (0.00055 gm/m^2)	91	55	0.609
Control	220	22	0.100

* Dose-response data and proportion mortality- Abbott's formula adjusted.

Table 6. Probit analysis of adjusted* dose-response data for *An. albimanus* using deltamethrin, 30-min exposure with 5 serial concentrations, presented as lethal dosage (LD) in gm/m^2 at 50 and 90% mortality, with 95% fiducial limits (p<0.05) and the slope and standard error (SE) of the estimate.

Species	LD_{50} gm/m^2 (% dose) (fiducial limits)	LD_{90} gm/m^2 (% dose) (fiducial limits)	Slope (SE)
Anopheles albimanus	0.0004147 (0.0011%) (0.00027-0.00054)	0.00180 (0.005%) (0.00147-0.00237)	2.009 (0.2717)

*Abbott's formula

Table 7. *P*roportion mortality and comparisons between *An. albimanus* and *An. vestitipennis*, 24-hr post-exposure to 0.025% (0.00925 gm/m^2) deltamethrin during 5 time periods using the standard WHO susceptibility test method.

***Anopheles albimanus*/deltamethrin**

Min.sec	N	Response	*P* mort.
60.00	98	98	1.0
30.00	59	59	1.0
15.00	82	82	1.0
7.30	56	49	0.875
3.15	77	42	0.545*
Control	116	02	0.017

***Anopheles vestitipennis*/deltamethrin**

Min.sec	N	Response	*P* mort.
60.00	56	56	1.0
30.00	84	84	1.0
15.00	93	93	1.0
7.30	91	70	0.769
3.15	63	07	0.111*
Control	173	02	0.011

*Significant difference $p<0.05$ χ^2 26.8 (p=0.0001)

Table 8. Proportion mortality and comparisons between *An. albimanus* and *An. vestitipennis*, 24-hr post-exposure to 0.00156% (0.00057 gm/m^2) deltamethrin during 4 time periods using the standard WHO susceptibility test method.

***Anopheles albimanus*/deltamethrin**

Min.sec	N	Response*	*P* mort.*
60.00	127	96	0.758
30.00	142	97	0.686[a]
15.00	112	62	0.554[b]
7.30	120	47	0.389[c]
Control	255	15	0.059

***Anopheles vestitipennis*/deltamethrin**

Min.sec	N	Response	*P* mort.
60.00	93	76	0.817
30.00	67	22	0.328[a]
15.00	75	15	0.200[b]
7.30	72	05	0.069[c]
Control	105	04	0.038

* Dose-response data and proportion mortality- Abbott's formula adjusted.
Significant difference between species $p<0.05$ **a**=χ^2 21.94 **b**=χ^2 21.75 **c**=χ^2 22.05

Table 9. Probit analysis of dose-response data to varying exposure times comparing *Anopheles* species with high and low concentrations of deltamethrin, presented as lethal time (LT) in minutes for 50 and 90% mortality, with 95% fiducial limits ($p<0.05$) and the slope and standard error (SE) of the estimate.

Species	LT_{50} (fiducial limits) minutes	LT_{90} (fiducial limits) minutes	Slope (SE)
	Concentration : 0.0925 gm/m² (0.025%)*		
Anopheles albimanus	3.085 (2.413-3.642)	7.238 (6.058-9.555)	3.459 (0.5441)
Anopheles vestitipennis	5.439 (4.908-5.969)	9.114 (8.146-10.596)	5.717 (0.6166)
	Concentration: 0.00057 gm/m² (0.00156%)**		
Anopheles albimanus◆	12.231 (8.386-15.816)	184.75 (104.92-***)	1.086 (0.1760)
Anopheles vestitipennis	32.474 (14.114-***)	99.02 (46.01-***)	2.647 (0.5353)

* exposure times 60, 30, 15, 7.5, 3.25 min. ** exposure times 60, 30, 15, 7.5-min.
*** high values omitted. ◆ response data- Abbott's formula adjusted.

Table 10. Probit analysis of dose-response data with exposure to high and low concentrations (0.025% and 0.00156%) of deltamethrin for 7.5-min, presented as lethal dose (LD) in gm/m^2 for 50 and 90% mortality, with 95% fiducial limits (p<0.05) and the slope and standard error (SE) of the estimate.

Species	LD_{50} gm/m^2 (% dose) (fiducial limits)	LD_{90} gm/m^2 (% dose) (fiducial limits)	Slope (SE)
Anopheles albimanus	0.00095 (0.0026%) (0.00061-00143)	0.0119 (0.032%) (0.00592-0.04515)	1.163 (0.1991)
Anopheles vestitipennis	0.00362 (0.0098%) (0.00263-0.00493)	0.01852 (0.05%) (0.01237-0.03324)	1.808 (0.2181)

Table 11. *P*roportion knockdown of *Anopheles* species* after 60-min exposure with 4 serial concentrations of DDT using the standard WHO susceptibility test method.

DDT	N	Response	*P* knockdown
4.0 % (2 gm/m^2)	518	502	0.969
2.0 % (1 gm/m^2)	594	543	0.914
1.0 % (0.5 gm/m^2)	548	257	0.469
0.25 % (0.125 gm/m^2)	542	39	0.073
Control	1188	28	0.023

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 12. *P*roportion knockdown of *Anopheles* species* after 60-min exposure with 5 serial concentrations of deltamethrin using the standard WHO susceptibility test method.

Deltamethrin	N	Response	*P* knockdown
0.025 % (0.00925 gm/m^2)	194	194	1.0
0.0125 % (0.0046 gm/m^2)	208	208	1.0
0.00625% (0.0023 gm/m^2)	210	206	0.981
0.003125% (0.00115 gm/m^2)	216	153	0.708
0.00156% (0.00057 gm/m^2)	208	116	0.558
Control	306	0.0	----

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 13. Probit analysis of dose-response data from insecticide susceptibility tests at 60-min exposure for *Anopheles* species*, presented as knockdown dosage (KD) in gm/m^2 for 50 and 90% affected, with 95% fiducial limits (p<0.05) and the slope and standard error (SE) of the estimate.

Insecticide	KD_{50} gm/m^2 (% dose) (fiducial limits)	KD_{90} gm/m^2 (% dose) (fiducial limits)	Slope (SE)
DDT	0.4424 (.88%) (0.1615-0.8059)	1.1879 (2.37%) (0.6782-7.920)	2.987 (0.4755)
Deltamethrin	0.00055 (0.0015%) (0.00007-0.00087)	0.00160 (0.0043%) (0.00102-0.01163)	2.793 (0.6744)

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 14. *P*roportion knockdown of *Anopheles* species* after 30-min exposure with 5 serial concentrations of deltamethrin using the standard WHO susceptibility test method.

Deltamethrin	N	Response	*P* knockdown
0.025 % (0.00925 gm/m^2)	110	94	0.854
0.0125 % (0.0046 gm/m^2)	109	89	0.816
0.00625% (0.0023 gm/m^2)	105	40	0.381
0.003125% (0.00115 gm/m^2)	102	16	0.157
0.00156% (0.00057 gm/m^2)	151	6	0.040
Control	207	3	0.015

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 15. Probit analysis of dose-response data for all *Anopheles* species* with 5 serial concentrations of deltamethrin for 30-min, presented as knockdown dosage (KD) in gm/m^2 for 50 and 90% affected, with 95% fiducial limits (p<0.05) and the slope and standard error (SE) of the estimate.

Species	KD_{50} gm/m^2 (% dose) (fiducial limits)	KD_{90} gm/m^2 (% dose) (fiducial limits)	Slope (SE)
Anopheles species.	0.00277 (0.0075%) (0.00193-0.00413)	0.00903 (0.0245%) (0.00564-0.02316)	2.5 (0.3109)

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 16. *Proportion* knockdown of *Anopheles* species* comparing 5 exposure times with 0.025 % (0.00925 gm/m^2) deltamethrin using the standard WHO susceptibility test method.

Min.sec	N	Response	*P* knockdown
60.00	175	161	0.920
30.00	178	142	0.797
15.00	192	159	0.828
7.30	161	35	0.217
3.15	154	7	0.045
Control	321	1	0.003

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 17. *P*roportion knockdown of *Anopheles* species* comparing 4 exposure times with 0.00156% (0.00057 gm/m^2) deltamethrin using the standard WHO susceptibility test method.

Min.sec	N	Response	*P* knockdown
60.00	172	67	0.389
30.00	209	37	0.177
15.00	187	22	0.117
7.30	174	15	0.086
Control	367	3	0.008

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*)

Table 18. Probit analysis of dose-response data* with varying exposure times using 0.025% and 0.00156% deltamethrin, presented as knockdown time (KDT) in minutes for 50 and 90% affected, with 95% fiducial limits ($p<0.05$) and the slope and standard error (SE) of the estimate.

Dose %	KDT_{50} (fiducial limits) minutes	KDT_{90} (fiducial limits) minutes	Slope (SE)
0.025	11.681 (2.121-28.831)	38.144 (18.500-***)	2.49 (0.6438)
0.00156	124.48 (48.78-***)	*** (***)	1.24 (0.2832)

* all species combined (*An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni* and *An. punctimacula*) *** high values excluded.

CHAPTER 3

Insecticide avoidance behavior in *Anopheles albimanus* and *Anopheles vestitipennis* (Diptera: Culicidae) in Caledonia, northern Belize, Central America, using excito-repellency chambers

ABSTRACT

Behavioral responses of *Anopheles albimanus* and *Anopheles vestitipennis* females to 3 different serial concentrations of DDT and deltamethrin were assessed in a series of excito-repellency tests. All mosquitoes tested were field-caught specimens from Caledonia Village, in Corozal District, northern Belize, Central America. Behavioral responses were measured using excito-repellency (ER) chambers (boxes) allowing either direct contact or noncontact to insecticide-impregnated papers to observe irritancy and/or repellency, respectively. Only unfed, nongravid female mosquitoes from populations known to be physiologically susceptible to both insecticides were used. A life table statistic (product-limit survival analysis) was used to generate expected probabilities of escape (exit) times (ET), and a Mantel-Haenzel log-rank test allowed comparisons of escape behavior patterns between species and test conditions. Excito-repellency responses in contact trials were significant. Strong behavioral avoidance responses due to contact irritancy with DDT and deltamethrin during 60-min exposure were observed for both *An. albimanus* and *An. vestitipennis* compared to controls and noncontact trials. Statistical comparisons between doses of DDT in contact and noncontact trials showed significantly stronger escape responses at higher concentrations. Deltamethrin produced similar irritancy and repellency ET between all three doses and a generally a more rapid escape response than DDT for *An. albimanus* in 60-min contact trials. Noncontact repellency during 60-min exposures was far less dramatic, but significantly different from controls at the diagnostic dosage for both chemicals. However, pronounced noncontact

repellency to DDT and deltamethrin were noted for both species during the extended 5-hr exposure period compared to controls. *Anopheles albimanus* had a significantly more rapid escape response in the 60-min contact tests than *An. vestitipennis* for the high and medium DDT doses. No differences in ET were seen between species in the 60-min noncontact tests. Conversely, 5-hr noncontact exposure indicated *An. vestitipennis* had a greater cumulative repellency response than *An. albimanus* with all 3 doses. This is the first study to document excito-repellency to insecticides by *An. vestitipennis* using an ER box. The utility of a recently improved test system and the use of survival analysis in the interpretation of data have been enormous improvements in the interpretation and utility of behavioral avoidance data. Further behavioral analyses are needed to more clearly define the nature of excito-repellency responses to different insecticides and to explain the inter- and intraspecific differences in response between geographical populations of species. This study underscores the importance of measuring behavioral responses of vectors to insecticides, and that the irritant and repellent qualities of particular insecticides remain important components of malaria control effectiveness.

INTRODUCTION

"*Clearly there is much that stands in need of investigation in the field of behaviour of mosquitos in relation to insecticides: this is a part of modern malariology which, no doubt, deserves greatest attention*" **de Zulueta, 1964.**

BEHAVIORAL and bionomic knowledge of disease vectors are essential to the understanding of disease transmission (Muirhead-Thomson, 1951; Mattingly, 1962). Information on behavioral responses of vectors to insecticides is critical to understanding the true effects of chemicals used in the control of vector-borne diseases (de Zulueta, 1964; Elliott, 1972). Data on the behavioral responses of neotropical anophelines, including *Anopheles albimanus*, remains fragmented and cursory, clearly indicating further field research is needed (Roberts & Andre, 1994).

Anopheles albimanus Wiedemann has long been recognized as a major vector of malaria throughout most of it's geographic range (Fredrickson, 1993). This species is widely distributed in the tropics and subtropics of Middle America and northern South America (Faran, 1980). Relative to other anophelines present in Central America, an impressive amount of published information has been generated on this important vector (Breeland, 1980; Fredrickson, 1993; PAHO, 1996). Physiological/biochemical resistance to a wide array of insecticides has been documented in scattered populations of this species (Brown, 1986). Behavioral avoidance to insecticides, in particular DDT, has been reported for *An. albimanus* populations in all studies that were designed to quantify

a behavioral response (Gabaldon, 1952; Trapido, 1952; Brown, 1958; Barrera, et al. 1959; Mancera & Hernandez, 1960; Hecht & Hernandez, 1960; Vigueras & Corzo, 1960; Rachou, et al. 1963, 1973; Duret, 1964; Coluzzi, 1963; Elliott, 1969; Vargas, 1976; Bown et al. 1987; Quinones & Suarez, 1989; Chareonviriyaphap, et al. 1997). Conversely, relatively little information has been gathered on the general bionomics and disease vector status of *Anopheles vestitipennis* (Roberts et al. 1993; Rejmankova et al. 1998). The geographical range of this species is similar to *An. albimanus*, although generally more focally restricted to lowland, coastal zones (Arredondo-Jimenez, et al. 1996). *Anopheles vestitipennis* has been strongly linked to malaria transmission in the northern part of its distribution (southern Mexico, Guatemala and Belize) (Roberts, et al. 1993). Little is known concerning this species' susceptibility status to insecticides and far less is known about its behavioral responses to chemicals used in vector control (Brown, 1986; Richards, et al. 1994).

The role of indoor insecticide residues for malaria control can be broadly partitioned into 3 categories: toxicity, irritancy and repellency. Roberts (1993) has reviewed the background and issues surrounding behavioral avoidance of mosquitoes to insecticides. Many terms (e.g., excitant, deterrent, contact 'repellent') have been applied, some with conflicting definitions, describing chemically-induced behaviors while insects are in motion or at rest (van Thiel, 1951; Dethier, et al. 1960; Kennedy, 1978; Lockwood, et al. 1984). The terms 'repellent', 'irritant' and 'excito-repellent' as defined by Roberts (1993) have been adopted in this study. Herein, avoidance behavior is defined as a series of responses stimulated by the combination and relative degree of irritancy and repellency (excito-repellency). Irritancy results after direct physical (tarsal) contact and repellency

occurs without actual physical contact with the insecticide residues (Roberts, 1993; Roberts & Andre, 1994).

In this study, two classes of compounds were evaluated for behavioral responses of mosquitoes to both irritancy and repellency- an organochlorine (DDT) and a fourth-generation synthetic pyrethroid (deltamethrin). Since the early 1950s, the use of residual applications of insecticides, primarily DDT, has remained one of the principal methods of malaria control worldwide (WHO, 1995). The influence of DDT on mosquito behavior was recognized before and shortly after its introduction into general malaria control programs (Kennedy, 1946; Ribbands, 1946a; Van Thiel, 1951; Gabaldon, 1952,1953; Muirhead-Thomson, 1960; de Zulueta, 1962, 1964; de Zulueta & Cullen, 1963; Ungureanu & Theodorescu, 1963). Decades later, this behavioral phenomenon of avoidance continues to spark controversy regarding its role in either reducing or exacerbating malaria transmission (Hamon et al. 1970; Roberts, 1993; Evans, 1993; Miller & Gibson, 1994; Roberts, et al. 1997a).

Synthetic pyrethroid analogues with improved residual formulations, including deltamethrin, have continued to gain considerable popularity as an operational alternative to DDT for vector control. Many pyrethroids irritate and repel insects, likely providing the same important attributes that are afforded by DDT in the control of malaria (Rani & Osmani, 1984; Threlkeld, 1985). Irritability and repellency in mosquitoes exposed to certain pyrethroids are clearly measurable responses and of importance in malaria transmission control (Ribbands, 1946b; Smith & Chadwick, 1964; Taylor et al. 1981; Darriet et al. 1984; Lines et al. 1987; Pell et al. 1989; Ree & Loong, 1989; Lindsay et al. 1991, 1992; Evans, 1993; Miller & Gibson, 1994; Chareonviriyaphap, et al. 1997).

The literature is fraught with apparent contradictory observations on avoidance behavior, possibly the result of experimental error or uncontrollable physiological, environmental and other biological factors. Considerable variations in excito-repellency among different natural field populations and within the same population have been reported (Coluzzi, 1963; Quinones & Suarez, 1989). Cullen and de Zulueta (1962) observed great individual variation in response even when conditions were carefully controlled (e.g., time of day of test, mosquito age and nutritional status). Such factors can be a particular problem when using heterogeneous wild-caught (field) mosquitoes of unknown age and physiological status. Problems may be compounded by inherent or unintentional sampling bias (e.g., exclusive use of human-landing collections or animal-baited traps). The interpretation becomes even more complex because of the degree of biological and behavioral variance that living organisms naturally possess superimposed on variations and influence of place, time and environmental conditions (Mattingly, 1962; Garrett-Jones, 1970; Bidlingmayer, 1985). In fact, differences in host preference and other behaviors are often the major attributes used to distinguish insect "biotypes" in nature (Diehl & Bush, 1984).

A common confounding factor of behavioral tests is often the wide variability of response between test subjects and an inherent biological difficulty to obtain more standardized and reproducible results between treatment trials, even under strict laboratory conditions (Busvine, 1964). This can be partly overcome by more stringent statistical treatments, including increased study sample size, use of random sampling and adequate replication of trials to increase the accuracy of information. In this study, survival analysis techniques were used for analysis of the behavior response data as

described by Roberts et al. (1997b) and Chareonviriyaphap et al. (1997). The use of escape probability estimates over time when comparing responses of different test populations under varying test conditions provides for a more powerful and comprehensive analysis of the data. One advantage of survival analysis over previous statistical methods is the minimal loss of important information. Use of survival analysis has added greatly to sound biological interpretation of excito-repellency test results.

Another obstacle to investigating irritancy and repellency has involved problems of accurately measuring and separating the two responses. Assays for evaluating behavioral responses of anophelines to pesticides have been reviewed (Muirhead-Thomson, 1960; Coluzzi, 1963; Busvine, 1964; Elliott, 1972; Elliott & de Zulueta, 1975; Roberts, 1993). Excito-repellency test systems have generally fallen into two general categories, both based on the direct or indirect measurement of flight activity and the combined effect of irritancy and repellency. Only recently has a system been developed to adequately assess the two phenomena separately (Roberts, et al. 1997b).

Historically, the principal test strategy for measuring behavioral responses and recommended by WHO (1960, 1970) involves the measurement of flight response or "excitation times" due to exposure and irritancy with a treated surface. This has involved the measurement of either the average time to first flight or by counting average number of flights ("take-offs"), generally over a 15-min time period, compared to paired untreated controls (Brown, 1958; Coluzzi et al. 1962; Cullen & de Zulueta, 1962; Elliott, 1964; Brown, 1964; WHO, 1970). Other methods also have been devised for measuring the flight activity and escape reactions using the standard WHO bioassay test kits and a twin-funnel apparatus (Gerold, 1970, 1977). A more elaborate system involving a wind

tunnel and host odor has been designed to assess mosquito responses to insecticide-impregnated bednets on videotape (Miller & Gibson, 1994).

The second system has involved the use of specially built chambers, excito-repellency boxes (ER boxes), that allows the mosquito a free choice of either remaining inside or attempting an escape from the box after contact with residual insecticides (Service, 1993). In a sense, the ER box serves as a miniature room fitted with an exit trap. Unlike irritability tests, the ER box can provide a better assessment of free-flying mosquito behavior once they have entered a sprayed structure. With the recent exception of Chareonviriyaphap et al. (1997), all previous efforts using ER boxes have described responses due to apparent physical contact or the combined response to irritancy and repellency and were not designed to discriminate separately noncontact repellency. One distinct advantage of the ER box system is the ability to observe mosquito behavior over a period of many hours, if necessary. Another advantage is the adaptability of using the box in the field. Although laboratory studies of behavioral avoidance may provide useful indications on the effects of insecticides, differences in response between laboratory colonies and field populations have made it clear that field investigations are necessary to evaluate the importance of mosquito behavior under natural conditions (WHO, 1960; Coluzzi, 1963).

Rachou et al. (1963) was one of the first to fully described a chambered ER device and its use, calling it an "excito-repellency test box". Numerous other excito-repellency test boxes or similar devices have been described (Hecht, et al. 1960, 1962; Elliott, 1964; Duret, 1964; Diaz Najera, 1964; Gerold & Laarman, 1967; Rachou, et al. 1973; Wilson et al. 1973; WHO, 1975; Charlwood & Paraluppi, 1978; Ungureanu & Gheorghiu, 1980;

Roberts, et al. 1984; Rozendaal, 1989; Ree & Loong, 1989; Evans, 1993). Some designs have included live bait in the compartments to evaluate response to insecticides in the presence of a host (Gheorghiu, et al. 1972). The ER box design used in this study is similar to the box described by Rachou et al. (1973) with appropriate design modifications that enables the assessment of both irritancy and repellency separately (Roberts et al. 1997b).

In previous attempts, most excito-repellency tests using the ER box involved the release of mosquitoes into enclosures containing insecticide (or a non-insecticide control) sprayed surfaces. Boxes are essentially lightproof except for the outward projecting baffles that permit the mosquitoes to escape into separate clean holding spaces. Numbers of mosquitoes escaping are counted and analyzed by time post-release. However, previous test systems have met with operational difficulties, principally, in ease of releasing mosquitoes into the boxes and removing live specimens at the conclusion of the test (Roberts, et al., 1984). In some cases, lack of a standardized insecticide dose into the test chambers was a deficiency. The recently described ER box by Roberts et al (1997b) has helped to overcome many of these previous obstacles. Using this improved test system, the behavioral responses of wild-caught anophelines from a site in northern Belize were compared using varying concentrations of DDT and deltamethrin. Both contact irritancy and noncontact repellency were evaluated.

MATERIALS AND METHODS

Study area: The country of Belize is located on the southeastern part of the Yucatan peninsula of Central America. Nearly half of this subtropical country is low-lying coastal plain, the remainder is hilly or mountainous. The rainy season, typically from May/June through November, affects the general abundance of mosquito vectors, species distribution and malaria transmission risk in the country (Roberts, et al. 1996). The expansive coastal plain region consists of lowlands with elevations generally less than 20 m above sea level. A substantial part of the lowlands is characterized by relatively undisturbed herbaceous wetlands providing important larval habitats for particular *Anopheles* species (Rejmankova, et al. 1993, 1998). Additional descriptions of the northern coastal plain and the wetland ecosystems where this study took place has been provided by Rejmankova et al. (1996).

All mosquito collections were conducted in Caledonia, a village in the northern coastal zone along the New River floodplain, in the Corozal District of northern Belize (18°13'78"N, 88°28'38"W). Besides native vegetation and wetlands, the general area of Caledonia is dedicated to seasonal sugarcane production. Malaria incidence in Caledonia has been among the highest in the northern sector of Belize since 1988 (VCP, unpublished report). In 1994, 100 cases (91/1000 incidence), primarily *Plasmodium vivax*, were recorded from the village. Regular indoor residual spraying (2-cycles/yr) with DDT only (75% wettable powder or technical grade product mixed in kerosene) had been conducted in Caledonia on a nearly continuous basis for 40 years at the time of this investigation. Prior to the tests described herein, a village-wide indoor application of

DDT was performed in March 1995 in response to the high number of malaria cases. Refer to Chapter 1 for further details on Belize and the study site. For purposes of discussion, this investigation will be referred to as the "Caledonia study".

Insecticides: Only analytical grade chemicals were used in the impregnation of test and control papers. DDT (1,1,1,-trichloro-2,2-bis [*p*-chlorophenyl] ethane), 99% pure *p,p'*-isomer, was provided by the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine (USACHPPM), Aberdeen Proving Ground, Maryland, USA. Deltamethrin (K-Othrine®, Decis®) [(S)-α-cyano-3-phenoxybenzyl (IR)-cis-trans-3-(2,2-dibromovinyl)-2, 2-dimethylcyclo-propane carboxylate], 99.7% pure, was kindly provided by AgroEvo Environmental Health (U.K.), United Kingdom in January 1994.

Test papers: Insecticide impregnated papers (MN261 Chromatography paper, Lot# SA231, Macherey-Nagel, Germany) were made to World Health Organization specifications by the USACHPPM, Entomological Sciences Division (Zeichner, 1992). The following concentrations were used (all mg and gm amounts expressed as active ingredients [AI] per ml or m^2): **DDT**: 4% (13.09 mg/ml or 2 gm/m^2), 1% (3.273 mg/ml or 0.5 gm/m^2), 0.25% (0.818 mg/ml or 0.125 gm/m^2); and **Deltamethrin**: 0.025% (0.0605 mg/ml or 00925 gm/m^2), 0.00625% (0.015125 mg/ml or 0.0023 gm/m^2), and 0.00156% (0.00378 mg/ml or 0.00057 gm/m^2). All 12 x 30 cm papers were prepared separately and treated at the rate of 5.5 ml of insecticide solution per 360 cm^2 of paper surface using the

WHO recommended gm/m^2 diagnostic dosage, with dose serial dilutions of 4 and 8-fold expressed as weight of insecticide per volume of carrier (WHO, 1981b). Trichloroethylene (TCE) was the solvent used to aid uniform dispersal of the solution onto the paper. The carrier for DDT was Semtol 85 (Witco) + TCE, and deltamethrin was dispersed using silicon fluid (Dow Corning #556) + TCE. Papers were separated based on chemical and concentration and hermetically sealed in airtight aluminum bags. Control papers were treated with Semtol or silicon fluid only, excluding each respective insecticide. All test papers were made and packaged on or about 13 May 1995 and stored at ambient temperatures (27 ± 5 °C).

Excito-repellency chambers: The excito-repellency test chamber (box) is illustrated and numbered in Figure 1, and the components and rationale for the box have been described by Roberts et al. (1997b). Briefly, each box (34 x 32 x 32 cm) was constructed of thin-gauge stainless steel to keep construction weight to a minimum, with an outer chamber (no. 4) and a smaller second screened inner chamber (no. 3) that can be inserted into the outer box. The rear removable inner panel (no.1) is constructed of clear Plexiglas® (31 x 30.5 cm) with a 15.5-cm diameter round access hole (no. 2). The hole is sealed with 2 pieces of vertical overlapping rubber latex (Dental Dam®, The Hygienic Corp., Akron, Ohio) forming an effective barrier against escaping mosquitoes. This self-sealed opening was included for delivering and removing test mosquitoes, preventing escape while inserting a mouth aspirator into the chamber. The rear of the box is a hinged door (no. 5), fitting tightly when closed over the outer frame. The front panel forms the outward

projecting exit funnel (louvre) (no. 6), forming the only means of escape for the mosquitoes through a 1.5 cm wide aperture. During the test period the only light entering the box is from the front escape portal. The frame of the inner chamber (no. 3) is constructed of 0.62 x 0.62 cm aluminum beams where the inner surface (29 x 28.5 x 28.5 cm) is covered with fine mesh metal screening (~20 cells/cm) on the top, bottom and sides. Rubber gaskets (0.62 cm wide) on the front and rear beams create an effective seal between the inner and outer chambers and the Plexiglas panel effectively preventing possible escape by test specimens. Importantly, the inner screen surface is a minimum of 0.62 cm from the surface of the test papers, preventing mosquitoes from making physical contact with the paper surface during noncontact tests. A 3.8-liter (1-gallon) cardboard ice cream carton with a screened top serves as a receiving cage for escaping mosquitoes. The cage fits over the outward projecting exit funnel (no. 6) using an attached orthopedic stocking covering a single opening on the side of the carton. Each carton has a single 2.5 cm opening covered with 2 pieces of slit rubber latex forming a one-way seal. This portal allows access for the mouth aspirator to remove escaped mosquitoes at intervals during the test period.

Mosquito collections and handling: All mosquito collections were conducted from the same study site location in Caledonia. Adult female anophelines were captured off exposed lower legs of human volunteers using mouth aspirators during early evening outdoor landing collections (WHO, 1975). Anophelines were pooled, unidentified, into a clean 3.8-liter cardboard ice cream container to allow random mixing. To reduce potential handling damage, mosquitoes were not sorted to species before testing.

Mosquitoes were kept overnight and supplied water only. Excito-repellency tests took place the following morning. Mosquitoes were not held for more than 12 hours before testing. Because the tests required replicates and sufficient controls, a relatively high number of mosquitoes were needed. Time and availability precluded use of mosquitoes other than unfed (no evidence of blood in the abdomen) and nongravid specimens for study. Only *An. albimanus* and *An. vestitipennis* were present in sufficient numbers to allow appropriate analysis and presentation. All field study populations are referred to as Caledonia (CAL) populations [e.g., *An. albimanus* (CAL)].

Behavioral test method: With only minor modifications, the general test approach and methodology follows Roberts, et al (1997b) and Chareonviriyaphap et al. (1997). The same person conducted all the tests during the study. Only 2 chambers (one with insecticide, one control) were used at a time to ensure accurate counting, removal and identification of escaped mosquitoes at 1-min time intervals. Generally contact and noncontact trials were conducted on alternate days. Paired controls were conducted with all trials. Each test was replicated a minimum of 3 times. Tests were performed to compare the 2 insecticides, the 3 concentrations of each insecticide, insecticide contact vs. noncontact, the 60-min (short) and 5-hr (long) noncontact exposure, and *An. albimanus* vs. *An vestitipennis* wild-caught populations.

For each surface (wall), two treated 12 x 30 cm (360 cm^2) test papers were joined together with metal paper clips to larger sheets of clean white photocopy paper the exact size of the inner wall of the outer chamber. Papers were attached to top, bottom and 2 side inner walls of the exposure chamber. Test papers were not positioned on the front

(exit portal) or back (Plexiglas) panels. Papers were secured to walls by pairing small magnets on the outside of the metal boxes with the paper clips positioned on the inside wall. Between alternating insecticide concentrations, chambers were carefully cleaned with 70% ethyl alcohol in distilled water and allowed to air dry.

All tests were conducted in the same indoor location on-site in Caledonia and during the cooler morning hours, generally between 0700-1000 hrs. Under the test conditions, mosquitoes are enclosed within the exposure chambers for periods of 1 to 5 hours. Each test involved placing unfed (blood or sugar) and nongravid female mosquitoes in a chamber lined with either insecticide treated or untreated (control) test papers, with or without an inner chamber. Approximately 25 mosquitoes, for each test chamber, were collected and placed in a 0.47-liter cardboard cup with moistened cotton for at least an one-half hour holding period to exclude dead, moribund or damaged specimens from the test. The exit apertures were sealed with close fitting Styrofoam® inserts before introducing the mosquitoes into the chambers. Mosquitoes were then carefully removed from the holding cups by mouth aspirator and gently blown into the test chamber through the back panel within 1-min of each other. The rear panel was then closed tightly and secured using an elastic cord. Mosquitoes were always placed in the control chamber first, followed by the insecticide exposure chamber. Released mosquitoes were permitted to 'adjust' to test chamber conditions for an approximate 3-min acclimation or "conditioning" period before beginning response measurements as described in similar tests (Busvine, 1964; Chareonviriyaphap et al. 1997).

After 3-min, the escape funnel was opened to begin the observation period. Mosquitoes escaping from the exposure chambers into the outside receiving cages were

recorded at 1-min intervals with the aid of a digital laboratory timer during the first 60-min. The same receiving cage remained in place throughout the test period. Individuals were carefully removed shortly after escape and quickly identified to species (Wilkerson, et al. 1990). Because only 5 species of *Anopheles* were captured at the study site, species identification using a 14x hand lens was adequate in most cases during the testing procedure. Identification of *An. vestitipennis* and *An. gabaldoni* required higher magnification. On a rare occasion, an escapee would reenter the test chamber before being removed from the receiving cage. For analysis, such mosquitoes were not recorded as 'escaped'. For the 5-hr noncontact trials, numbers escaping were also recorded at 1-min intervals for the first hour of exposure. Afterwards, escapees were counted and removed each subsequent hour. In 60-min contact tests, escapees were variously pooled into groups; two escape time intervals (1-30 min and 30-60 min), those remaining within the chambers (nonescapees), and paired controls, and held for 24-hr to observe mortality. Similarly, noncontact tests and controls were grouped by 1-30 min, 30-60 min, 2-hr, 3-hr, 4-hr, 5-hr exposure intervals for escapees and post 5-hr nonescapees. Holding cups were provided with 10% sucrose solution soaked on cotton as a food source. Cups were placed upright in a large plastic ice chest on plastic pans over a layer of water to thwart scavenging ants. After 24-hr post-exposure, mortality was recorded and each specimen identified to species using a 40-100x dissecting microscope and a prepared key (see Appendix I).

Analysis and interpretation: Figure 2 provides a simple flow diagram summarizing the excito-repellency tests performed. Test data with or without the inner

chambers, for either insecticide treatment or control papers, (herein referred to as contact trials or noncontact trials) were subjected to a Kaplan-Meier product-limit survival analysis (life table method) for estimates of 'survival' distribution, estimating the cumulative rates of mosquitoes escaping from chambers as described by Roberts, et al. (1997b), using SAS/STAT 6.04 (SAS® Institute, Inc. Cary, N.C., USA) LIFETEST procedure. The mosquito escape rate was estimated at 1-min intervals for 60-min in both contact and noncontact trials. Noncontact tests were extended for 4 additional hours of observation and escape responses were recorded at each hour. Mosquitoes that escape were treated as "deaths" and those that remained in the chambers as "survivals". Specimens that remained in the exposure chamber at the end-point of interest (60-min or 5-hr) were treated as "censored" data points and included in the survival time analysis (Lee, 1992, Collett, 1994). The time in minutes for 50 and 90% of the test population to escape (=death) was estimated with the life table method to derive estimates of "escape time" summary statistics. Analytical results are presented as proportions escaping from exposure chambers.

The log-rank (Mantel-Haenzel) test, a nonparametric method for comparing survival distributions, was used to compare patterns of escape behavior between species, chemicals, concentrations (doses) and contact and noncontact tests (Mantel & Haenzel, 1959). In the rare event when the difference in compared escape rates between 2 tests was not consistent over time (proportional hazard rate), a more appropriate statistic, the χ^2 Wilcoxon test, was used instead (Lee, 1992). In either case, statistical significance level was set at p-value less than 0.05. To test the null hypothesis, observed and expected

escape (=death) for each 1-min interval are calculated to compute the overall measure of deviation of observed from expected escape for each interval. The significance level of experiment-wise comparisons (all doses within a contact or noncontact test series) was $p<0.05$ and comparison-wise analysis (between doses within a test series) was $p<0.02$ (Milliken & Johnson, 1992). The log-rank test has a chi-square (χ^2) distribution with k degrees of freedom, where k is the number of groups analyzed - 1. Roberts (1993) defined most ethological (behavioral) terminology used in this study.

RESULTS

Escape responses of *An. albimanus* and *An. vestitipennis* to DDT were tested in contact and noncontact exposure chambers using 3 different serial concentrations of insecticide impregnated on papers. Limited availability of *An. vestitipennis* specimens precluded testing this species with deltamethrin. Only *An. albimanus* was tested using DDT and deltamethrin. Other *Anopheles* species observed during the trials were limited in number and could not be meaningfully analyzed. Tests with or without inner chambers for treatments and controls will be herein referred to as either contact (no inner chamber) or noncontact (with inner chamber) trials. For enhanced accuracy, only 2 boxes were operated at a time, precluding contact and noncontact tests using a particular chemical and concentration from being temporally paired. Figure 2 provides a flow diagram summarizing the excito-repellency tests as performed herein. Statistical comparisons are provided by species, chemical, dose and test condition (contact or noncontact).

The 3 insecticide concentrations were based on serial 4 and 8-fold dilutions of the recommended operational diagnostic dosage for 4% DDT (2 gm/m^2) and 0.025% deltamethrin (0.00925 gm/m^2) (WHO, 1992). The diagnostic (target or discriminating) dosage is 2-fold higher than the experimentally derived 100% lethal dosage (LD_{100}) value (WHO, 1981). The timing of this study prevented attempts to establish an estimated LD_{50} or LD_{90} values before test papers were impregnated. Fortunately, the 4-fold dilution of DDT (0.5 g/m^2) came reasonably close to the LD_{50} (0.44 g/m^2) established for *An. albimanus* (CAL) during susceptibility testing at the study site (see Chapter 2). However,

a DDT dose near the LD_{90} (0.99 g/m^2) for *An. albimanus* was not available for testing. Similarly, the 4-fold (0.0023 g/m^2) and 8-fold (0.00057 g/m2) dilutions of deltamethrin agreed well with the subsequent established LD_{90} (0.0018 g/m^2) and LD_{50} (0.00042 g/m^2) respective values for *An. albimanus* (CAL). These 2 doses also corresponded well with established concentrations used by Chareonviriyaphap, et al. (1997) for *An. albimanus* in Belize. For brevity, the concentrations of DDT (2.00, 0.5, 0.125 g/m^2) and deltamethrin (0.00925, 0.0023, 0.00057g/m^2) will be referred to as high, medium and low doses, respectively, unless otherwise indicated.

Mosquito mortality after a 24-hr holding period, from contact and noncontact trials, are given in Tables 1-3. Mosquitoes were grouped as either 'escapees' (those that entered the receiving cage) or 'nonescapees' (those that remained in the exposure chamber). For analysis, all escapees were grouped together because no significant difference in mortality (p>0.05) was seen by time of escape (1-30 min or 30-60 min). For both species, higher mortality was observed in DDT contact trials compared to noncontact trials and untreated controls. For deltamethrin, the difference in mortality was less pronounced between doses for contact trials, possibly the result of a more rapid escape from the exposure chambers compared to DDT trials (Table 7; Figs. 15-17). For both chemicals, higher 24-hr mortality was observed among nonescapees compared to escapees, presumably reflecting longer contact exposure with the toxicant or possibly some inherent difference in fitness between the 2 outcome groups. Post-exposure knockdown was more frequent at the higher doses for both compounds. Differences in percentage mortality between contact trials and controls decreased as exposure dosage decreased. Contact trials with high DDT gave 37.5% and 22.8% mortality following a

24-hr holding period for *An. albimanus* and *An. vestitipennis* escapees, respectively. Medium DDT dosage produced near identical mortality among escapees of both species. At the low DDT dosage, no mortality was seen in escapees. Deltamethrin produced high 24-hr mortality in nonescapees for all 3 concentrations (>87%), while mortality among escapees ranged from 17.4% at the high dose to 7.0% at the lowest. In most cases, only slightly higher mortality was seen between noncontact trials and controls at both 60-min and 5-hr exposures, indicating that contact with the chemical was the primary cause of mortality, further suggesting low airborne toxicity of both insecticides.

Times in minutes for mosquitoes to escape from treated (contact) chambers are given in Table 4. The escape time (ET) was defined as times observed for 50 and 90% of the test population to escape the treated or control chambers (ET_{50} and ET_{90}). Escape times were not adjusted to account for numbers of control escapees. For high dose DDT, *An. albimanus* had an ET_{50} of 18 min and an ET_{90} of 46 min. The *An. vestitipennis* population had a comparable ET_{50} (20 min) at the high dose, but never attained 90% escape within 60 min. At the medium DDT dose, *An. albimanus* and *An. vestitipennis* had an ET_{50} of 25 and 47 min, respectively. Estimates of ET_{90} at medium dose and ET_{50} and ET_{90} values for low dose DDT were not possible as less than 50% of the test population escaped within the 60-min exposure period. *Anopheles albimanus* had a more rapid contact escape response with deltamethrin than with DDT. Estimates of ET_{50} and ET_{90} were available for all 3 deltamethrin concentrations. From high to low dose, the ET_{50} varied from 7 to 10 min, the ET90 from 14 to 31 min. There was no significant difference (p>0.02) in escape time probabilities between the middle and low dose deltamethrin (Table 7).

Multiple statistical comparisons within species among the different test dosages for contact, noncontact, and paired control trial escape time responses are shown in Tables 5-9. The patterns of escape probabilities by dose (high, medium, low and zero-control) were treated using a log-rank method, with significance set at $p< 0.05$ and $p< 0.02$ level for experiment-wise and comparison-wise tests, respectively. Table 5 compares escape responses in contact vs. control, contact vs. noncontact and noncontact vs. control for the 60-min trials. For both species, marked differences were seen in the 60-min contact vs. control and contact vs. noncontact trials for all doses of DDT and deltamethrin. The single exception was the contact vs. control low dose DDT test with *An. vestitipennis*. For both species, only the high dose DDT and high dose deltamethrin noncontact vs. control trials were significantly different in escape response. All doses of deltamethrin did not differ significantly from paired controls in noncontact tests over the 60-min exposure period. Table 6 compares within species differences between high, medium and low doses of DDT and escape response for contact and noncontact trials. Both species had significant ($p< 0.05$) experiment-wise (all doses within a test series) responses among the 3 doses in contact and noncontact test series. The comparison-wise tests (between doses within a test series) showed clear differences ($p< 0.02$) between all paired doses, except for *An. vestitipennis* in the medium-low (0.5-0.125 gm/m^2) dose comparisons for both contact and noncontact trials. Table 7 compares the experiment and comparison-wise responses for *An. albimanus* and deltamethrin. Compared to DDT, deltamethrin produced a more dramatic and rapid escape response in the contact tests with all 3 doses. There was experiment-wise significance; however, unlike DDT, the only statistical comparison-wise difference was between the high and medium dose

response in the contact tests. Experiment-wise, there was no difference between escape responses and deltamethrin doses in the 60-min noncontact trials.

Tests observing escape response with 5-hr exposures in noncontact trials were conducted. Table 8 provides a log-rank comparison of escape responses in noncontact vs. control for 60-min and 5-hr trials. Compared to the response of the first 1-hr exposure, the extended test detected pronounced differences in repellency compared to paired controls. The additional 4 hours exposure produced significant differences in response at the high and medium DDT doses for both species. Whereas 60-min noncontact exposure to deltamethrin failed to evoke significant responses in *An. albimanus*, the complete 5-hr test found all 3 doses had escape probabilities significantly higher than controls. Within species differences for escape probabilities comparing different chemical doses of DDT and deltamethrin during 5-hr noncontact trials is given in Table 9. Both species had significant ($p< 0.05$) experiment-wise responses between the 3 doses. With DDT, the comparison-wise tests showed clear differences ($p< 0.02$) between all paired doses except for *An. vestitipennis* in the high-medium (2-0.5 gm/m2) dose comparisons. *Anopheles albimanus* only showed a statistical difference between the high and medium doses of deltamethrin.

The log-rank method was used to compare escape responses between species in contact and noncontact trials using DDT at the 3 varying concentrations for 60-min and 5-hr tests. Significant differences ($p< 0.05$) were noted between species comparing escape probabilities for contact and 5-hr noncontact trials (Table 10). *Anopheles albimanus* had a significantly greater escape response than *An. vestitipennis* during the high and medium DDT contact tests. There was no apparent difference in response

between species in the 60-min noncontact trials. However, *An vestitipennis* had a marked delayed escape pattern, significant at all 3 dosages (p=0.0001), as proportions that escaped increased over the remaining 4 hours of noncontact exposure compared to *An. albimanus* (Fig. 21).

Figures 3-21 show the proportions of mosquitoes remaining in the exposure chambers under different test conditions. These proportions are used to illustrate patterns of escape rates, which indicate escape probabilities from contact and noncontact trials using 2 different insecticides at 3 different doses with appropriate controls. Figures 3 and 4 illustrate contact escape responses at 1-min intervals for 60-min with high, medium and low doses of DDT for *An. albimanus* and *An. vestitipennis*, respectively. Percentage escape at the end of 1-hr is provided on Tables 1 and 3 for each species. Overall, *An. albimanus* showed a more rapid and greater overall escape response for all 3 doses of DDT compared to *An. vestitipennis*. Under the same test conditions using deltamethrin, *An. albimanus* shows (Fig. 5) an even greater escape response compared to DDT in both time and overall percentage of escape, all 3 doses being very similar (see Table 4). Only the high and medium doses of deltamethrin were found significantly different from one another (Table 7). In all comparisons, but one (*An. vestitipennis* with low dose DDT), there was significant differences between contact trials and controls (see Table 5).

Figures 6-8 present proportions of *Anopheles* remaining in exposure chambers during 5-hr noncontact exposure vs. controls for DDT and deltamethrin, respectively (Table 8). Tables 2-3 provide overall percentage escape at the end of 5-hr exposure for both species. Escape response increased significantly ($p< 0.05$) over the 5-hr period with the high and medium doses of DDT compared to controls for both species (Figs. 6-7).

Nearly all doses differed from one another in ET (Table 9). All doses of deltamethrin with *An. albimanus* were significantly different ($p< 0.05$) from noncontact controls (Fig. 8), yet differed significantly ($p< 0.02$) between high and medium doses only (Table 9).

Figures 9-11 and 12-14 show escape patterns for DDT and *An. albimanus* and *An. vestitipennis*, respectively, comparing contact and noncontact trials and controls for each of the 3 doses over a 60-min observation period. Figures 15-17 shows escape patterns under the same comparisons and conditions using deltamethrin and *An. albimanus* for high, medium and low doses, respectively. Contact trials clearly show strong irritancy compared to controls in all tests with only one exception (low dose DDT and *An. vestitipennis*). This is particularly evident with the high and medium doses of DDT and all doses of deltamethrin. Significant differences are also seen between all contact vs. noncontact test comparisons at the same dosage. As noted earlier, the 60-min observation period produced significant differences between noncontact and control trials only at the high DDT dose, all other comparisons were statistically similar (Table 5).

Comparisons between species and 60-min contact and noncontact escape responses to the 3 doses of DDT are presented in figures 18-20. At all doses, *An. albimanus* had a faster ET than *An. vestitipennis*, being significant at high and medium doses. No significant differences were seen between species and 60-min noncontact trials (Table 10). Figure 21 compares both species during the extended 5 hr noncontact trials and the 3 DDT doses. *An vestitipennis* had a marked delayed escape pattern compared to *An. albimanus*, significantly different at all 3 doses (p=0.0001).

DISCUSSION

With the exception of *Anopheles gambiae* in Africa, *An. albimanus* has been the most commonly evaluated anopheline regarding behavior and the impact of insecticides (Roberts, 1993). The behavior of *An. albimanus* in response to insecticides, particularly DDT, was noted shortly after the introduction of residual house spraying in Panama (Trapido, 1946, 1952). Excito-repellency to DDT in *An. albimanus* has been reported over most of its geographic range, from Mexico to Colombia (Fredrickson, 1993). In laboratory investigations conducted in Panama and El Salvador, Brown (1958) and Rachou et al. (1963) showed that DDT had powerful behavioral effects on this vector that also varied by strain. Using ER boxes, Rachou, et al. (1973) found 82-92% of *An. albimanus* escaped when exposed to 2gm/m^2 DDT for 1 hr. Recent tests carried out in Belize, using excito-repellency boxes, had shown a pronounced escape response by 2 field populations (Chareonviriyaphap, et al. 1997). The Caledonia study further supported the dramatic behavioral avoidance responses that DDT and deltamethrin elicit from *An. albimanus* populations.

Far less is known about *An. vestitipennis* regarding susceptibility and response to insecticides. DDT resistance has been documented in a few localities (WHO, 1992; Mekuria et al. 1990). Richards, et al. (1994) found a marked reduction in the indoor resting population in houses that had permethrin-impregnated bed nets compared to control households, suggesting that permethrin had both a repellency and toxic effect on the population. The paucity of information on insecticide susceptibility and bionomics of

An. vestitipennis is likely a reflection of poor sampling efforts and an under-appreciation of this species' vector importance. The Caledonia study is the first documented account of excito-repellency to insecticides by *An. vestitipennis* using an ER box.

The behavior of mosquitoes in general and the effects of insecticides that influence feeding and resting behavior are critical in the understanding and control of vector-borne diseases (Mattingly, 1962; de Zulueta, 1962, 1964; Pampana, 1963; Hamon, et al. 1970; Elliott, 1972; Elliott & de Zulueta, 1975; Gillies, 1988; Klowden, 1996). The refractory behavior of mosquitoes in response to various chemicals has been known for decades (Gahan et al. 1945; Metcalf et al. 1945; Muirhead-Thomson, 1947, 1950; Downs & Bordas, 1951; Davidson, 1953; de Zulueta et al. 1961; Kuhlow, 1962). The phenomenon of excito-repellency, especially irritancy, has generated both research and controversy in terms of measurement, mechanisms of genetic expression, interpretation and significance in control of vectors and/or disease (Gabaldon, 1953; Muirhead-Thomson, 1960; de Zulueta, 1962; Busvine, 1964; Elliott, 1969; Roberts, 1993; Roberts & Andre, 1994). Behavioral avoidance (excito-repellency) is clearly defined as the ability of an insect to detect and avoid an insecticide-treated surface by contact irritancy (i.e., with physical tarsal contact) and/or non-contact repellency (i.e., without tarsal or other physical contact).

The strong excito-repellency shown by most mosquitoes to DDT and various other chemicals has been considered a detrimental property and a potential obstacle to effective vector and malaria control (Muirhead-Thomson, 1951; Slooff, 1964; Bruce-Chwatt, 1970; Miller & Gibson, 1994). However, others have advocated a serious reexamination of this perception in light of the evidence (Roberts & Andre, 1994;

Roberts, et al. 1997a). Contrary to most conventional thought, effective control is still being achieved by the regular use of DDT in areas that have documented resistance and/or excito-repellency behavior in the local vectors. Roberts et al. (unpub. doc.) has recently developed a stochastic model addressing the impact of vector behavior in response to insecticide residues and malaria control. Supported by analyses of selected field data on DDT, this model indicated that irritancy and repellency predominate in importance, quantitatively, over the actual toxic properties of DDT as the primary means of reducing indoor human-vector contact. This is probably true for most other insecticides having significant excito-repellency properties. In short, the behavioral impact of DDT residues on altering normal indoor feeding and resting activities of vectors helps explain the continued effectiveness of many spraying programs despite the presence of high physiological resistance in the mosquito populations (Roberts & Andre, 1994).

The development of resistance to synthetic pyrethroids in the Americas has raised public health concern about the future utility of these newer chemicals (Beach, et al, 1989a; Cordon-Rosales et al. 1992). However, resistance to pyrethroids may have limited operational impact on their continued effectiveness either as residual insecticides applied in homes or as impregnated on bednets and curtains, provided a vector-human barrier persists in the form of behavioral avoidance (Rishikesh et al. 1979; Taylor et al. 1981; Darriet et al. 1984; Miller, et al. 1991; Lindsey, et al. 1991, 1992; Arredondo-Jimenez, et al. 1997; Chareonviriyaphap, et al., 1997).

In this study, excito-repellency responses were significant. *Anopheles albimanus* and *An. vestitipennis* showed strong behavioral avoidance responses due to contact

irritancy with DDT and deltamethrin during 60-min exposure compared to controls and noncontact trials. Statistical comparisons between doses of DDT in contact and noncontact trials showed significantly stronger escape responses as serial concentrations increased. All 3 doses of deltamethrin produced a more rapid and dramatic escape response than DDT for *An. albimanus* in contact trials.

Noncontact repellency during 60-min exposures was far less dramatic than irritancy, but still significantly different from controls at the diagnostic dosage of both chemicals. More pronounced noncontact repellency to DDT and deltamethrin were noted for both species during the extended 5-hr exposure period compared to controls. *Anopheles albimanus* had a significantly more rapid escape response in the 60-min contact tests than *An. vestitipennis* for the high and medium DDT doses. Conversely, 5-hr noncontact exposure indicated *An. vestitipennis* had a significantly greater repellency response than *An. albimanus* with all 3 doses. No significant differences in ET were seen between species in the 60-min noncontact tests. Overall, deltamethrin at the diagnostic dose (0.125 g/m^2) appeared more repellent than DDT (2 gm/m^2) with *An. albimanus* in the 5-hr trials. However, DDT produced a greater proportion escape response than deltamethrin during the 60-min noncontact exposures. Chareonviriyaphap, et al., 1997 concluded that 30-min noncontact exposure was inadequate to derive meaningful assessment of repellency. Given the differences in repellency response seen in my study, it appears the 60-min exposure may be only marginally sufficient an observation period to assess repellency. More work is needed to standardize an appropriate noncontact time.

Deltamethrin and DDT produced different response patterns of irritancy and repellency in *An. albimanus* depending on concentration. Changes in DDT dose altered

the threshold of toleration of contact with the treated surface (Fig. 3), whereas deltamethrin, appeared to stimulate the mosquitoes to escape, more equally between concentrations than DDT, with no appreciable dose effect except at the high dose (Fig. 5). This was equally true for toxicity, i.e., the test doses being too toxic to provide any clear dose response LD scale (Chapter 2). All 3 comparison-wise tests were found significant between doses of DDT in contact tests. Although repellency escape response at 60-min was much weaker than the 60-min irritant effects, noncontact tests gave similar patterns of response for the 2 chemicals. DDT showed a significant repellency escape-response between doses at 60-min exposure, whereas deltamethrin did not. Repellency tests at 5-hr showed significantly greater escape with time of exposure for both insecticides compared to controls. DDT, again showed an apparent dose relationship between concentrations, whereas deltamethrin indicated a dose response between the high and medium concentrations only ($p< 0.05$). The specific responses produced between DDT and deltamethrin are not directly comparable at the different doses as each differed considerably in tested concentration and also significantly in relative toxicity as defined with susceptibility tests.

The presence of physiological/biochemical resistance to insecticides has often been cited as an important reason for the apparent or predicted failures to control malaria in Central America (Busvine & Pal, 1969; Brown & Pal, 1971; Beach, et al. 1989; Fredrickson, 1993). The excito-repellent effects of insecticides and exophilic behavior of vectors after blood feeding have also been identified as important impediments to conventional malaria control (Gillies, 1956; Bruce-Chwatt, 1970; Brown et al. 1976; WHO, 1995). Although, behavioral avoidance has been clearly documented in a number

of arthropods, the importance of these behavioral responses in suppressing disease transmission and its possible role in the selection or non-selection of insecticide resistance in vectors has been controversial. (Muirhead-Thomson, 1960; Roberts & Andre, 1994). The interrelationship between insecticide susceptibility (physiological resistance patterns) and excito-repellency has been indicated for some insects, including mosquitoes, but remains unclear (Hooper & Brown, 1965; Lockwood, et al. 1984; Sparks, et al. 1989). Experimental evidence has indicated greater irritability among some mosquito strains resistant to DDT compared to strains showing less physiological resistance (Gaaboub & Dawood, 1974), and laboratory susceptible colonies of *An. albimanus* have been found less irritable compared to wild-caught anophelines (Brown, 1958; Coluzzi, 1963). However, contradictory observations with *An. culicificies* and *An. sacharovi* have shown lower irritability in a DDT-resistant strains compared to susceptible populations (de Zulueta, 1959; Bhatia & Deobhankar, 1962). Chareonviriyaphap et al. (1997) has shown significantly less irritability to DDT, permethrin and deltamethrin in a susceptible long-term laboratory colony of *An. albimanus* compared to susceptible and resistant wild-caught specimens, indicating a possible loss of capability, in colonies over time, to respond to insecticides. In El Salvador, Rachou, et al. (1965), observed 8 different wild-caught populations of *An. albimanus* exhibiting marked excito-repellency regardless of there susceptibility status. Overall, Chareonviriyaphap, et al. (1997) concluded that there appeared to be no genetic relationship between physiological and behavioral responses among the populations tested. *Anopheles albimanus* and *An. vestitipennis* in Caledonia were found completely susceptible to the standard target dosages of DDT and deltamethrin (see Chapter 2).

Interesting parallels and differences can be seen when comparing the Caledonia results with Central American *An. albimanus* strains used by Chareonviriyaphap, et al. (1997), two from colonized material from El Salvador and Guatemala and 2 wild-caught populations from southern and northern Belize, respectively. Comparing field populations, contact escape response times ($ET_{50/90}$) with deltamethrin were very similar between both studies. A rapid exit was noted with both medium (~LD_{90}) and low (~LD_{50}) doses. Interestingly, both studies noted a faster exit time with the low versus medium dose. My results also showed a greater percentage escape (99%) for the low compared to the medium and high (92%) doses. Mortality data in both studies would indicate that increased knockdown and subsequent mortality at the medium dose reduced the number that were able to escape the toxic effects. The same effect would apply at the high dose in my study. Table 1 shows this relationship. At the high (diagnostic) dose of deltamethrin (not used by Chareonviriyaphap, et al.), total percentage escape was similar to the medium dose, yet more rapid than either of the 2 lower doses. These observations would indicate that there is an interplay of knockdown (toxicity) and behavioral avoidance in these wild-caught populations. At the high and medium doses, contact knockdown influenced the ability to escape. Standard susceptibility tests (Chapter 2) produced nearly 100% knockdown and complete mortality at the 2 higher doses; whereas, the low dose produced less knockdown and lower mortality rates. Consequently, the lower dose, resulting in reduced knockdown, provided sufficient irritation to produce an effective and rapid escape response.

Physiologically, the higher the dosage of toxicant the greater the mortality per unit time of exposure compared to lesser concentrations for susceptible populations.

Alternatively, the greater amount of exposure time to a particular dosage, the greater the likelihood of accumulated dosage of insecticide contributing to mortality (Ariaratnam & Brown, 1969). Intuitively, the same may apply to the irritant and repellent properties of certain insecticides. Irritation has been shown to increase as the dose of DDT increased; however, usually by only moderate levels (Brown, 1958; Cullen & de Zulueta, 1962). Others report no differences in irritability between different DDT concentrations (Brown 1964; Busvine, 1964). Ree & Loong (1989) observed graded irritability responses to increasing doses of permethrin in *Anopheles maculatus*, but not in two other species of mosquitoes. The Caledonia findings suggest, that at increasing doses of DDT, repellency becomes more significant in behavioral avoidance, resulting in a more rapid escape response. This dose-response phenomenon was not seen in repellency trials using deltamethrin, as knockdown and mortality among nonescapees was similar to paired controls. As reported by Chareonviriyaphap, et al. (1997), deltamethrin did not produce "greater escape activity" at higher doses in the 30-min or 4-hr repellency tests. The *An. albimanus* (CAL), 60-min noncontact trials showed no dose effect (comparison-wise test), whereas 5-hr exposures produced a significant difference between the high and medium doses.

The DDT noncontact repellency trials gave a different picture than that seen by Chareonviriyaphap, et al. (1997). Different DDT concentrations significantly influenced repellency escape responses in *An. albimanus* (CAL) during the 60-min tests. These apparent intraspecific differences between the two studies may have been species strain related, differing physiology or seasonal differences in the populations (Rachou, 1963). The dose range between high and low concentrations of DDT used by

Chareonviriyaphap, et al. (1997) may have been too small (< 2-fold difference) to detect a dose effect. Another likely possibility is the lower exposure time used. Those authors concluded that 30-min exposure was not adequate for a meaningful test of noncontact repellency compared to their 4-hr test results.

Numerous studies have reported substantial interspecific differences in excito-repellency (Coluzzi, 1963; Busvine, 1964; Charlwood & Paraluppi, 1978; Ree & Loong, 1989). The behavioral patterns between *Anopheles albimanus* and *An. vestitipennis* using DDT differed noticeably (deltamethrin was not tested between species) in contact and 5-hr noncontact tests. The differences in escape patterns may have been influenced by differences in physiological age of female mosquitoes, the natural species variability of innate activity patterns, or responses to ambient test conditions (Busvine, 1964). However, in my study, nutritional and gonotrophic states (unfed, nongravid) were controlled as best as possible for all tests.

It is always prudent to take into account a number of potentially confounding factors, including the test system, when evaluating and extrapolating the impact of insecticides on vector behavior. In artificial test systems, like the ER box, mosquitoes are intentionally introduced and kept reasonably close to the insecticide providing a uniformity of exposure that likely maximizes the expected and observed impact. However, unknown confounders in the test data can affect analysis. For example, based on analysis of excito-repellency test components (e.g., diameter of escape hole), Gerold & Laarman (1967) concluded escape behavior of mosquitoes exposed to an insecticide consists of independent components with increased activity (excito-repellency) not necessarily leading to escape through an exit portal (the outcome measure). Regardless,

mosquitoes undoubtedly have a different pattern of contact with insecticides under natural conditions, within sprayed structures and when they are near host stimuli. However, the ER box allows for a range of options, including flight escape and the ability to rest on inside surfaces in the box, that can provide better indications of natural responses inside sprayed houses.

Within panmictic (random mating) populations, behavioral heterogeneity of preferred resting sites has been reported for a number of important vectors (Hii, 1985; Smits et al. 1996). This intraspecific genetic variability supports the contention that non-uniform (non-random) exposure of a given spatial population, made up of two or more genetically defined subpopulations, is more plausible under many natural conditions (Molineaux et al. 1978) than uniform (random) exposure implicit in many models of malaria transmission and control (Macdonald, 1957). This may be valid; however, exposure and subsequent toxicity to an insecticide is not an issue when examining excito-repellency and its impact on vectorial capacity. If the assumption remains that all mosquitoes must enter a house to obtain a blood meal, then regardless if a particular portion of the population is endophilic or exophilic, they must first obtain a blood meal before moving to their preferred resting site. However, in the sequence of events leading to a bloodmeal, many vectors may actually rest indoors before moving to a host. DDT and other insecticides can have a marked effect on altering normal resting behavior (Wilson et al. 1973; Roberts et al. 1987). The point is that excito-repellency serves to interrupt or prevent blood feeding, independent of toxicity (but not necessarily insecticide concentration). The ER box measures avoidance response to a chemical as an indicator of potential reduction in human-vector contact. In other words, most transmission and

control models have been based on the toxic impact of insecticides and have neglected the importance of avoidance behavior in the equation. Only recently has a model been developed to incorporate vector avoidance as a measured impact on transmission (Roberts et al., unpublished data).

Intrinsic factors concerning the mosquito and environmental conditions can effect irritability. Besides species, the influence of physiological variability (e.g., age, nutritional and gonotrophic status) in test populations and response to insecticides remains an important consideration in the interpretation of excito-repellency test results (Hecht, et al. 1960; Hamon & Eyraud, 1961; Cullen & de Zulueta, 1962; Coluzzi, 1963; Elliott & Ochoa-Aguirre, 1974; Roberts, et al. 1984; Gaaboub & Dawood, 1974). In general, there is a progressive increase in susceptibility with advancing age in mosquitoes (Raffaele et al. 1958). However, Hamon & Eyraud (1961) have found older anophelines less irritable than younger ones. In many cases, blooded mosquitoes show less irritability than unfed females (Barrera et al. 1959; Hecht et al. 1960; Qutubuddin, 1967; Roberts et al. 1984) which may cause delayed escape responses.

The influence of the actual test conditions (e.g., time of day, illumination, ambient temperature and humidity, exposure time, crowding) also can effect response outcome (Kartman & da Silveira, 1946; Raffaele, et al. 1958; Hecht, et al. 1962). Coluzzi (1963) showed increased "excitability" of *Anopheles maculipennis* during evening hours; whereas, laboratory strains exhibited less irritability during the evening compared to morning periods. DDT-irritated mosquitoes have been found attracted to light (Kennedy, 1946; Trapido, 1952); whereas, Hecht et al. (1960) found no increased phototactic response. The number of mosquitoes used in a test can influence the degree of activity by

"mutual activation", i.e., increased numbers lead to greater disturbance (Brown, 1958). Insecticides and particular formulations can vary under actual test conditions. DDT, for example, is generally more toxic at lower temperatures (Davidson & Zahar, 1973) and temperature variations can influence irritability with DDT (Kaschef, 1970). In general, increased temperature and lower humidity have been associated with increased adult activity (Busvine, 1964).

The heterogeneity in responses between similar tests and controls were expected. Variation was dependent on test conditions and physiological characteristics of the population used. With very few exceptions, control mortality was not significantly different ($p > 0.05$) between trials. Some tests had higher control mortality (>5%) than would have been desired or expected (Tabs. 1-3). When encountered, excess control mortality in the excito-repellency boxes possibly reflected a combination of natural causes and possible ill-effects in handling methods and holding conditions over the 24-hr period. Increased control mortality may have resulted from the intentional withholding of sugar prior to testing. Sugar feeding has been found to significantly reduce mortality compared to unfed mosquitoes (Elliott & Ochoa-Aguirre, 1974). Mosquitoes recently blood-fed are normally recommended for standard contact susceptibility testing as they generally have lower natural mortality over the period of the observation than unfed mosquitoes (WHO, 1981). For the purposes of this study, it was considered more appropriate to use unfed/nongravid specimens as a closer measure of the insecticidal impact of excito-repellency on hungry (potentially host-seeking) females. Moreover, because the age of adult mosquitoes could not be controlled, field-collected adults were

selected for testing on the basis of as uniform a nutritional (non-bloodfed) status as possible.

Interpretation of results can be complicated by variations in response among individual insects in a population (Hoskins & Gordon, 1956). So-called 'vigor tolerance' may impact susceptibility test results, and appears influenced by body size, physiological and nutritional states, overcrowding during larval development, and seasonality (Busvine, 1956; Raffaele et al. 1958; Gordon, 1961; Gilotra, 1966; Hadaway & Barlow, 1956). Vigor tolerance applies to strains with inducible secondary (indirect) physiological mechanisms due to selective pressure. The effects of tolerance on refractory behavior are not clear. Cullen & de Zulueta (1962) and Coluzzi (1963) have shown that if mosquitoes are repeatedly exposed to DDT they become less irritable; although notions of developing physiological tolerance during the life span of an insect due to previous insecticide exposure has been refuted (Brown & Pal, 1971). The effects of seasonal climatic changes are assumed diminished in the subtropical climes; however, seasonality with respect to agricultural use of insecticides in Central America has been associated with changes in local vector susceptibility (Georghiou et al. 1973; Bown, 1987) which might also influence mosquito behavioral responses to various stimuli (Coluzzi, 1963). Obviously, many of these factors are difficult to control or measure accurately in the field, yet test interpretations must be made with these variables in mind.

Irritability is a general property of most insects and represents one of the principal chemoreceptive responses to disagreeable external stimuli (Chapman, 1982). It is well known that DDT deposits exert a direct irritant effect on adult mosquitoes (Kennedy, 1946). DDT contact has been shown to elicit specific effects on insect chemoreceptors

and sensory hairs (Smyth & Roys, 1955; Soliman & Cutkomp, 1963). Compared to irritancy, the repellent effect of insecticides has been more difficult to measure objectively. Shortly after DDT and BHC (benzene hexachloride) were introduced for house spraying, the 'fumigant' effect or repellent properties of both chemicals had been advanced (Gabaldon, 1952; Field, 1950; van Thiel, 1951; Muirhead-Thomson, 1951). Field studies from Africa, Asia and the Americas have clearly demonstrated strong behavioral avoidance of sprayed walls by anophelines under controlled experimental hut studies (Muirhead-Thomson, 1960; Smith, 1963; Roberts, 1993). In the Americas, repellency had been implicated as an important factor in the reduction of house entering and indoor human blood feeding by *Anopheles pseudopunctipennis* in Mexico and *Anopheles darlingi* in Brazil and Surinam (Downs & Bordas, 1951; Rozendaal, et al. 1989; Loyola, et al. 1990; Roberts & Alecrim, 1991).

The two most common quantitative methods for assessing insecticides and vector behavior involve specially constructed experimental houses (huts), designed with entrance and exit traps to sample mosquito populations, and the use of various excito-repellency test systems. Unfortunately, no single method, design or analysis for the study of behavior has yet been widely accepted, resulting in difficulties for interpreting and comparing excito-repellency data (Rachou, et al. 1965; Roberts et al. 1984). Roberts et al. (1997b) has advocated the continued use and development of excito-repellency test boxes as a method to standardized test procedures and measure graded behavioral avoidance to insecticides under differing conditions. In principal, the box design is analogous to a sprayed one-room house wherein mosquitoes are provided free behavioral options depending on their innate predilections to either remain inside or take flight to

escape. A chamber, as designed by Roberts et al. (1997b) has been successfully used by Chareonviriyaphap et al. (1997) proving the utility of excito-repellency boxes in the evaluation of behavioral responses to insecticides in laboratory and field settings. The same set of boxes was used in the Caledonia study. The improved design allowed for a highly standardized means of objectively measuring both irritancy and repellency responses (with and without physical contact with insecticides), among populations, insecticides and various concentrations of chemicals over different exposure time.

The use of the ER box in laboratory and field settings, augmented by careful experimental hut studies, can provide valuable data to determine the role of behavioral avoidance in vector control under operational conditions. Experimental huts have played an important role in assessing new insecticides and vector behavior (Muirhead-Thomson, 1960, Smith, 1963, 1964; Service, 1993). For reasons that are unclear, in recent decades a diminishing number of studies using experimental hut techniques have been published. The use of specially constructed experimental huts, fitted with a variety of entry and exit traps fitted to windows, doors or eaves, have held considerable importance in the evaluation of the efficiency of spraying houses with residual insecticides or using insecticide-impregnated bed nets (Muirhead-Thomson, 1968; Smith & Webley, 1969; WHO, 1975; Service, 1993b). However, such experiments are more expensive and difficult to carry out. Huts are also subject to high degrees of variability because of exposure to a large number of natural factors, and the greater difficulty of evaluating causes of accidental error compared to ER boxes (Cullen & de Zulueta, 1962; Coluzzi, 1963).

In this study, both contact irritability and noncontact repellency responses were seen with *An. albimanus* and *An. vestitipennis* in the presence of DDT and deltamethrin. These results from field-caught mosquitoes in Caledonia clearly demonstrated the utility of the ER box in the field to measure and show clear differences in the escape responses of these two vectors. The use of survival statistics and the escape probability estimates greatly enhanced the presentation and interpretation of the data. A limitation to earlier attempts at evaluating excito-repellency has been the lack of a more stringent and comprehensive method of data analysis. This new test system, using survival analysis of the response data, provides more useful information and interpretation over previous efforts (Roberts et al. 1997b; Chareonviriyaphap et al. 1997).

The implications of excito-repellency and our understanding of vector-borne disease control are enormous, because of the inter- and intraspecific differences in vector response to insecticides. As with physiological/biochemical resistance patterns, behavioral aspects must be evaluated as discreet and separate populations to determine their specific role in the transmission of disease pathogens and the assessment of control methods. Problems in the interpretation of behavioral observations still exist because of the difficulty in accurately measuring and analyzing behavioral responses in populations and the paucity of quality field studies.

The tendency of a mosquito to avoid insecticide-treated surfaces appears to be a general phenomenon. Behavioral avoidance, has been documented for decades, and is regarded as an important component in interrupting human-vector contact and malaria transmission (Gabaldon, 1953; Evans, 1993; Roberts & Andre, 1994). Further behavioral studies are needed to more clearly describe the nature of the response to different

insecticides and to clarify the inter- and intraspecific differences in response between geographically different populations. It would be an important step if international health organizations would endorse more studies on the behavior of vectors.

REFERENCES CITED

Ariaratnam, V. and A.W.A. Brown. 1969. Exposure time versus concentration in the WHO standard test for mosquito resistance to chlorohydrocarbon insecticides. Bull. Wld. Hlth. Organ. 40: 561-567.

Arredondo-Jimenez, J.I., J. Gimnig, M.H. Rodriguez and R.K. Washino. 1996. Genetic differences among *Anopheles vestitipennis* subpopulations collected using different methods in Chiapas state, southern Mexico. J. Am. Mosq. Control Assoc. 12:396-401.

Arredondo-Jimenez. J.I., M.H. Rodriguez, E.G. Loyola and D.N. Bown. 1997. Behavior of *Anopheles albimanus* in relation to pyrethroid-treated bednets. Med. Veter. Entomol. 11:87-94.

Barrera, A., O. Hecht and O. Mancera. 1959. Estimacion cuantitativa de la excitacion producida por el contact con superficies tratades con DDT en tres especies de anofelinos mexicanos. [Quantitative estimation of the irritation produced by contact with surfaces treated with DDT in three species of Mexican anophelines.] In: Trop. Dis. Bull. 1960, 57:788-789.

Beach, R.F., C. Cordon-Rosales and W.G. Brogdon. 1989. Detoxifying esterases may limit the use of pyrethroids for malaria vector control in the Americas. Parasitol. Today. 5:326-327.

Bhatia, S. C. & Deobhankar, R. B. 1962. Irritability of susceptible and resistant field populations of *Anopheles culicifacies* in Maharashtra state, India. WHO/MAL. Oct 12: 9 pp.

Bidlingmayer, W.L. 1985. The measurement of adult mosquito population changes: some considerations. J. Am. Mosq. Control Assoc. 1:328-348.

Bown, D.N. 1987. Agricultural use of pesticides and their effect on vector borne disease transmission in the WHO region of the Americas. In: *Effects of Agricultural Development on Vector-borne Diseases*. Food & Agric. Organ. AGL/MISC/12/87. pp. 53-56.

Bown, D.N., E.C. Fredrickson, G. del A. Cabanas and J.F. Mendez. 1987. An evaluation of bendiocarb and deltamethrin applications in the same Mexican village and their impact on populations of *Anopheles albimanus*. Bull. Pan Am. Hlth. Organ. 21:121-135.

Breeland, S. 1980. A bibliography to the literature of *Anopheles albimanus* (Diptera: Culicidae). Mosq. Syst. 12:50-150.

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

Brown, A.W.A. 1964. Experimental observations governing the choice of a test method for determining the DDT-irritability of adult mosquitos. Bull. Wld. Hlth. Organ. 30:97-111.

Brown, A.W.A. and R. Pal. 1971. *Insecticide Resistance in Arthropods*. Wld. Hlth Organ. Monogr. Series No. 38. WHO, Geneva. 491 pp.

Brown, A.W.A., J. Haworth and A.R. Zahar. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13:1-25.

Brown, A.W.A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2:123-140.

Bruce-Chwatt, L.J. 1970. Global review of malaria control and eradication by attack on the vector. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):7-27.

Busvine, J.R. 1956. The significance of insecticide-resistant strains, with special reference to pests of medical importance. Bull. Wld. Hlth Organ. 15:389-401.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitoes. Bull. Wld. Hlth. Organ. 31:645-656.

Busvine, J.R. and R. Pal. 1969. The impact of insecticide-resistance on control of vectors and vector-borne diseases. Bull. Wld. Hlth. Organ. 40:731-744.

Chapman, R.F. 1982. *The Insects: Structure and Function.* 3ed. Harvard Univ. Press, Cambridge. Chapter 30: Chemoreception. pp. 736-755.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.H. Harlan, S. Manguin and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus*, a malaria vector in the Americas. J. Am. Mosq. Control Assoc. 13:171-183.

Charlwood, J.D. and N.D. Paraluppi. 1978. O uso de caixas excito-repelentes com *Anopheles darlingi* Root, *A. nuneztovari* Gabaldon e *Culex pipiens quinquefasciatus* Say, obtidos em areas perto de Manaus, Amazonas. [The use of excito-repellency boxes with *Anopheles darlingi* Root, *A. nuneztovari* Gabaldon and *Culex pipiens quinquefasciatus* Say, obtained from areas near Manaus,Amazonas]. Acta Amazonica. 8:605-611. [In Portuguese]

Collett, D. 1994. *Modelling Survival Data in Medical Research.* Chapman & Hall, New York.

Coluzzi, A., Cullen, J. R. & de Zulueta, J. 1962. A field trial in Uganda of a new method for determining the irritability of mosquitoes to insecticides (preliminary report). WHO/Insecticides/2. 9 pp.

Coluzzi, M. 1963. Studies on irritability of DDT to anopheline mosquitoes. WHO/Vector Control/33. 22 pp.

Cordon-Rosales, C., N. Padilla and R. Zeissig. 1992. Impact of partial deltamethrin resistance in *Anopheles albimanus* on the efficacy of intradomiciliary spraying for malaria control. J. Am. Mosq. Control Assoc. 8:308-309.

Cullen, J.R. and J. De Zulueta. 1962. Observations on the irritability of mosquitos to DDT in Uganda. Bull. Wld. Hlth. Organ. 27:239-250.

Darriet, F., V. Robert, N. Tho Vien and P. Carnevale. 1984. Evaluation of the efficacy of permethrin impregnated intact and perforated mosquito nets against vectors of malaria. WHO/VBC/84.899.

Davidson, G. 1953. Experiments on the effect of residual insecticides in houses against *Anopheles gambiae* and *A. funestus*. Bull. Entomol. Res. 44:231-254.

Davidson, G. and A.R. Zahar. 1973. The practical implications of resistance of malaria vectors to insecticides. Bull. Wld. Hlth. Organ. 49:475-483.

De Zulueta, J. 1959. Insecticide resistance in *Anopheles sacharovi*. Bull. Wld. Hlth Organ. 20:797-822.

De Zulueta, J., G.W. Kafuko, J,R, Cullen and C.K. Pedersen. 1961. The results of the first year of a malaria eradication pilot project in northern Kigezi (Uganda). E. Afr. Med. J. 38:1-26.

De Zulueta, J. 1962. The irritability of mosquitos to DDT and its importance in malaria eradication. Riv. Malariol. 41:169-178.

De Zulueta, J. and J.R. Cullen. 1963. Deterrent effect of insecticides on malaria vectors. Nature, Lond. 200(4909):860-861.

De Zulueta, J. 1964. Ethological changes in malaria vectors. A review of the situation in the light of recent findings. Riv. Malariol. 43:29-36.

Dethier, V. G., Browne, L. B. & Smith, C. N. 1960. The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Entomol. 53: 134-36.

Diaz Najera, A. 1964. Nueva tecnica para ensayos de formulaciones de insecticidas sobre *Anopheles*. [A new method for the testing of insecticides on *Anopheles*.] Rev. Inst. Salubr. Enferm. Trop. (Mex.). 24(1-4):31-42.

Diehl, S.R. and G.L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Annu. Rev. Entomol. 29:471-504.

Downs, W.G. and E. Bordas. 1951. Control of *Anopheles pseudopunctipennis* in Mexico with DDT residual sprays applied in buildings. Part V: Effectiveness of residual applications of DDT and gammexane up to one year after application under controlled conditions. Am. J. Hyg. 54: 150-156.

Duret, J.P. 1964. Pruebas de excito-repelencia de algunos anofelinos de Panama. [Excito-repellency tests for some anophelines from Panama] Bol. Of. Sanit. Panamerica. 57:259-267.

Elliott, R. 1964. Studies on the kinetic response of mosquitos to chemicals. Bull. Wld. Hlth. Organ. 31:657-667.

Elliott, R. 1969. Ecology and behaviour of malaria vectors in the american region. Cah. ORSTOM., ser. Ent. Med. et Parasitol. 7:29-33.

Elliott, R. 1972. The influence of mosquito behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21:755-763.

Elliott, R. and O. Ochoa-Aguirre. 1974. Overnight access to sugar and response to DDT in *Anopheles albimanus* Wied. Bull. Wld. Hlth Organ. 51:311-313.

Elliott, R. and J. de Zulueta. 1975. Ethological resistance in malaria vectors. Behavioral response to intradomestic residual insecticides. WHO\VBC\75.569: 15 pp.

Evans, R.G. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrine and DDT to *Anopheles gambiae*. J. Am. Mosq. Control Assoc. 9:285-293.

Faran, M. E. 1980. Mosquito studies (Diptera: Culicidae) 34. A revision of the Albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. 15:1-215.

Field, J.W. 1950. Fumigant and repellent effects of BHC (Gammexane) and DDT upon *Anopheles*. Trans. Roy. Soc. Trop. Med. Hyg. 43:547-548.

Fredrickson, E.C. 1993. Bionomics and control of *Anopheles albimanus*. Tech. Paper 34. Pan Am. Hlth. Organ. Washington. D.C. 76 pp.

Gaaboub, I.A. and M.R. Dawood. 1974. Irritability status of adults of *Culex pipiens* L. under selection pressure with lethal concentrations of DDT and malathion. Zeitschrift fur Angewandte Entomologie. 77:126-132.

Gabaldon, A. 1952. The effect of DDT on the population of anopheline vectors in Venezuela. Riv. Parassitol. 13:29-41.

Gabaldon, A. 1953. Possible effects of residual insecticides on the interruption of malaria transmission. Riv. Malariol. 32:155-171.

Gahan, J.B., B.V. Travis, A.W. Lindquist. 1945. DDT as a residual-type spray to control disease-carrying mosquitoes: Laboratory tests. J. Econ. Entomol. 38:236-240.

Garrett-Jones, C. 1970. Problems of epidemiological entomology as applied to malariology. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):168-180.

Gerold, J.L. and J.J. Laarman. 1967. Behavioural responses to contact with DDT in *Anopheles atroparvus*. Nature. 215:518-520.

Gerold, J.L. 1970. Selection and analysis of the disturbed behaviour after contact with DDT of *Anopheles atroparvus*. Acta Leiden. 37:1-94.

Gerold, J.L. 1977. Behavioural differences in field populations of *Anopheles sacharovi* in Turkey and in selected strains of *Anopheles atroparvus*. Acta Leiden. 45:67-77.

Georghiou, G.P., S. Breeland and V. Ariaratnam. 1973. Seasonal escalation of organophosphorus and carbamate resistance in *Anopheles albimanus* by agricultural sprays. Environ. Entomol. 2:369-374.

Gheorghiu, T., E.M. Ungureanu and C. Garrett-Jones. 1972. An apparatus for the study of the behavior patterns of mosquitoes under the influence of insecticides. Bull. Wld. Hlth. Organ. 46:122-126.

Gillies, M.T. 1956. The problems of exophily in *Anopheles gambiae*. Bull. Wld. Hlth. Organ. 15:437-449.

Gillies, M.T. 1988. Anopheline mosquitoes: vector behaviour and bionomics. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer & I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 453-485.

Gilotra, S. 1966. Effects of larval environments on adult resistance in *Anopheles albimanus* Wiedemann. Mosq. News 26:26-30.

Gordon, H. 1961. Nutritional factors in insect resistance to chemicals. Annu. Rev. Entomol. 6:27-54.

Hadaway, A.B. and F. Barlow. 1956. Effects of age, sex and feeding on the susceptibility of mosquitoes to insecticides. Ann. Trop. Med. Parasitol. 50:438-443.

Hamon, J. and M. Eyraud. 1961. Etude des facteurs physiologiques conditionnant, chez les *Anopheles*, l' irritabilite au DDT. [Study of physiological factors conditioning in *Anopheles* irritability to DDT]. Riv. Malariol. 40:219-242.

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. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):28-41

Hecht, O. and J. Hernandez. 1960. Resena de las investigaciones sobre la irritacion de Anofelinos por el contacto con superficies cubiertas con DDT. [Review of investigations on the irritation of anophelines by contact with DDT-treated surfaces.] Bol. Com. Nac. Errad. Paludismo. 4:93-106.

Hecht, O., O. Mancera and A. Barrera. 1960. Relation of DDT-irritation threshold to knockdown of three species of anopheline mosquitoes. J. Econ. Entomol. 53:634-640.

Hecht, O., H. Chapa-Saldana and J. Hernandez-Corzo. 1962. The influence of illumination upon the direction of the escape flights of anophelines irritated by DDT. Verhandl 11 Kongr. Entomol. Wien. [XI Int. Congr. Entomol.] 1960. 3:79-81.

Hii, J.L.K. 1985. Evidence for the existence of genetic variability in the tendency of *Anopheles balabacensis* to rest in houses and to bite man. Southeast Asian J. Trop. Med. Publ. Hlth. 16:173-182.

Hooper, G.H.S. and A.W.A. Brown. 1965. Development of increased irritability to insecticides due to decreased detoxication. Entomol. Exp. Appl. 8:263-270.

Hoskins, W.M. and Gordon, H.T. 1956. Arthropod resistance to chemicals. Annu. Rev. Entomol. 1:89-122.

Kartman, L. and M.M. Silveira da. 1946. Effect of short contact with DDT residues on *Anopheles gambiae*. J. Econ. Entomol. 39:356-359.

Kaschef, A.H. 1970. Effects of temperature on the irritability caused by DDT and DDT analogues in anopheline mosquitoes. Bull. Wld Hlth. Organ. 42:917-930.

Kennedy, J.S. 1946. The excitant and repellent effects on mosquitos of sublethal contacts with DDT. Bull. Entomol. Res. 37:593-607.

Kennedy, J.S. 1978. The concepts of olfactory 'arrestment' and 'attraction'. Physiol. Entomol. 3:91-98.

Klowdon, M.J. 1996. Vector Behavior. In: *The Biology of Disease Vectors*. B.J. Beaty & W.C. Marquardt (Eds.). University Press, Colorado. 632 pp.

Kuhlow, F. 1962. Field experiments on the behaviour of malaria vectors in an unsprayed hut and in a hut sprayed with DDT in northern Nigeria. Bull. Wld. Hlth Organ. 26:93-102.

Lee, E.T. 1992. *Statistical Methods for the Survival Data Analysis*. John Wiley, New York.

Lindsey, S.W., J.H. Adiamah, J.E. Miller and J.R.M. Armstrong. 1991. Pyrethroid treated bednet effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. Med. Veter. Entomol. 5:477-483.

Lindsay, S.W., J.H. Adiamah and J.R.M. Armstrong. 1992. The effect of permethrin impregnated bednets on house entry by mosquitoes (Diptera: Culicidae) in The Gambia. Bull. Entomol. Res. 82:49-55.

Lines, J.D., J. Myamba and C.F. Curtis. 1987. Experimental hut trials of permethrin impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. Med. Veter. Entomol. 1:37-51.

Lockwood, J.A., T.C. Sparks and R.N. Story. 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30:41-51.

Loyola, E.G., M.H. Rodriquez, L. Gonzalez, J.L. Arredondo, D.N. Bown and M.A. Vaca. 1990. Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of *Anopheles pseudopunctipennis* in Mexico. J. Am. Mosq. Control Assoc. 6:635-640.

Mancera, V.O. and C.J. Hernandez.. 1960. El efecto irritante en mosquitos de algunos insecticides de contacto. Pruedas con *Anopheles (N.) albimanus*. [The irritant effect of some contact insecticides on mosquitos. Test with *Anopheles (N.) albimanus*.] Bol. Com Nac. Errad. Paludismo. 4:82-85. In: Trop. Dis. Bull. 1960, 57:175.

Mantel, N. and W. Haenzel. 1959. Statistical aspects of the analysis of data from retrospective studies of diseases. J. Nat. Cancer Inst. 22:719-748.

Macdonald, G. 1957. *The Epidemiology and Control of Malaria*. Oxford Univ. Press.

Mattingly, P.F. 1962. Mosquito behaviour in relation to disease eradication programmes. Annu. Rev. Entomol. 7:419-436.

Mekuria, Y., D.C. Williams, M.A. Tidwell and T.A. Santana. 1990. Studies of the susceptibility of *Anopheles albimanus* and *Anopheles vestitipennis* from Dajabon, Dominican Republic, to insecticides. J. Am. Mosq. Control Assoc. 6: 645-650.

Metcalf, R.L., A.D. Hess, C.E. Smith, G.M. Jeffrey and G.L. Ludwig. 1945. Observations on the use of DDT for the control of *Anopheles quadrimaculatus*. U.S. Publ. Hlth. Rep. 60:753-774.

Miller, J.E., S.W. Lindsay and J.R.M. Armstrong. 1991. Experimental hut trials of bednets impregnated with synthetic pyrethroid and orgnophosphate insecticides for mosquito control in The Gambia. Med. Vet. Entomol. 5:465-476.

Miller, J.E. and G. Gibson. 1994. Behavioral response of host-seeking mosquitoes (Diptera: Culicidae) to insecticide-impregnated bed netting: a new approach to insecticide bioassays. J. Med. Entomol. 31:114-122.

Milliken, G.A. and D.E. Johnson. 1992. *Analysis of Messy Data*. Van Nostrand Reinhold, New York. 473 pp.

Molineaux, L., G.R. Shidrawi, J.L. Clarke, J.R. Boulzaguet and T.S. Ashkar. 1979. Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition. Bull. Wld. Hlth. Organ. 57:265-274.

Muirhead-Thomson, R.C. 1947. The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. Bull. Entomol. Res. 38:449-464.

Muirhead-Thomson, R.C. 1950. DDT and Gammexane as residual insecticides against *Anopheles gambiae* in African houses. Trans. Roy. Soc. Trop. Med. Hyg. 43:401-412.

Muirhead-Thomson, R.C. 1951. *Mosquito Behaviour in Relation to Malaria Transmission and Control in the Tropics*. Edward Arnold, London. 219 pp.

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. Wld. Hlth. Organ. 22:721-734.

Muirhead-Thomson, R.C. 1968. *Ecology of Insect Vector Populations*. Academic Press, London. 174 pp.

Pell, J.K., M.A. Spinney and K.J. Ward. 1989. Observations on the behaviour of adult *Anopheles gambiae* encountering residual deposits of lambda-cyhalothrin compared with other major classes of commercially available insecticides. Proc. 4th Annu. Conf. Soc. Vector Ecol. Eur. Region. p. 18.

[PAHO] Pan American Health Organization. 1996. Biology and ecology of *Anopheles albimanus* Weidmann in Central America. Pan Am. Hlth. Organ. Tech. Rep. Ser. No. 43. Washington, D.C. 44 pp.

Pampana, E. 1963. *A Textbook of Malaria Eradication*. Oxford, London. 508 pp.

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

Qutubuddin, M. 1967. Irritability of *Anopheles pharoensis* Theobald to different insecticides as observed in laboratory experiments. Sudan Med. J. 5:18-28.

Rachou, R.G., M. Moura-Lima, J.P. Duret, J.A. Kerr. 1963. Experiments with the excito-repellency test box--model OPS. Proc. 50th Annu. Mtg. N.J. Mosq. Exter. Assoc./19th Annu. Mtg. Am. Mosq. Control Assoc. pp. 442-447.

Rachou, R.G., G. Lyons, M. Moura-Lima and J.A. Kerr. 1965. Synoptic epidemiological studies of malaria in El Salvador. Am. J. Trop. Med. Hyg. 14:1-62.

Rachou, R.G., L.A. Schinazi and M.M. Lima. 1973. An extensive epidemiological Study of the causes for the failure of residual DDT-spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages on the coastal plain of El Salvador, Central America. Rev. Bras. Malariol. Doencas Trop. 25:3-293.

Raffaele, G., A. Coluzzi and J. de Zulueta. 1958. Observations on the effect of numbers and age on the susceptibility of mosquitoes to DDT. Bull. Wld. Hlth. Organ. 18:464-468.

Rani, P.U. and Z. Osmani. 1984. Studies on the toxic, repellent and attractant properties of certain insecticides towards the housefly, *Musca domestica nebulo* (L.). Inter. Pest Control. May/June, pp. 72-75.

Ree, H. I. and K.P. Loong. 1989. Irritability of *Anopheles farauti*, *An. maculatus* and *Culex quinquefasciatus* to permethrin. Jap. J. Sanit. Zool. 40:47-51.

Rejmankova, E., D.R. Roberts, R.E. Harbach, J. Pecor, E.L. Peyton, S. Manguin, R. Krieg, J. Polanco and L. Legters. 1993. Environmental and regional determinants of *Anopheles* (Diptera: Culicidae) larval distribution in Belize, Central America. Environ. Entomol. 22:978-992.

Rejmankova, E., D.R. Roberts, S. Manguin, K.O. Pope, J. Komarek and R.A. Post. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. Environ Entomol. 25:1058-1067.

Rejmankova, E., K.O. Pope, D.R. Roberts, M.G. Lege, R. Andre, J. Greico and Y. Alonzo. 1998. Characterization and detection of *Anopheles vestitipennis* and *Anopheles punctimacula* (Diptera: Culicidae) larval habitats in Belize with field survey and SPOT satellite imagery. J. Vect. Ecol. 23:74-88.

Ribbands, C.R. 1946a. The use of residual films of DDT and Gammexane in malaria control. Bull. Entomol. Res. 37:567-592.

Ribbands, C.R. 1946b. Repellency of pyrethrum and lethane sprays to mosquitoes. Bull. Entomol. Res. 37:163-172.

Richards, F.O., Jr., R.Z. Flores, J.D. Sexton, R.F. Beach, D.L. Mount, C. Cordon Rosales, M. Gatica and R.E. Klein. 1994. Effects of permethrin-impregnated bed nets on malaria vectors in northern Guatemala. Bull. Pan Am. Hlth. Organ. 28:112-121.

Roberts, D.R., W.D. Alecrim, A.M. Tavares and K.M. McNeill. 1984. Influence of physiological condition on the behavioral response of *Anopheles darlingi* to DDT. Mosq. News 44:357-362.

Roberts, D.R., W.D. Alecrim, A.M. Tavares and M.G. Radke. 1987. The house frequenting, host-seeking and resting behavior of *Anopheles darlingi* in southeastern Amazonas, Brazil. J. Am. Mosq. Control Assoc. 3:433-441.

Roberts, D.R. and W.D. Alecrim. 1991. Behavioral response of *Anopheles darlingi* to DDT-sprayed house walls in Amazonia. Bull. Pan Am. Hlth. Organ. 25:210-217.

Roberts, D.R., O. Chan, J. Pecor, E. Rejmankova, S. Manguin, J. Polanco and L.J. Legters. 1993. Preliminary observations on the changing roles of malaria vectors in southern Belize. J. Am. Mosq. Control Assoc. 9:456-459.

Roberts, D.R. 1993. Insecticide repellency in malaria vector control: a position paper. VBC report no. 81131. U.S. Agency for International Development, Arlington, VA. 72 pp.

Roberts, D.R. and R.G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. J. Am. Trop. Med. Hyg. 50(Suppl.):21-34.

Roberts, D.R., J.F. Paris, S. Manguin, R.E. Harbach, R. Woodruff, E. Rejmankova, J. Polanco, B. Wullschleger and L.J. Legters. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. Am. J. Trop. Med. Hyg. 54:304-308.

Roberts, D.R., L.L. Laughlin, P. Hshieh and L.J. Letgers. 1997a. DDT: global strategies, and a malaria control crisis in South America. Emerg. Inf. Dis. 3:295-302.

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

Roberts, D.R., W.D. Alecrim, R.G. Andre and P. Hshieh. A stochastic model of vector behavior: Effects of DDT repellency, irritancy and toxicity in malaria control. Emerg. Infect. Dis. (unpub. doc.)

Rozendaal, J.A., J.P.M. van Hoof, J. Voorhamand B.F.J. Oostburg. 1989. Behavioral responses of *Anopheles darlingi* in Suriname to DDT residues on house walls. J. Am. Mosq. Control Assoc. 5:339-350.

Service, M.W. 1993. *Mosquito Ecology, Field Sampling Methods*. 2ed. Elsevier, London. 988 pp.

Slooff, R. 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *Anopheles punctulatus* group. Leyden: A.W. Sythoff. 144 pp.

Smith, A. 1963. Deterrent effect of insecticides on malaria vectors. Nature. 200:861 862.

Smith, A. 1964. A review of the origin and development of experimental hut techniques used in the study of insecticides in East Africa. E. Afr. Med. J. 41:361-374.

Smith, A. and P.R. Chadwick. 1964. A reassessment of pyrethrum against *Anopheles gambiae* with particular reference to its action as a residual repellent. E. Afr. Med. J. 41:137-141.

Smith, A. and D.J. Webley. 1969. A verandah-type hut for studying the house frequenting habits of mosquitoes and for assessing insecticides. III. The effect of DDT on behaviour and mortality. Bull. Entomol. Res. 59:33-46.

Smits, A., P. Roelants, W. Van Bortel and M. Coosemans. 1996. Enzyme polymorphisms in the *Anopheles gambiae* (Diptera: Culicidae) complex related to feeding and resting behavior in the Imbo Valley, Burundi. J. Med. Entomol. 33:545-553.

Smyth, T., Jr. and C.C. Roys. 1955. Chemoreception in insects and the action of DDT. Biol. Bull. 108:66-76.

Soliman, S.A. and L.K. Cutkomp. 1963. A comparison of chemoreceptor and whole fly response to DDT and parathion. J. Econ. Entomol. 56:492-494.

Sparks, T.C., J.A. Lockwood, R.L. Byford, J.B. Graves and B.R. Leonard. 1989. The role of behavior in insecticide resistance. Pestic. Sci. 26:383-399.

Taylor, P., M.J. Crees and K. Hargreaves. 1981. Duration of *Anopheles arabiensis* control in experimental huts sprayed with DDT and Decamethrin. Trans. Zimbabwe Scient. Assoc. 61:1-13.

Thiel, P.H. van. 1951. The repellent effect of insecticides, especially of contact insecticides. Doc. Neerl. Indones. Morb. Trop. 3:117-125.

Threlkeld, S.F.H. 1985. Behavioral responses in *Drosophilia melanogaster* associated with permethrin and ectiban. In: Proceedings of the Thirty-second Annual Meeting, Canadian Pest Management Society, Charlottetown, Prince Island, 24-26 June, 1985. Can. Pest Manag. Soc. pp. 29-36.

Trapido, H. 1946. The residual spraying of dwellings with DDT in the control of malaria transmission in Panama, with special reference to *Anopheles albimanus*. Am. J. Trop. Med. 26:383-415.

Trapido, H. 1952. Modified response of *Anopheles albimanus* to DDT residual housing spraying in Panama. Am. J. Trop. Med. Hyg. 1:853-861.

Ungureanu, E. M. and Gheorghiu, T. 1980. Comparative studies on the behaviour of a population of *Anopheles maculipennis* under the influence of DDT and malathion, using the multiple-response test-apparatus. WHO/VBC/80.774:1-7.

Ungureanu, E. M. and Teodorescu, C. 1963. Observations on the irritability of mosquitoes to DDT. Ind. J. Malariol. 17:47-53.

Vargas, L. 1976. Criterio epidemiologico para valorar las pruebas de susceptibilidad de *Anopheles* a insecticidas y la importancia del fenomeno de la excitation que produce el DDT [Epidemiological criteria for assessing the susceptibility of *Anopheles* to insecticides and the significance of the irritant effect of DDT]. Bol. Dir. Malariol. Saneam. Ambient. 16:363-366.

Vigueras, O.M. and J.H. Corzo. 1960. El efecto irritante en mosquitoes de algunos insecticidas de contacto. Pruebas con *Anopheles (N.) albimanus*, Wied. Comision Nat. Errad. Paludismo Bol. 4:82-85.

[WHO] World Health Organization. 1960. Expert Committee on Insecticides. Tentative method of determining the irritability of adult mosquitoes to insecticides. Wld. Hlth. Organ. Tech. Rep. Ser. 191:46-49.

[WHO] World Health Organization. 1970. Insecticide resistance and vector control. Instructions for determining the irritability of adult mosquitoes to insecticide, Wld. Hlth. Organ. Tech. Rep. Ser. 433:158-163.

[WHO] World Health Organization. 1975. Manual on practical entomology in malaria. Part II. methods and techniques. WHO Offset Pub. No, 13. Geneva. 191 pp.

[WHO] World Health Organization. 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organphosphate and carbamate insecticides. Diagnostic Test. WHO/VBC/81.806. Geneva. 6 pp.

[WHO] World Health Organization. 1992. Vector resistance to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 818. 62 pp.

[WHO] World Health Organization. 1995. Vector control for malaria and other mosquito-borne diseases. Wld. Hlth. Organ. Tech. Rep. Ser. No. 857. 91 pp.

Wilkerson, R.C., D. Strickman and T.R. Litwak. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. J. Am. Mosq. Control Assoc. 6:7-34.

Wilson, H.G., D.E. Weidhaas and G.C. LaBrecque. 1973. Toxicity of insecticide residues to *Anopheles quadrimaculatus* and effects on resting behavior. Mosq. News 33:539-542.

Zeichner, B. 1992. Procedures for the diagnostic dose resistance test kits for mosquitoes, body lice, and beetle pests of stored products. U.S. Army Environ. Hyg. Agency Tech. Guide No. 189. 39 pp.

Table 1. Summary percentage mortality of *Anopheles albimanus* females from Caledonia, Belize following 24-hr post - exposure* to DDT or deltamethrin in excito-repellency CONTACT trials.

Insecticide gm/m²	N		Escape (%)		Mortality (%)		No escape (%)		Mortality (%)	
	E	C	E	C	E	C	E	C	E	C
DDT										
2.0	180	194	168(93.3)	35(18.0)	63(37.5)	2(5.7)	12(6.7)	159(82.0)	11(91.6)	14(8.8)
0.5	148	142	107(72.3)	21(14.7)	21(19.6)	1(4.7)	41(27.7)	121(85.3)	13(31.7)	5(4.1)
0.125	146	148	63(43.1)	27(18.2)	0(0.0)	1(3.7)	83(56.8)	121(81.8)	12(14.2)	1(0.8)
Deltamethrin										
0.00925	100	99	92(92.0)	17(17.2)	16(17.4)	0(0.0)	8(8.0)	82(82.8)	8(100)	2(2.4)
0.0023	98	100	90(91.8)	12(12.0)	8(8.9)	0(0.0)	8(8.2)	88(88.0)	7(87.5)	5(5.7)
0.00057	100	100	99(99.0)	23(23.0)	7(7.0)	1(4.3)	1(1.0)	77(77.0)	1(100)	2(2.6)

*exposure/escape period: 0-60 min. N=sample size, E=test exposure, C=control

Table 2. Summary percentage mortality of *Anopheles albimanus* females from Caledonia, Belize following 24-hr post-exposure* to DDT or deltamethrin in excito-repellency NONCONTACT trials.

Insecticide gm/m²	N		Escape (%)		Mortality (%)		No escape (%)		Mortality (%)	
	E	C	E	C	E	C	E	C	E	C
DDT										
2.0	140	139	76(54.3)	55(39.6)	2(2.6)	0(0.0)	64(45.7)	84(60.4)	1(1.6)	2(2.4)
0.5	100	98	30(30.0)	16(16.3)	0(0.0)	0(0.0)	70(70.0)	82(83.7)	4(5.7)	3(3.7)
0.125	95	97	10(10.5)	11(11.3)	0(0.0)	1(9.1)	85(89.5)	86(88.7)	3(3.5)	4(4.6)
Deltamethrin										
0.00925	75	74	54(72.0)	17(23.0)	3(5.6)	0(0.0)	21(28.0)	57(77.0)	1(4.7)	2(9.5)
0.0023	73	75	39(53.4)	17(22.7)	2(5.1)	0(0.0)	34(46.6)	58(77.3)	2(5.9)	4(6.9)
0.00057	73	75	41(56.2)	20(26.6)	0(0.0)	0(0.0)	32(43.8)	55(73.4)	6(18.7)	4(7.3)

*exposure/escape period: 5-hr. N=sample size, E=test exposure, C=control

Table 3. Summary percentage mortality of *Anopheles vestitipennis* females from Caledonia, Belize following 24-hr post-exposure to DDT excito-repellency CONTACT* and NONCONTACT trials.**

DDT gm/m²	N		Escape (%)		Mortality (%)		No escape (%)		Mortality (%)	
	E	C	E	C	E	C	E	C	E	C
Contact										
2.0	84	85	70(83.3)	27(31.7)	16(22.8)	1(8.3)	14(16.7)	58(68.3)	10(71.4)	1(1.7)
0.5	117	118	66(56.4)	23(19.5)	13(19.7)	0(0.0)	51(43.6)	95(80.5)	12(23.5)	5(5.3)
0.125	56	59	22(39.3)	17(28.8)	0(0.0)	0(0.0)	34(60.7)	42(71.2)	3(8.8)	1(2.4)
Noncontact										
2.0	62	69	54(87.1)	31(44.9)	3(5.5)	0(0.0)	8(12.9)	38(55.1)	1(12.5)	1(2.6)
0.5	77	75	66(85.7)	45(60.0)	8(12.1)	4(8.8)	11(14.3)	30(40.0)	8(72.7)	8(26.6)
0.125	65	69	25(38.5)	22(31.9)	1(4.0)	2(9.1)	40(61.5)	47(68.1)	1(2.5)	4(8.5)

*exposure/escape period: 0-60 min. ** exposure/escape period: 5-hr. N=sample size, E=test exposure, C=control

Table 4. Contact escape (ET) time from 0-60 min for 50 and 90% of *Anopheles* to exit from excito-repellency chambers treated with either DDT or deltamethrin.

Species	DDT					
	(2.0 g/m^2)		(0.5 g/m^2)		(0.125 g/m^2)	
	ET_{50}	ET_{90}	ET_{50}	ET_{90}	ET_{50}	ET_{90}
Anopheles albimanus	18	46	25	***	***	***
Anopheles vestitipennis	20	***	47	***	***	***
	Deltamethrin					
	(0.00925 g/m^2)		(0.0023 g/m^2)		(0.00057g/m^2)	
	ET_{50}	ET_{90}	ET_{50}	ET_{90}	ET_{50}	ET_{90}
Anopheles albimanus	7	14	10	31	9	24

***overall percentage escape not attained within allotted time.

Table 5. Log-rank comparison* of excito-repellency escape responses at 60-min by chemical concentration.

Species	Contact vs. Control gm/m^2	(χ^2)	Contact vs. Noncontact gm/m^2	(χ^2)	Noncontact vs. Control gm/m^2	(χ^2)
DDT						
An. albimanus	2.00◆	(233.48)	2.00◆	(105.04)	2.00◆	(15.28)
	0.5◆	(112.93)	0.5◆	(56.93)	0.5	(2.65)
	0.125◆	(20.73)	0.125◆	(33.34)	0.125	(0.194)
An. vestitipennis	2.00◆	(42.24)	2.00◆	(23.63)	2.00◆	(18.94)
	0.5◆	(33.14)	0.5◆	(21.94)	0.5	(0.029)
	0.125	(1.17)	0.125✱	(8.88)	0.125	(0.038)
Deltamethrin						
An. albimanus	.00925◆	(142.06)	.00925◆	(102.56)	.00925	(2.72)
	.0023◆	(148.11)	.0023◆	(113.69)	.0023	(0.006)
	.00057◆	(193.09)	.00057◆	(129.71)	.00057	(0.84)

*Significant difference at $p<0.05$ ◆χ^2 $p=0.0001$ ✱χ^2 $p=0.0005$

Table 6. Within species log-rank comparison of 60-min excito-repellency escape responses using 3 concentrations of DDT for contact and noncontact trials.

Condition	Species	Comparison	χ^2	(p=)
Contact				
	An. albimanus ❖	2.0 vs. 0.5◆	25.18	(0.0001)
	χ^2 104.67 (p<0.0001)	2.0 vs. 0.125◆	105.83	(0.0001)
		0.5 vs. 0.125◆	27.15	(0.0001)
	An. vestitipennis❖	2.0 vs. 0.5◆	27.03	(0.0001)
	χ^2 43.41 (p<0.0001)	2.0 vs. 0.125◆	29.56	(0.0001)
		0.5 vs. 0.125	3.58	(0.0585)
Noncontact				
	An. albimanus ❖	2.0 vs. 0.5◆	6.97	(0.0082)
	χ^2 30.98 (p<0.0001)	2.0 vs. 0.125◆	28.88	(0.0001)
		0.5 vs. 0.125◆	9.57	(0.0020)
	An. vestitipennis❖	2.0 vs. 0.5◆	12.66	(0.0004)
	χ^2 22.64 (p<0.0001)	2.0 vs. 0.125◆	16.92	(0.0001)
		0.5 vs. 0.125	0.68	(0.4092)

❖ Significant difference experiment-wise, χ^2 log-rank test (df=2), p<0.05
◆ Significant difference comparison-wise, χ^2 log-rank test (df=1), p<0.02

Table 7. Within species log-rank comparison of 60-min excito-repellency escape responses using 3 concentrations of deltamethrin for contact and noncontact trials.

Condition	Species	Comparison	χ^2	(p=)
Contact				
	An. albimanus ❖	.00925 vs .0023◆	7.26	(0.0070)
	χ^2 7.17 (p<0.027)	.00925 vs .00057	2.84	(0.0916)
		.0023 vs .00057	0.92	(0.3371)
Noncontact				
	An. albimanus	.00925 vs .0023	3.35	(0.0673)
	χ^2 3.54 (p<0.17)	.00925 vs .00057	0.10	(0.7510)
		.0023 vs .00057	2.31	(0.1285)

❖ Significant difference experiment-wise, χ^2 log-rank test (df=2), p<0.05
◆ Significant difference comparison-wise, χ^2 log-rank test (df=1), p<0.02

Table 8. Log-rank comparison of noncontact vs. control excito-repellency escape responses for 3 concentrations of DDT and deltamethrin at 60-min and 5-hr.

Insecticide gm/m^2	Species	Noncontact vs. Control 60-min	(χ^2)	5-hr	(χ^2)
DDT					
	An. albimanus	2.00◆	(15.28)	2.00◆	(12.86)
		0.5	(2.65)	0.5◆	(5.15)
		0.125	(0.194)	0.125	(0.04)
	An. vestitipennis	2.00◆	(18.94)	2.00◆	(30.25)
		0.5	(0.029)	0.5◆	(13.22)
		0.125	(0.038)	0.125	(0.60)
Deltamethrin					
	An. albimanus	.00925	(2.72)	0.00925◆	(33.52)
		.0023	(0.006)	0.0023◆	(11.67)
		.00057	(0.84)	0.00057◆	(11.66)

◆ Significant difference at $p < 0.05$

Table 9. Within species comparison of noncontact escape responses for 3 doses of DDT and deltamethrin in excito-repellency trials at 5-hr exposure.

Insecticide	Species	Comparison	χ^2	$p=$
DDT				
	An. albimanus ❇	2.0 vs. 0.5◆	10.51	0.0012
	χ^2 43.11 ($P<0.0001$)	2.0 vs. 0.125◆	41.23	0.0001
		0.5 vs. 0.125◆	11.41	0.0007
	An. vestitipennis❇	2.0 vs. 0.5	4.04	0.0443
	χ^2 57.67 ($P<0.0001$)	2.0 vs. 0.125◆	47.66	0.0001
		0.5 vs. 0.125◆	39.48	0.0001
Deltamethrin				
	An. albimanus ❇	0.00925 vs .0023◆	6.27	0.0123
	χ^2 6.47 ($P<0.039$)	0.00925 vs .00057	3.05	0.0804
		0.0023 vs .00057	0.43	0.5121

❇ Significant difference experiment-wise using a χ^2 log-rank test (df=2), $p<0.05$
◆ Significant difference comparison-wise using a χ^2 log-rank test (df=1), $p<0.02$

Table 10. Log-rank comparison of escapes responses between species in contact and noncontact excito-repellency trials using DDT at 3 different concentrations for 60-min (contact & noncontact) and 5-hr (noncontact).

	An. albimanus vs. *An. vestitipennis*		
Concentration	Contact	Noncontact	Noncontact▼
gm/m^2	χ^2	χ^2	χ^2
2.0	❇ 5.29 (p=0.021)	- 0.47 (p=0.49)	❇ 23.63 (p=0.0001)
0.5	❇ 12.12 (p=0.0005)	- 0.24 (p=0.62)	❇ 45.61 (p=0.0001)
0.125	- 0.31 (p=0.57)	- 2.48 (p=0.11)	❇ 16.85 (p=0.0001)

❇ Significant difference at p<0.05 (df=1) ▼ Noncontact 5-hr test.

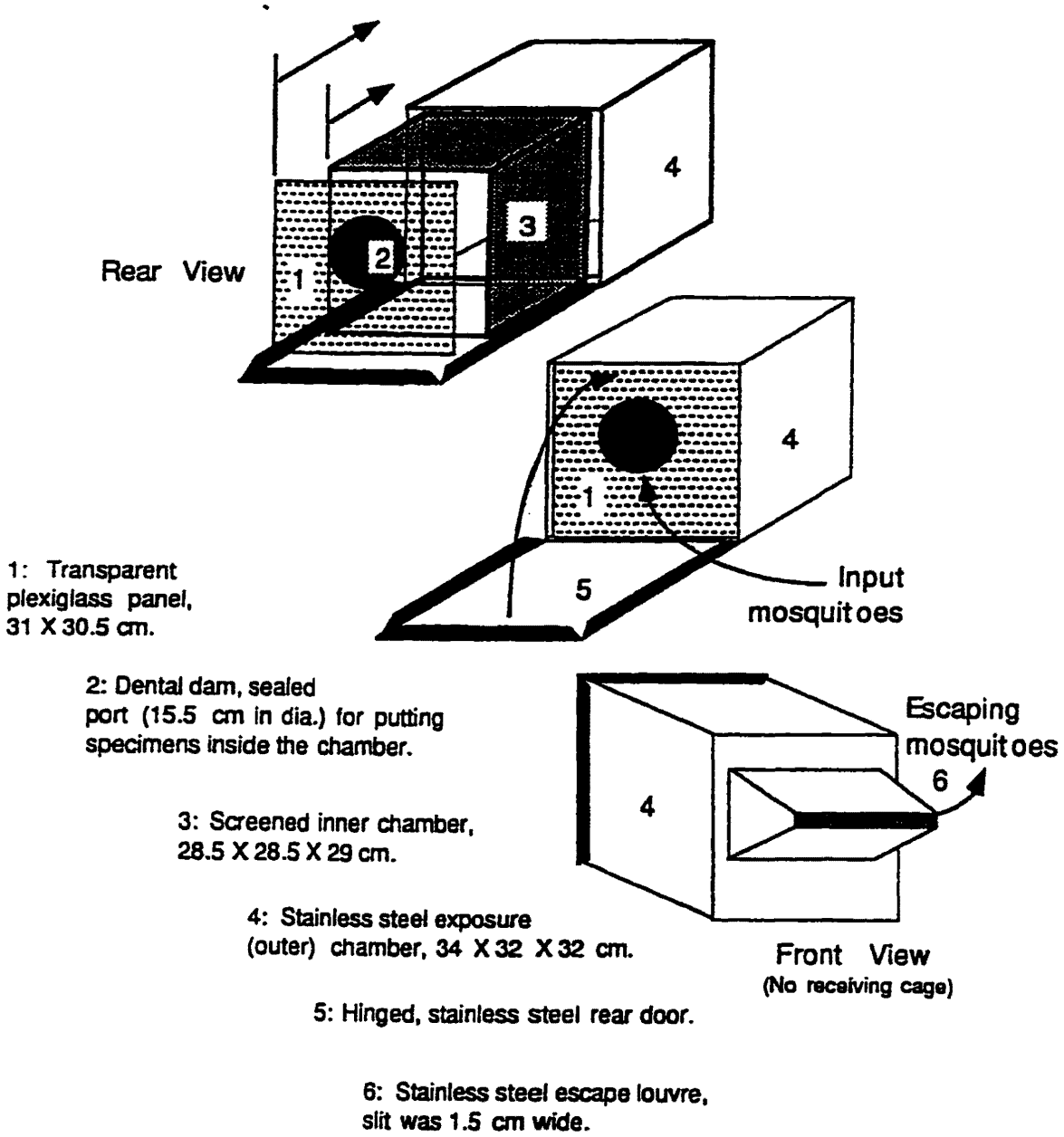


Figure 1. An excito-repellency test box for study of the behavioral responses of mosquitoes to insecticides. (Roberts et al. 1997)

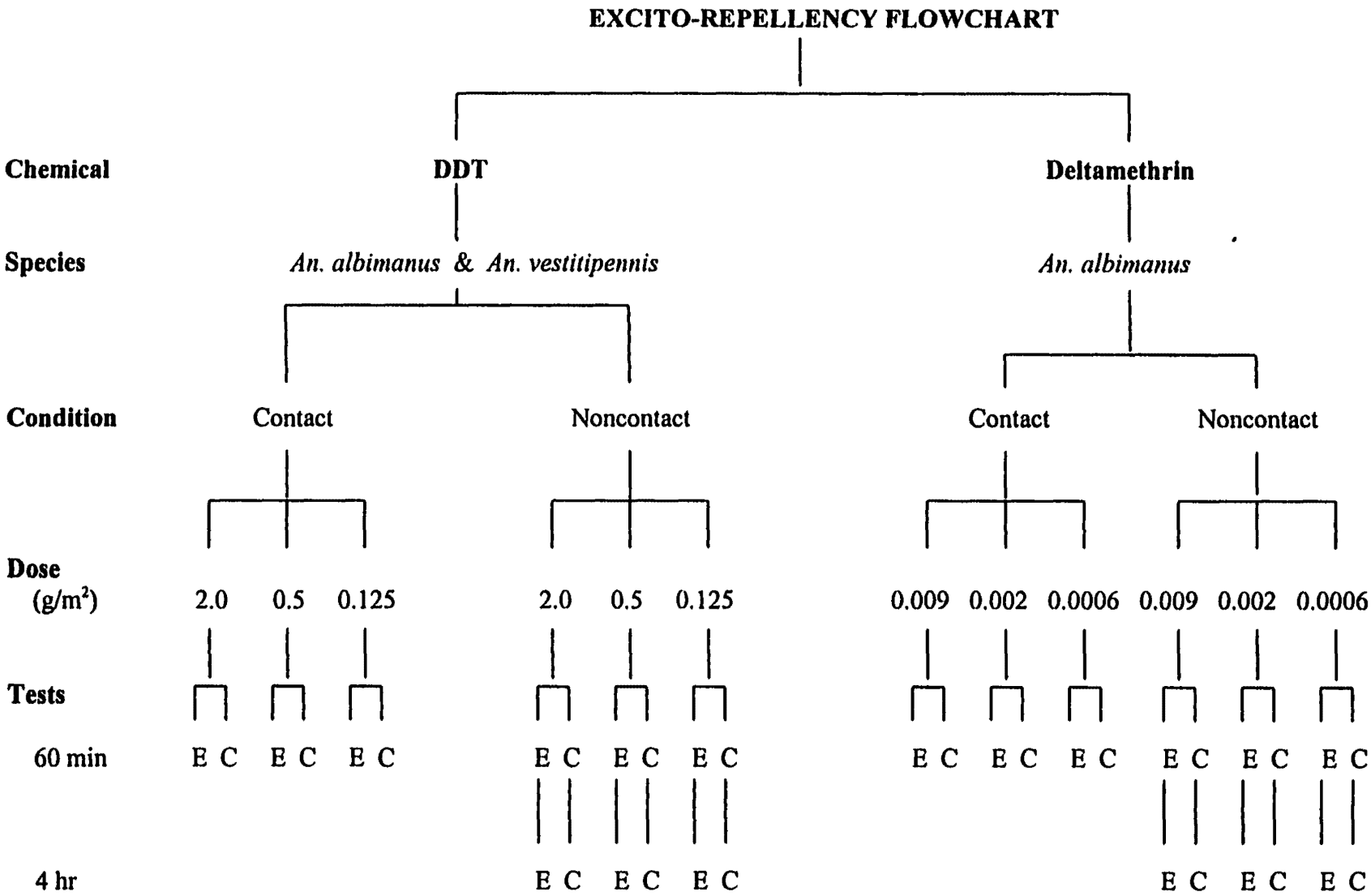

EXCITO-REPELLENCY FLOWCHART
Chemical
DDT
Deltamethrin
Species
An. albimanus & An. vestitipennis
An. albimanus
Condition
Contact
Noncontact
Contact
Noncontact
Dose
(g/m²)
2.0 0.5 0.125
2.0 0.5 0.125
0.009 0.002 0.0006
0.009 0.002 0.0006
Tests
60 min
E C E C E C
E C E C E C
E C E C E C
E C E C E C
4 hr
E C E C E C
E C E C E C

Figure 2. Flow diagram of excito-repellency study conditions for contact and noncontact tests listed by chemical, *Anopheles* species, condition, dose, and exposure time. C= paired control; E= insecticide exposure.

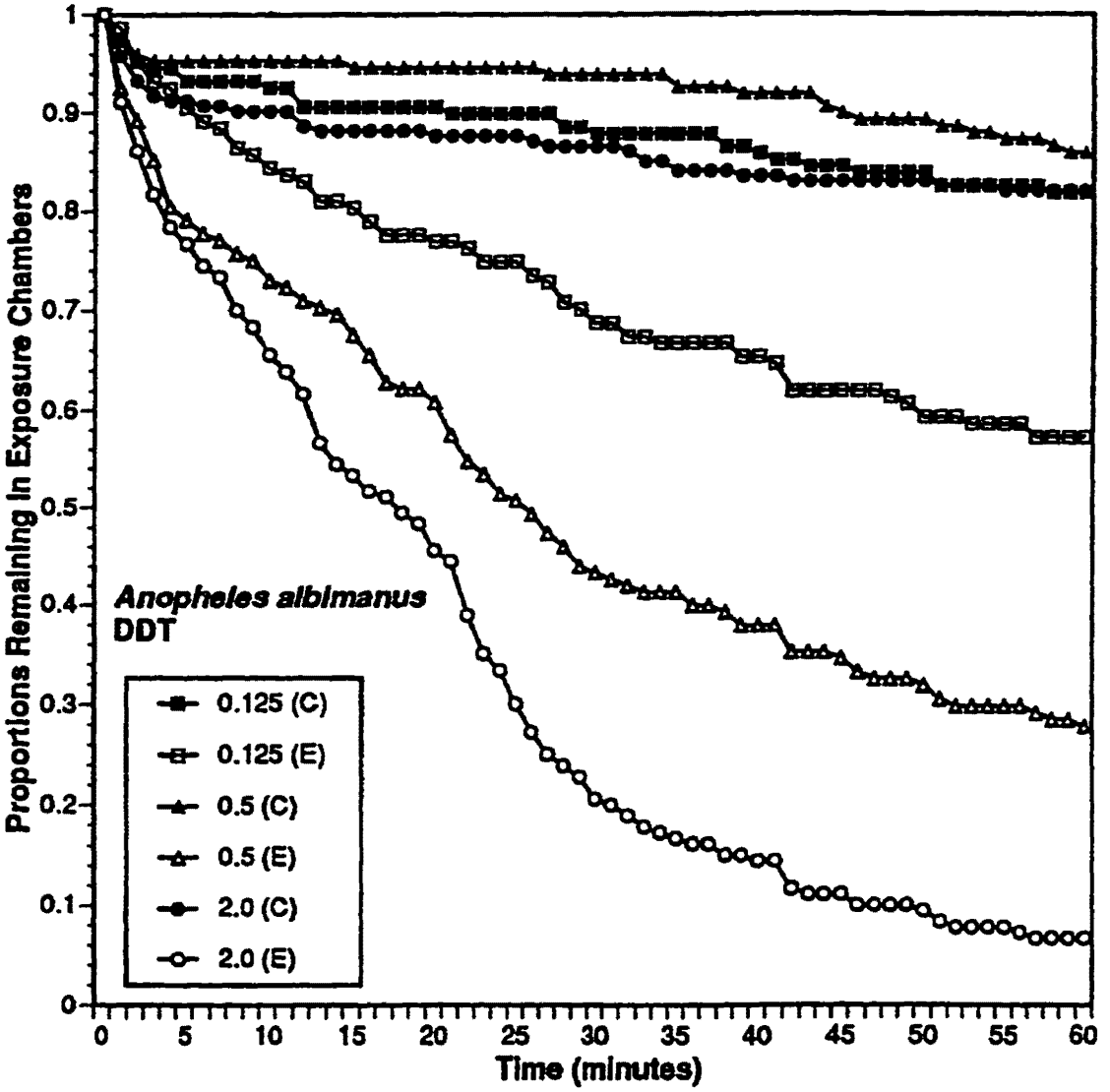


Figure 3. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. control trials with 3 different concentrations of DDT (gm/m^2) during 60-min exposure. (C) Control; (E) Exposure to insecticide.

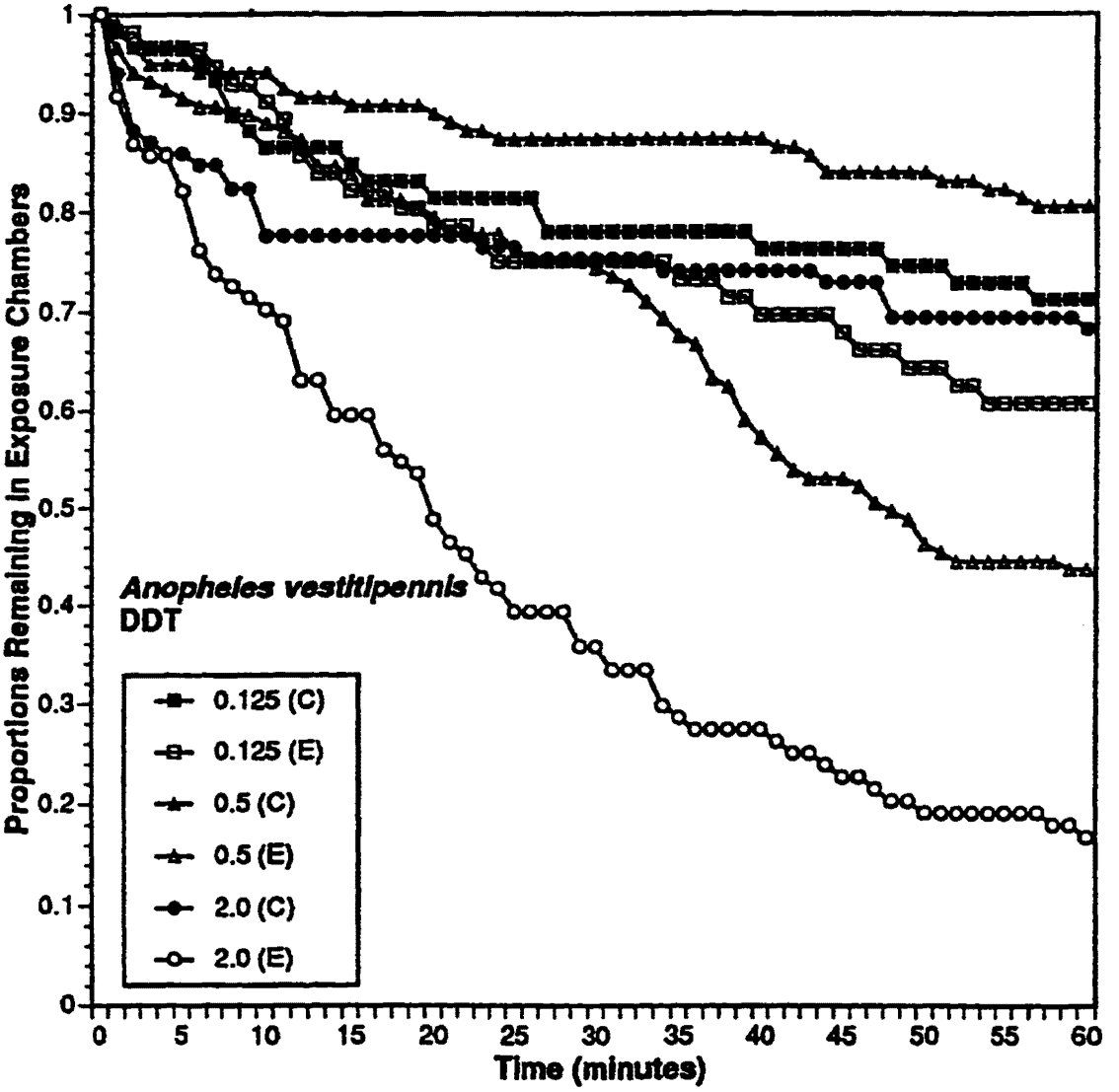


Figure 4. Proportions of *Anopheles vestitipennis* females remaining in exposure chambers in contact vs. control trials with 3 different concentrations of DDT (gm/m^2) during 60-min exposure. (C) Control; (E) Exposure to insecticide.

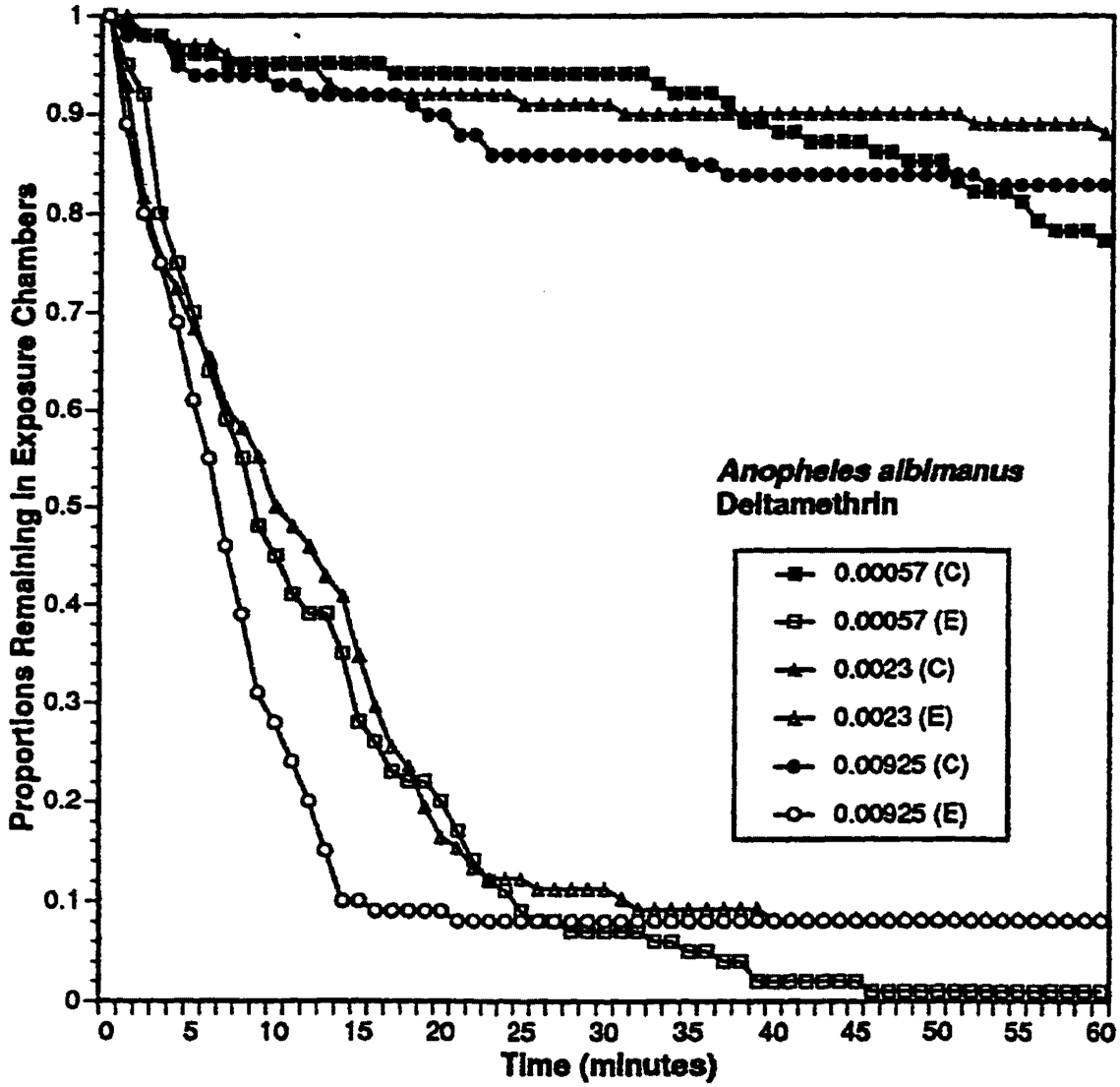


Figure 5. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. control trials with 3 different concentrations of deltamethrin (gm/m^2) during 60-min exposure. (C) Control; (E) Exposure to insecticide.

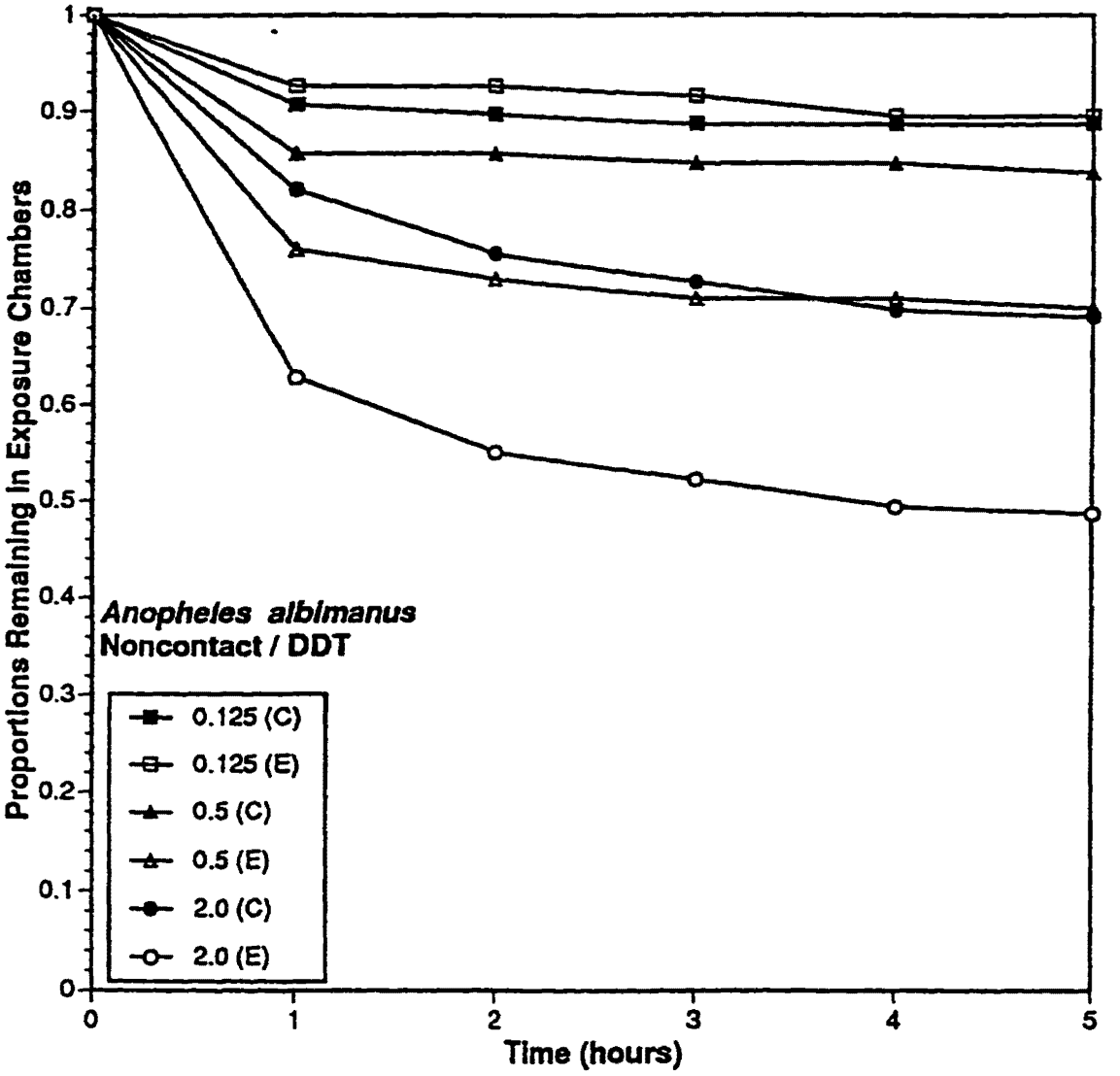


Figure 6. Proportions of *Anopheles albimanus* females remaining in exposure chambers in noncontact vs. control trials with 3 different concentrations of DDT (gm/m^2) during 5-hr exposure. (C) Control; (E) Exposure to insecticide.

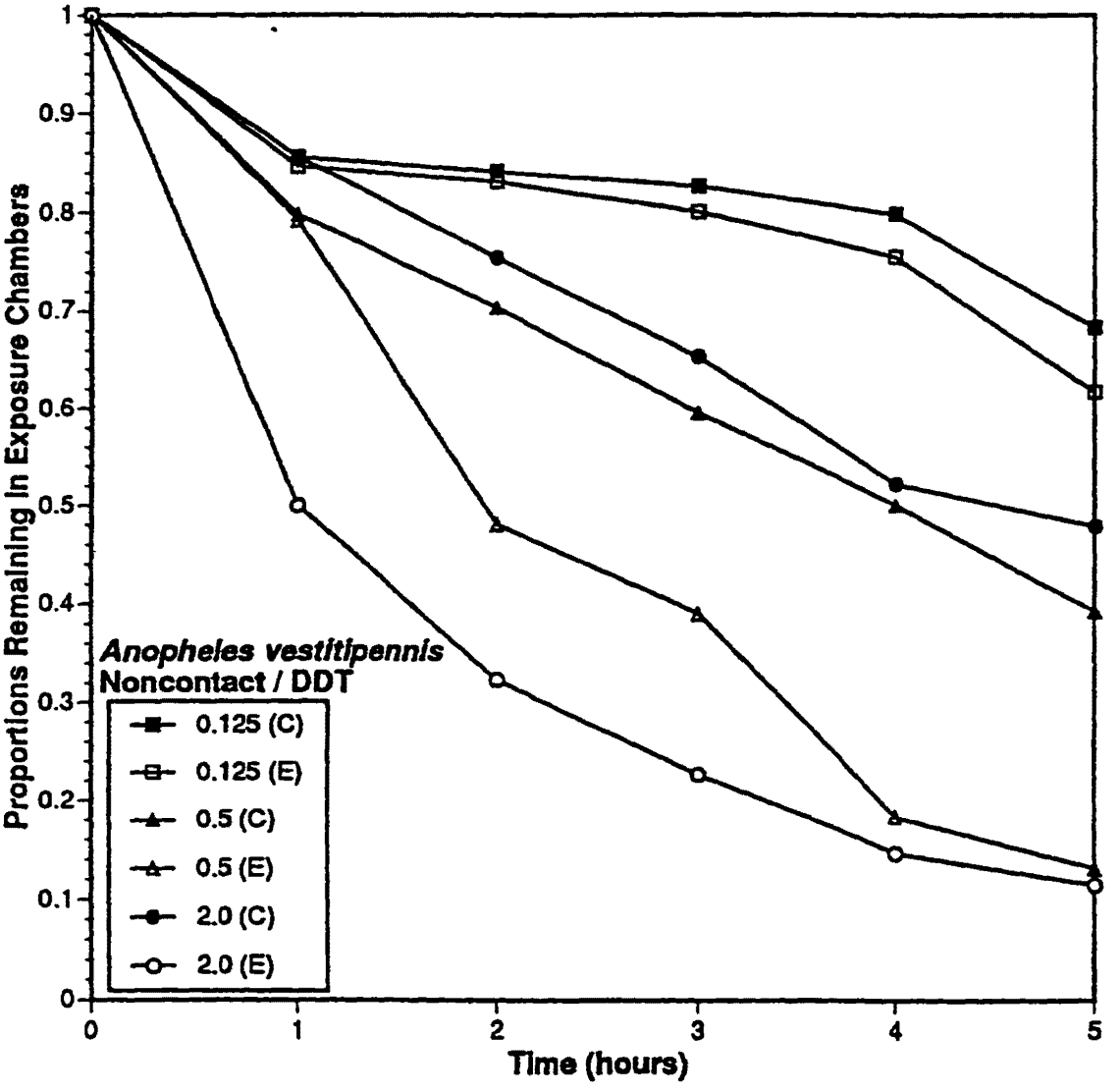


Figure 7. Proportions of *Anopheles vestitipennis* females remaining in exposure chambers in noncontact vs. control trials with 3 different concentrations of DDT (gm/m^2) during 5-hr exposure. (C) Control; (E) Exposure to insecticide.

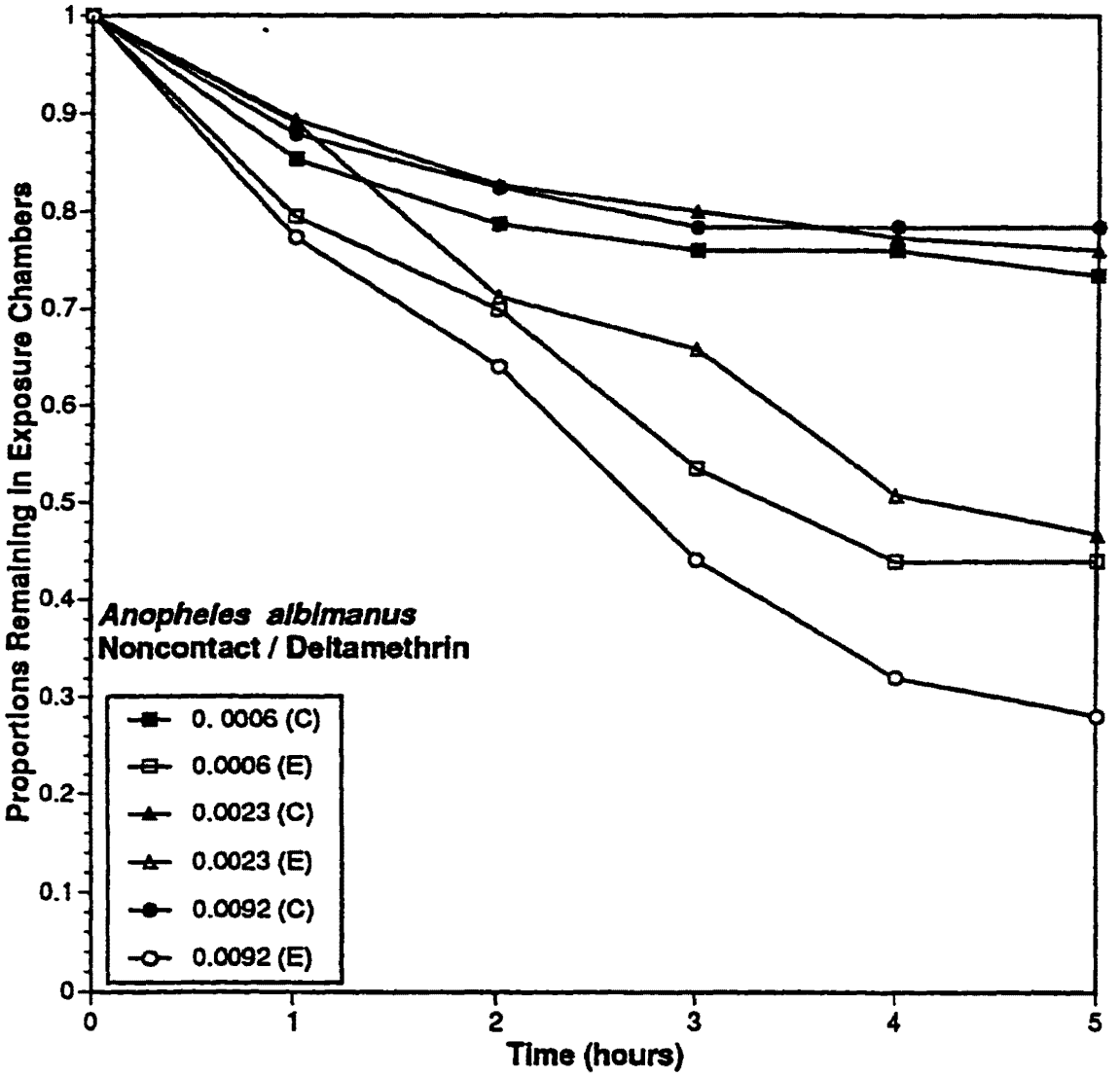


Figure 8. Proportions of *Anopheles albimanus* females remaining in exposure chambers in noncontact vs. control trials with 3 different concentrations of deltamethrin (gm/m^2) during 5-hr exposure. (C) Control; (E) Exposure to insecticide.

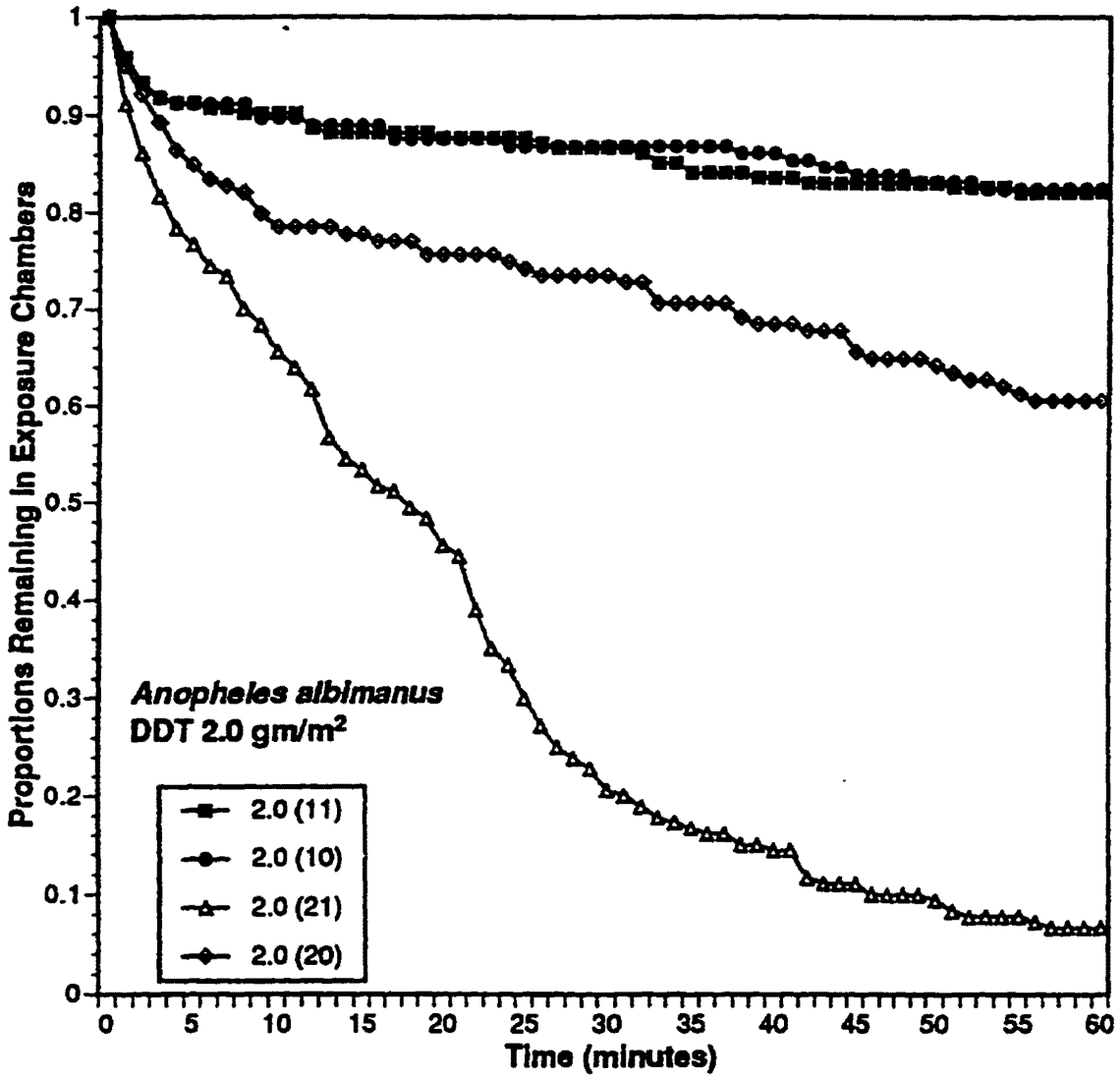


Figure 9. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 2 gm/m^2 DDT during 60-min exposure. (11= Contact Control; 10= Noncontact Control; 21= Contact; 20= Noncontact).

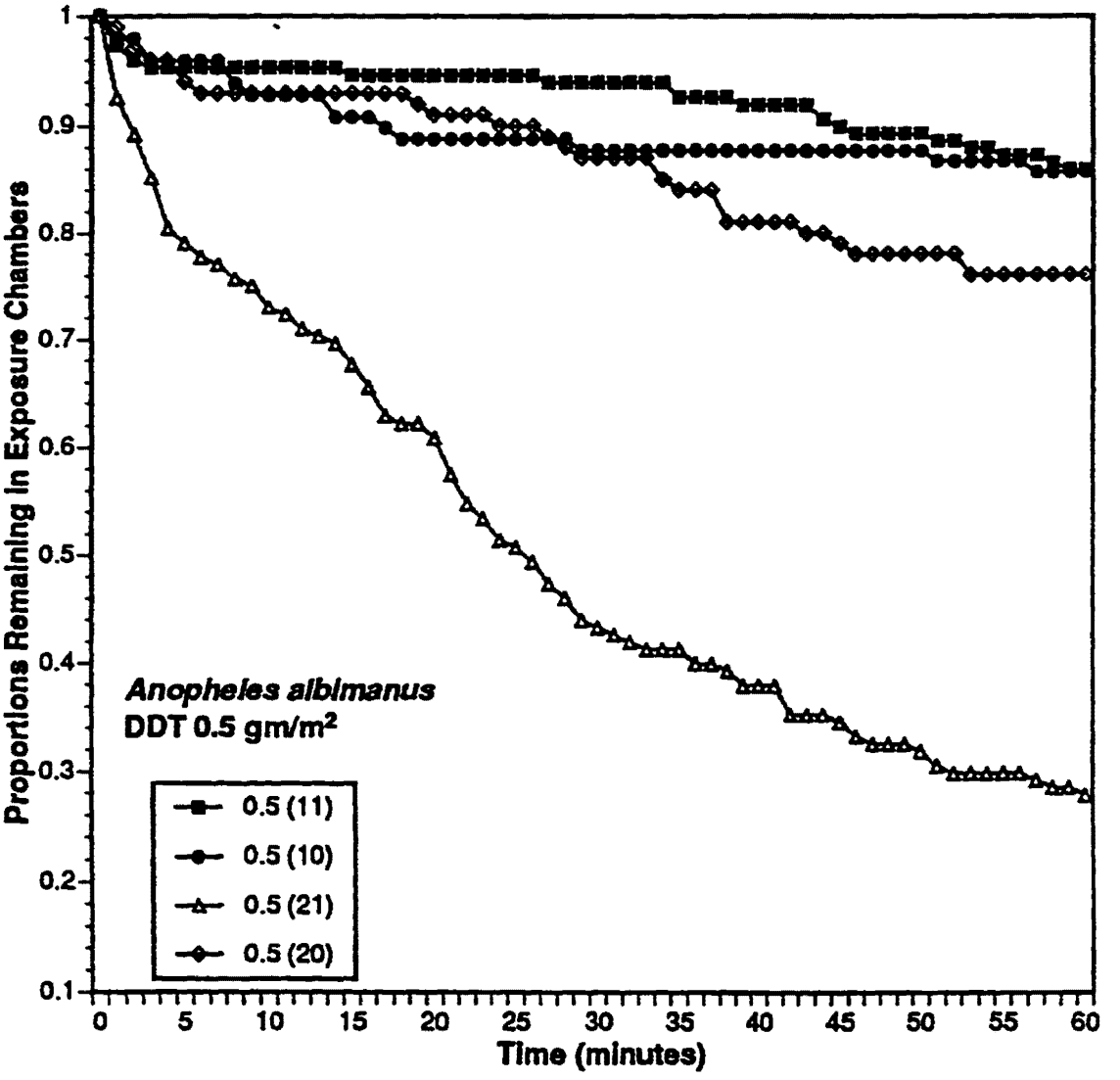


Figure 10. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 0.5 gm/m^2 DDT during 60-min exposure. (11= Contact Control; 10= Noncontact Control; 21= Contact; 20= Noncontact).

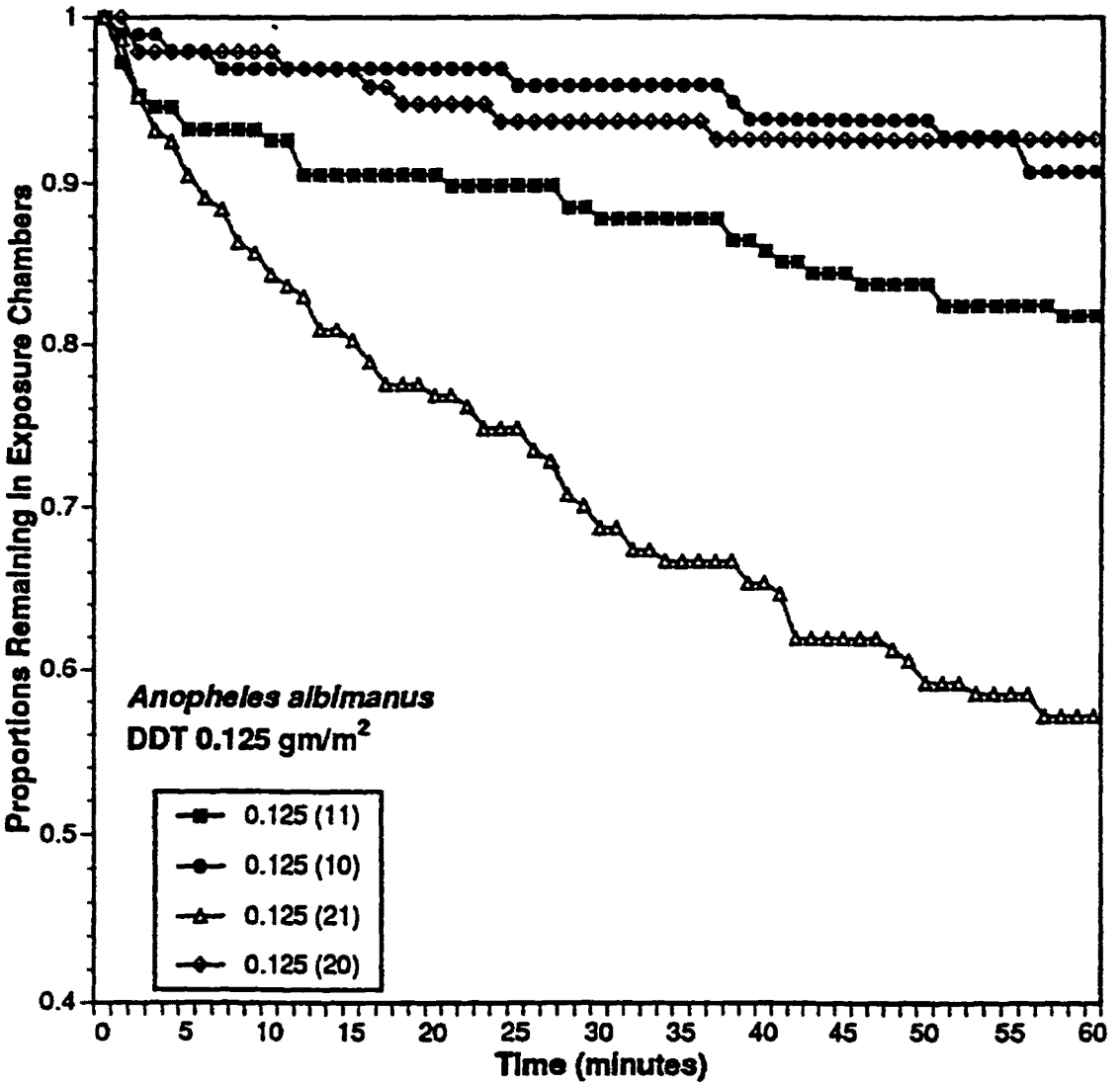


Figure 11. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 0.125 gm/m^2 DDT during 60-min exposure. (11= Contact Control; 10= Noncontact Control; 21= Contact; 20= Noncontact).

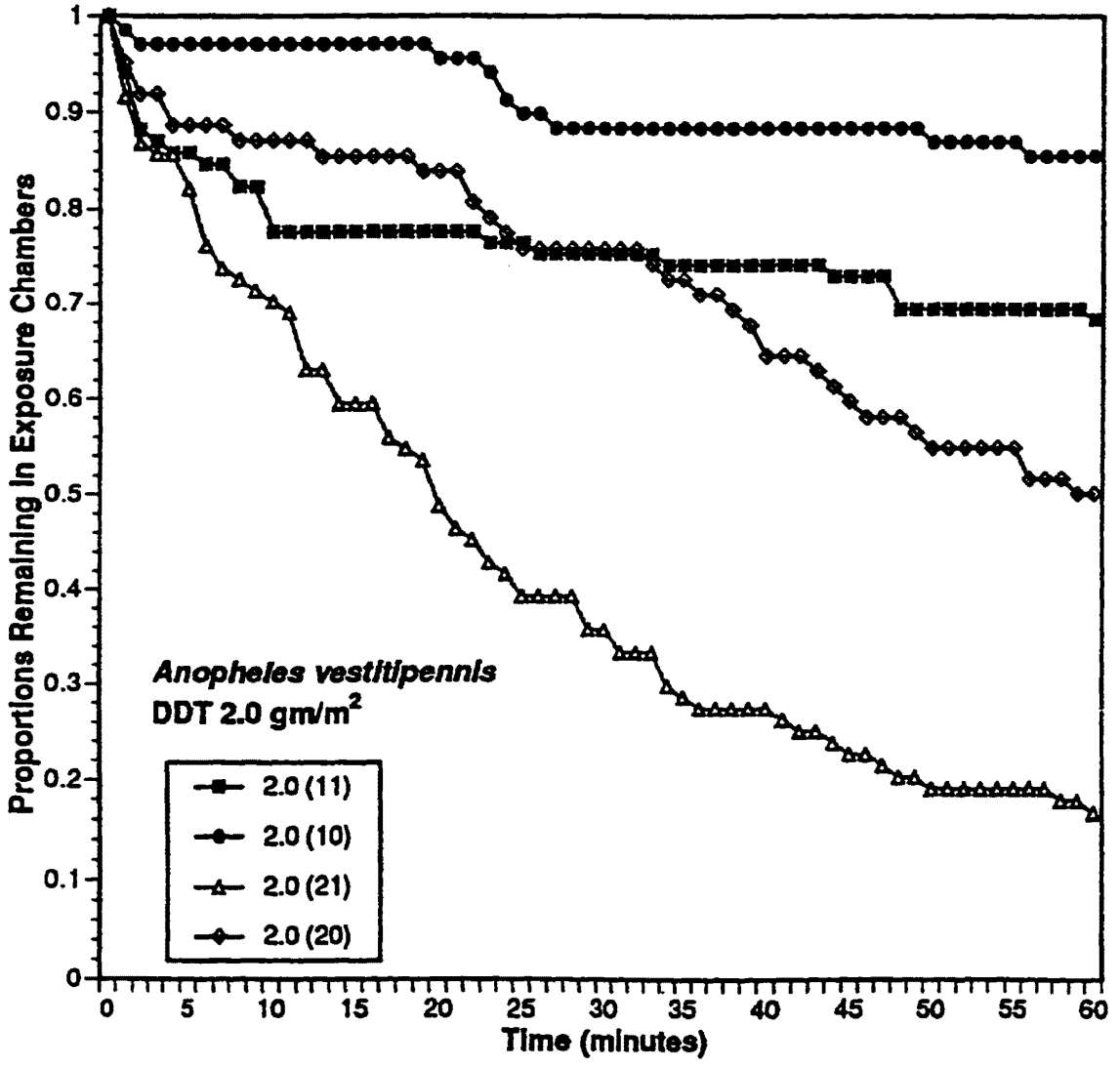


Figure 12. Proportions of *Anopheles vestitipennis* females remaining in exposure chambers in contact vs. noncontact trials with 2 gm/m^2 DDT during 60-min exposure. (11= Contact Control; 10= Noncontact Control; 21= Contact; 20= Noncontact).

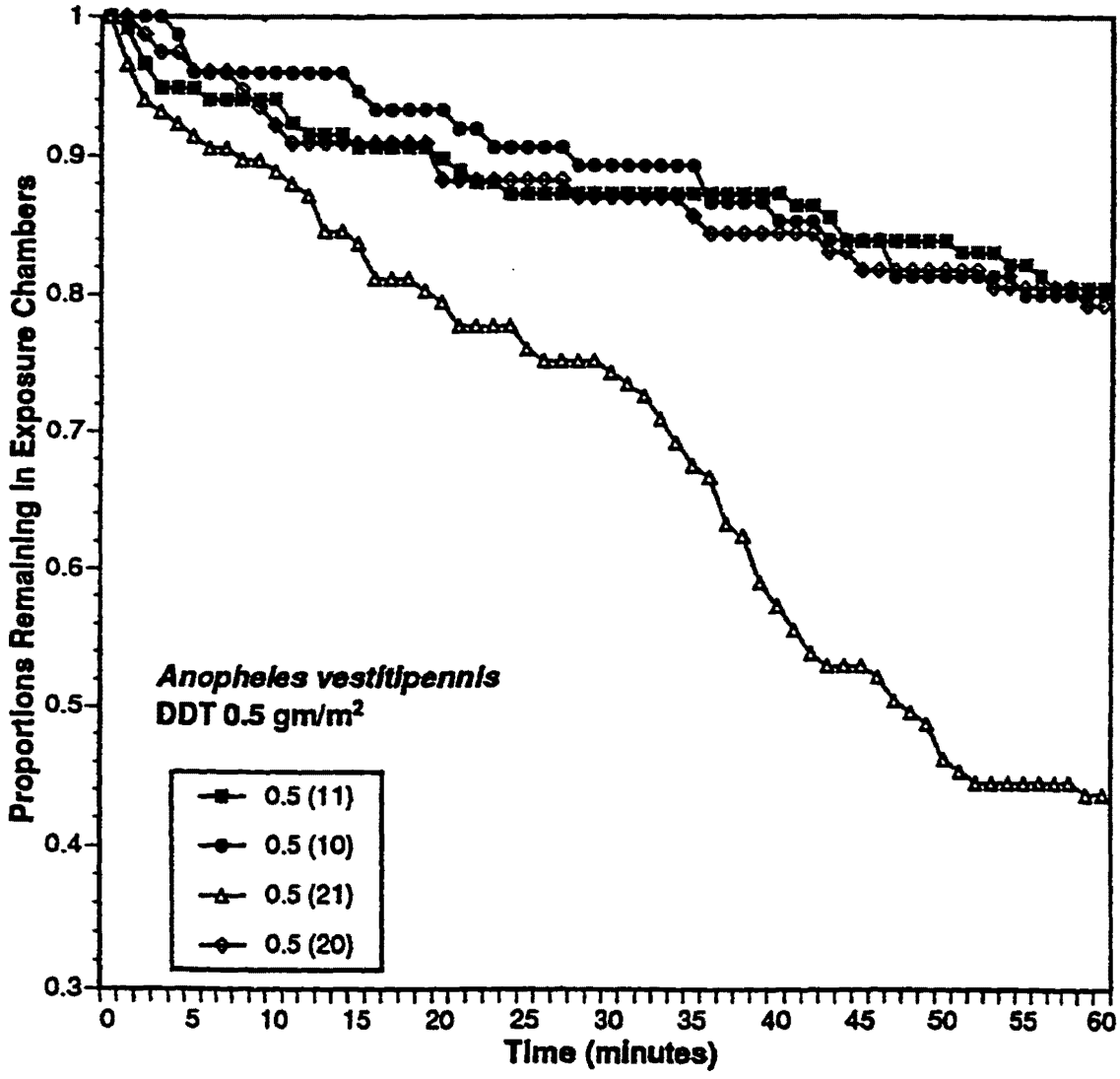


Figure 13. Proportions of *Anopheles vestitipennis* females remaining in exposure chambers in contact vs. noncontact trials with 0.5 gm/m^2 DDT during 60-min exposure. (11= Contact Control; 10= Noncontact Control; 21= Contact; 20= Noncontact).

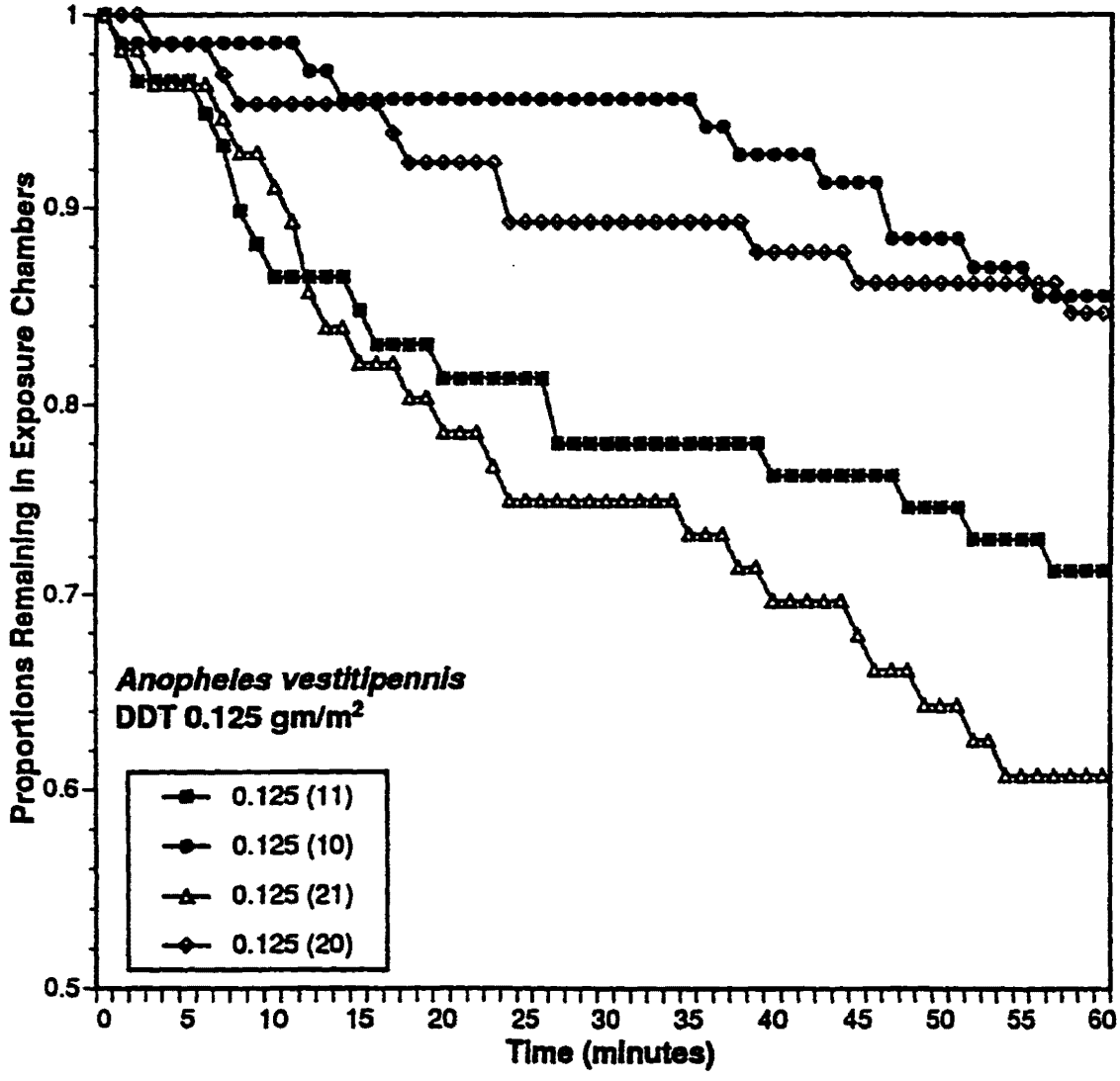


Figure 14. Proportions of *Anopheles vestitipennis* females remaining in exposure chambers in contact vs. noncontact trials with 0.125 gm/m^2 DDT during 60-min exposure. (11= Contact Control; 10= Noncontact Control; 21= Contact; 20= Noncontact).

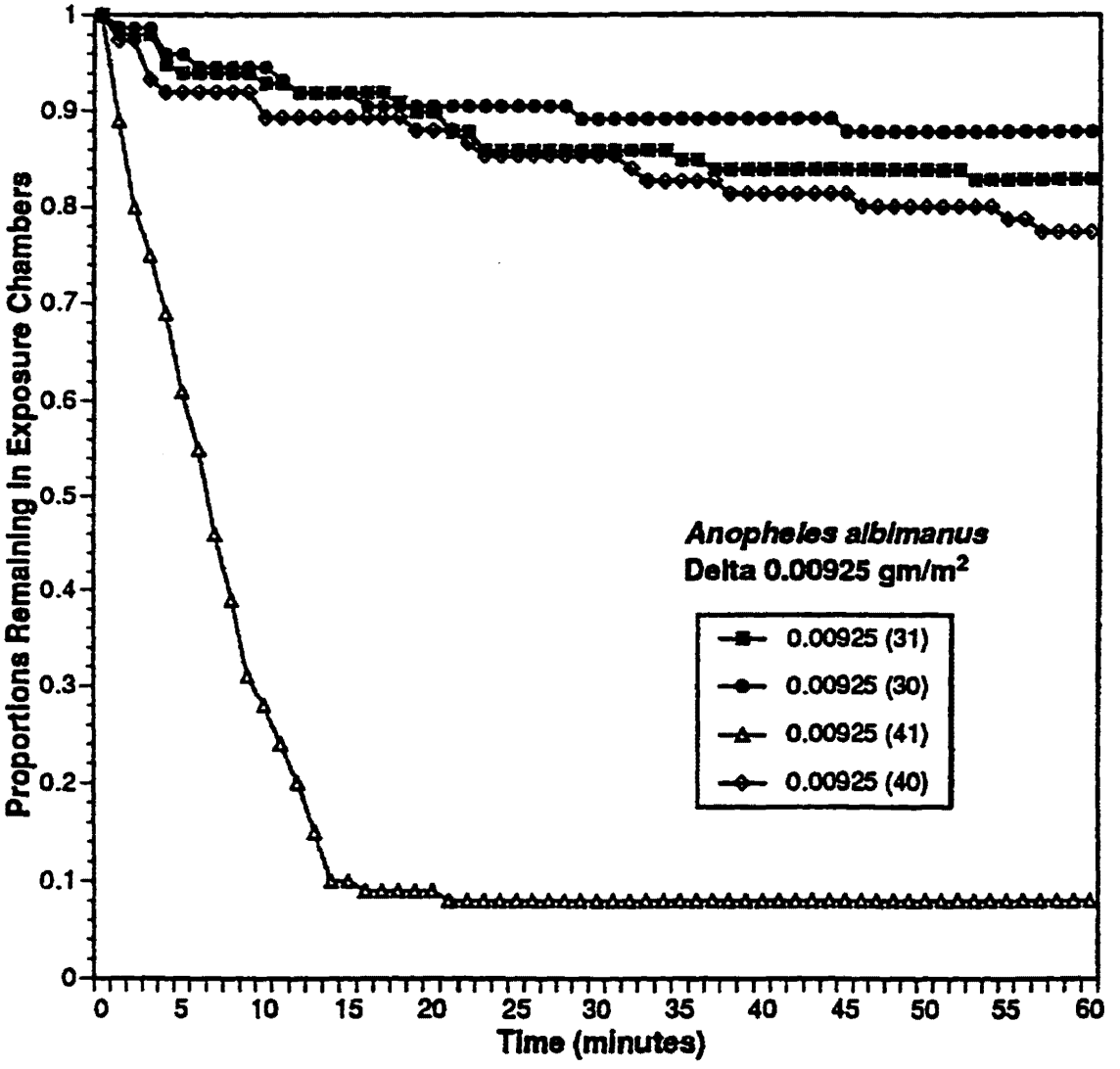


Figure 15. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 0.00925 gm/m^2 deltamethrin during 60-min exposure. (31= Contact Control; 30= Noncontact Control; 41= Contact; 40= Noncontact).

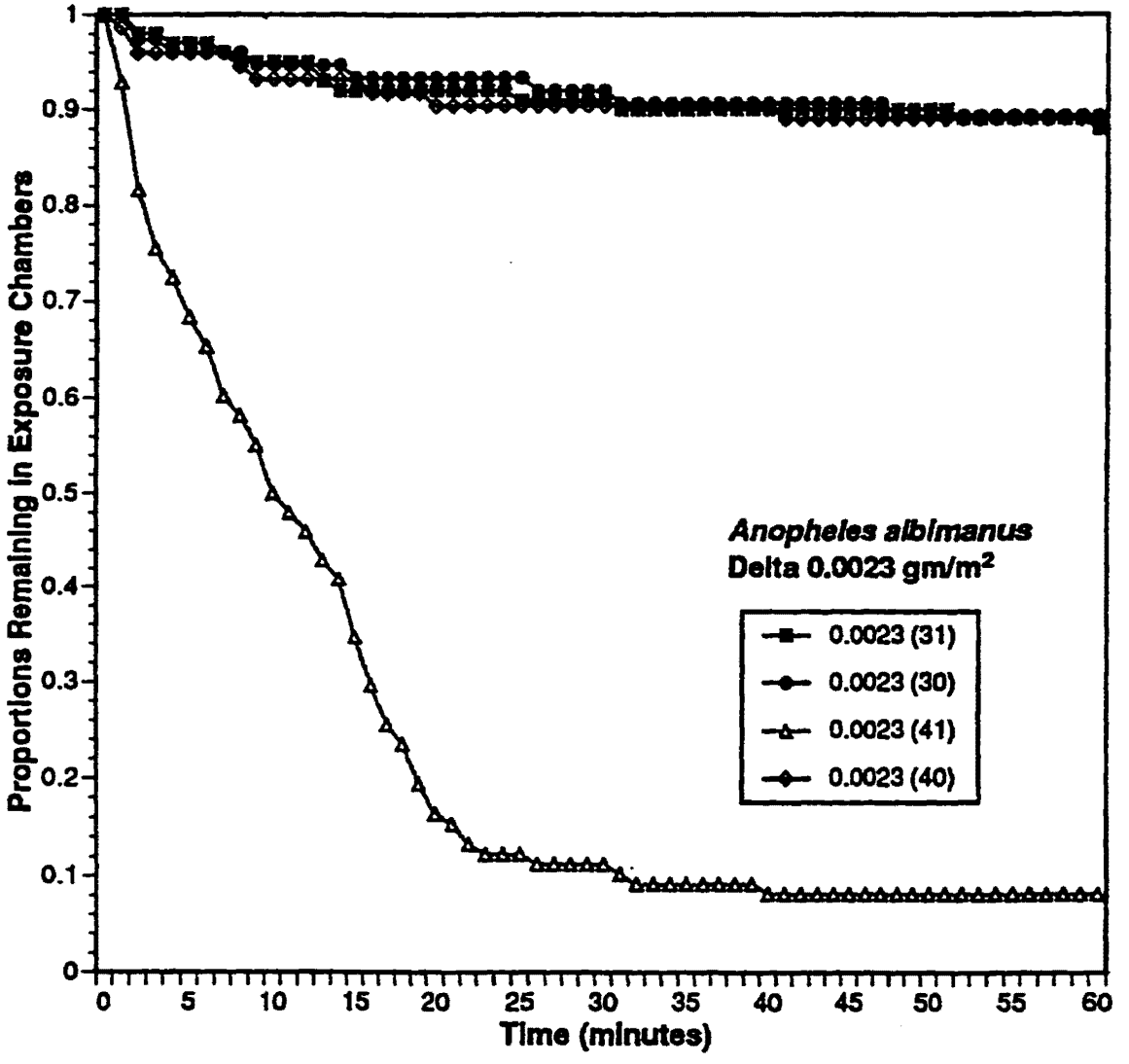


Figure 16. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 0.0023 gm/m^2 deltamethrin during 60-min exposure. (31= Contact Control; 30= Noncontact Control; 41= Contact; 40= Noncontact).

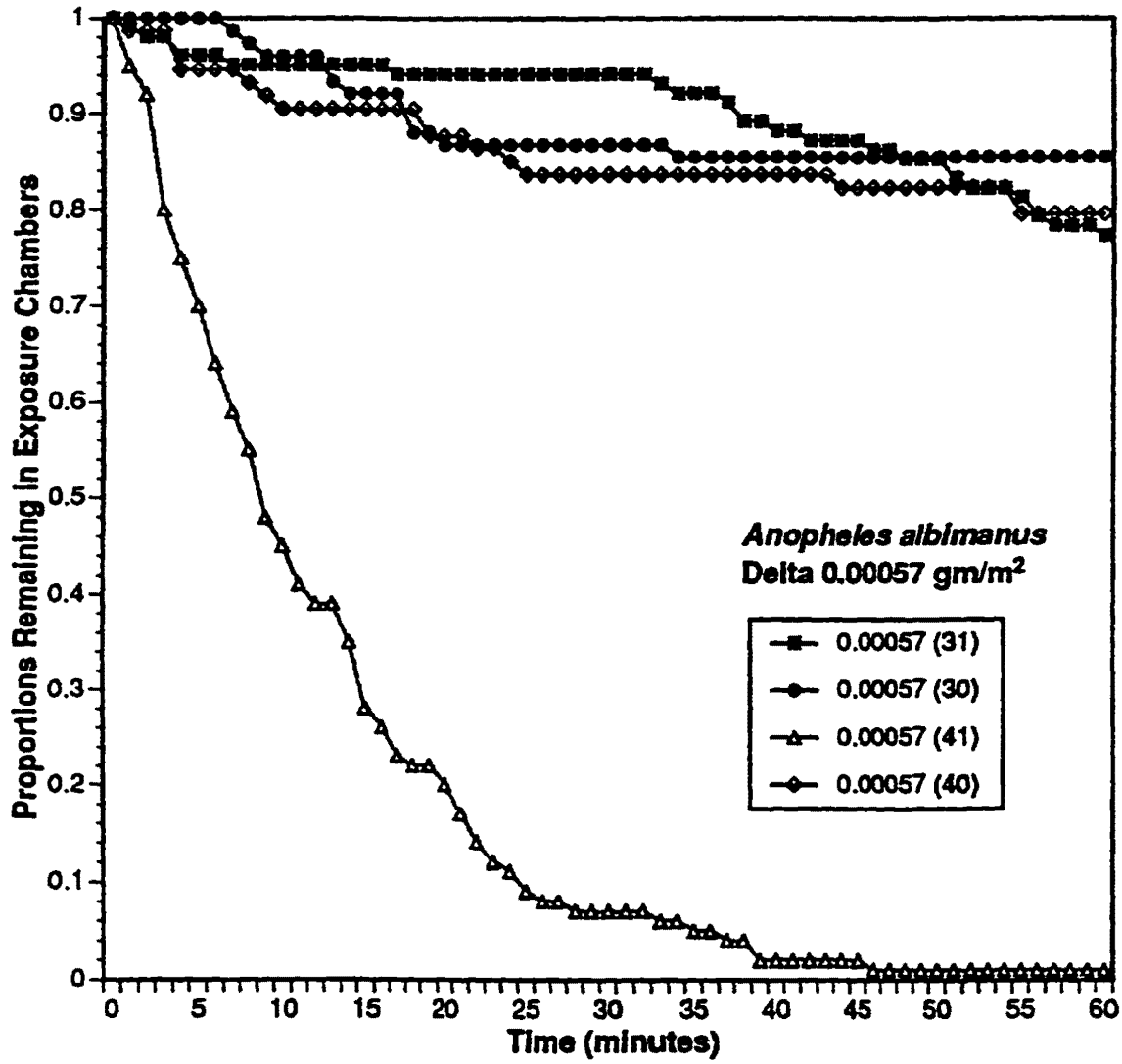


Figure 17. Proportions of *Anopheles albimanus* females remaining in exposure chambers in contact vs. noncontact trials with 0.00057 gm/m^2 deltamethrin during 60-min exposure. (31= Contact Control; 30= Noncontact Control; 41= Contact; 40= Noncontact).

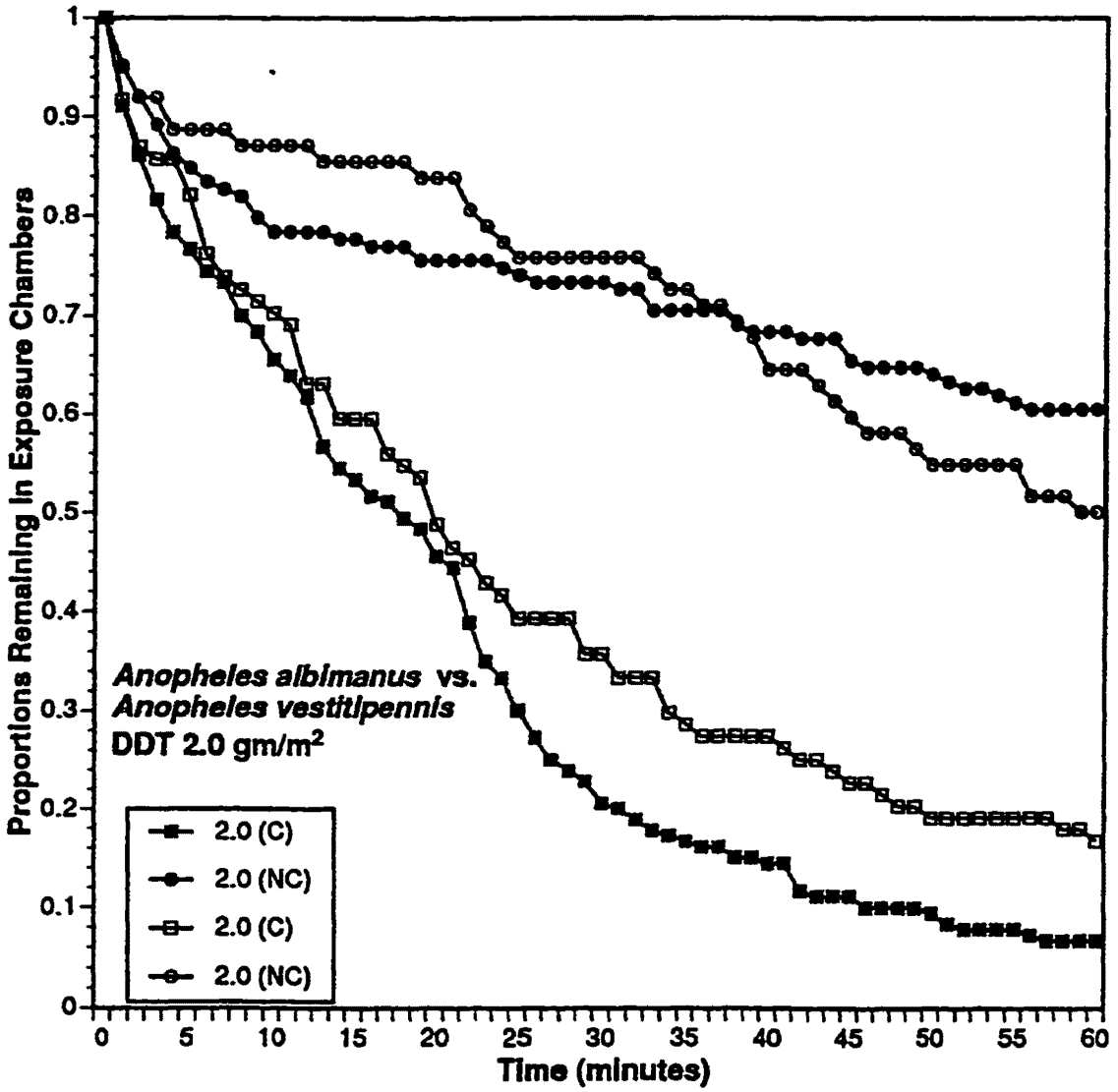


Figure 18. Proportions of *Anopheles albimanus* (●■) vs. *Anopheles vestitipennis* (○□) females remaining in exposure chambers in contact (C) and noncontact (NC) trials with 2 gm/m^2 during 60-min exposure.

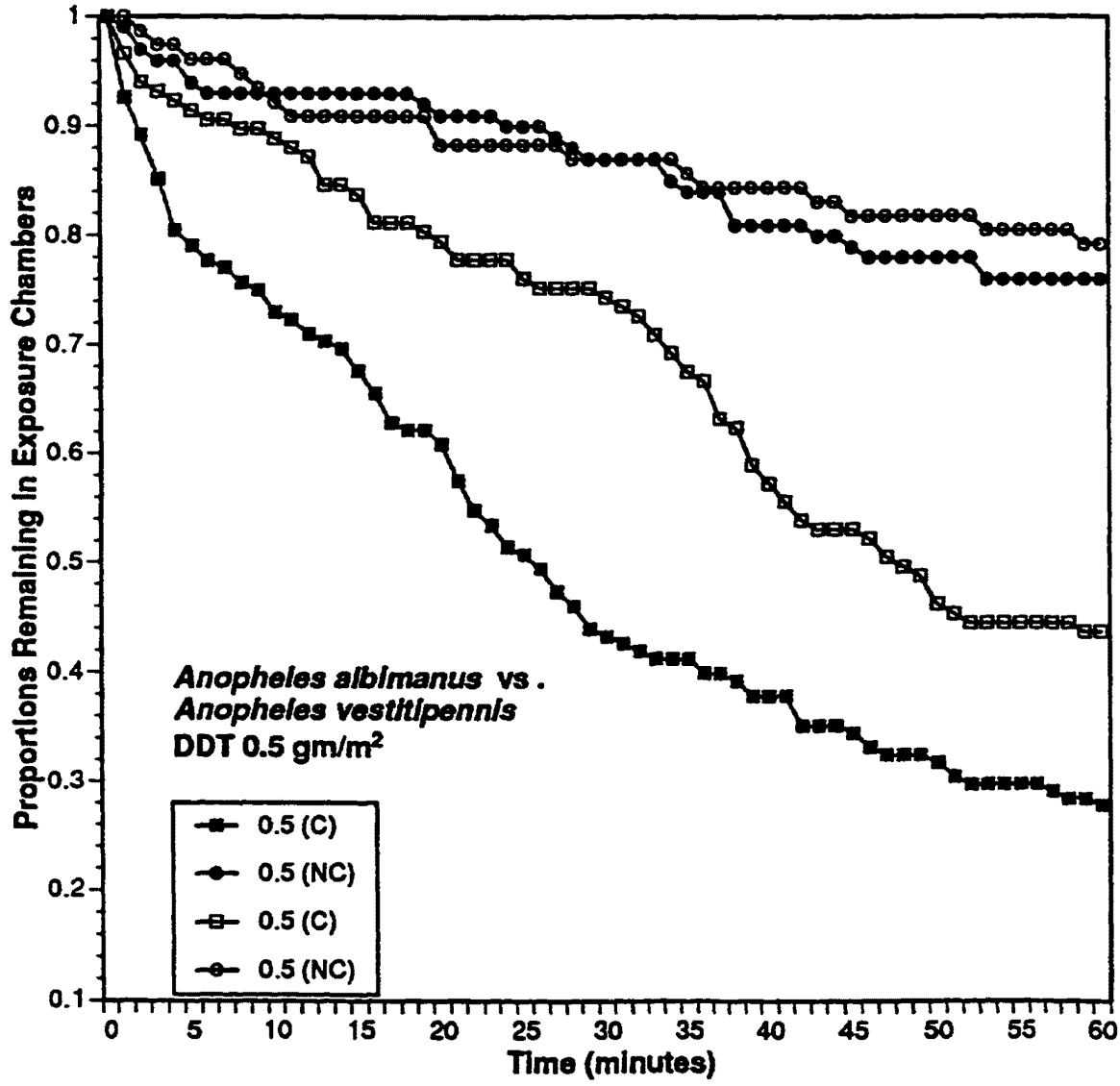


Figure 19. Proportions of *Anopheles albimanus* (●■) vs. *Anopheles vestitipennis* (○□) females remaining in exposure chambers in contact (C) and noncontact (NC) trials with 0.5 gm/m^2 during 60-min exposure.

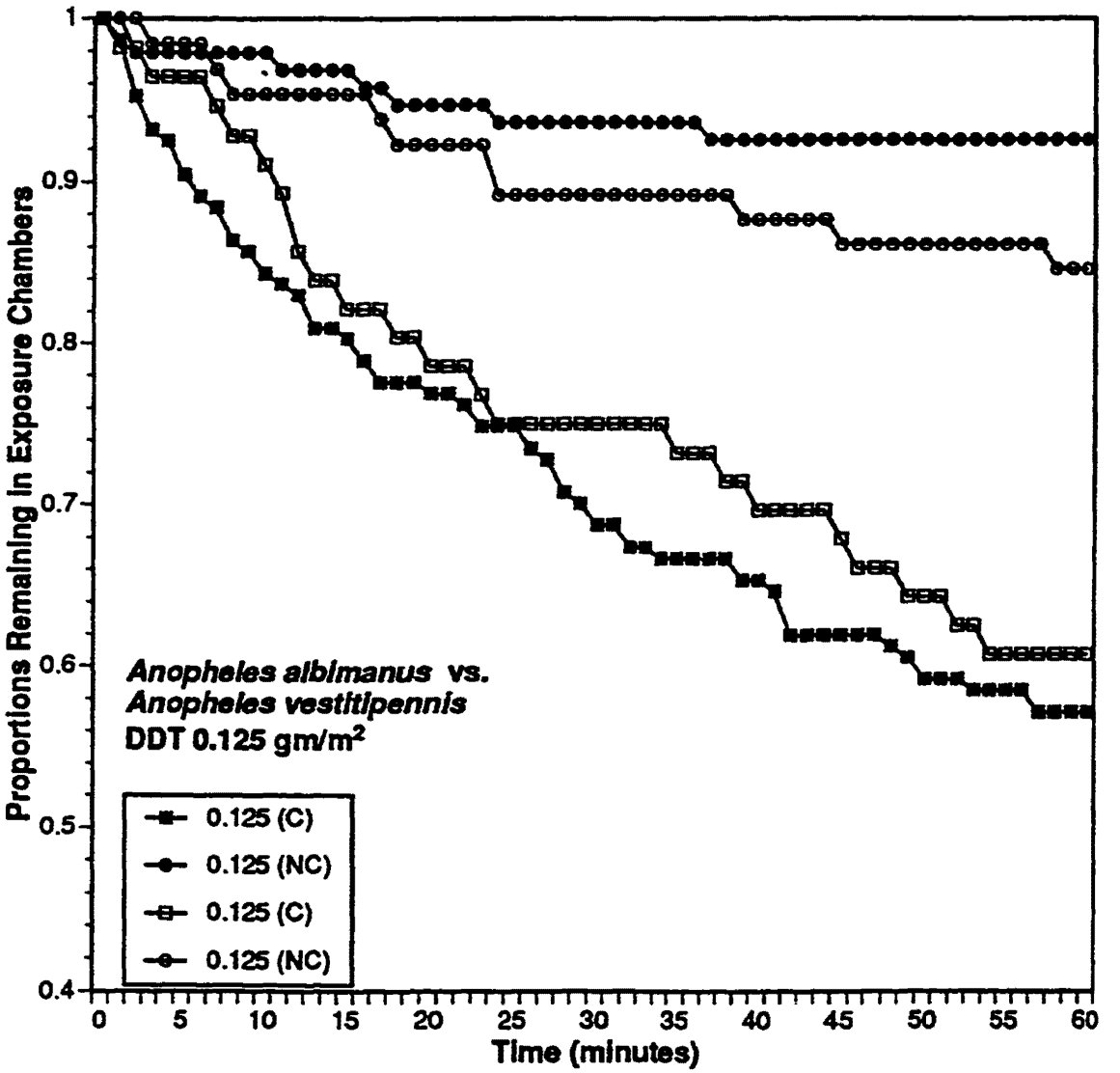


Figure 20. Proportions of *Anopheles albimanus* (●■) vs. *Anopheles vestitipennis* (○□) females remaining in exposure chambers in contact (C) and noncontact (NC) trials with 0.125 gm/m^2 during 60-min exposure.

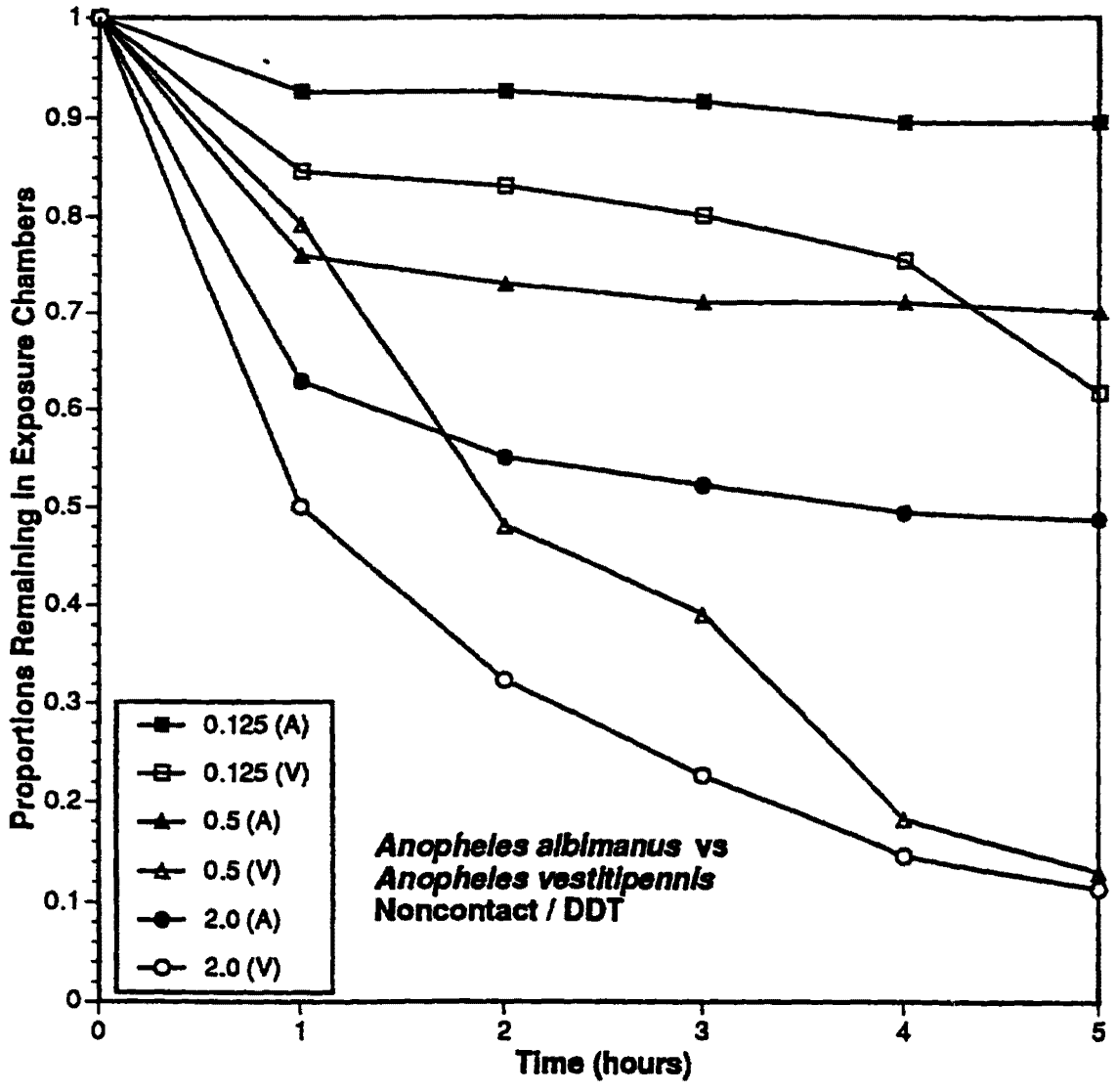


Figure 21. Proportions of *Anopheles albimanus* (A) vs. *Anopheles vestitipennis* (V) females remaining in exposure chambers in noncontact trials with 3 different concentrations of DDT (gm/m^2) during 5-hr exposure.

CHAPTER 4

Behavioral response of *Anopheles albimanus* and *Anopheles vestitipennis* (Diptera: Culicidae) to DDT using experimental huts in Caledonia, northern Belize, Central America

ABSTRACT

A series of experiments, using experimental huts, examined the behavioral response of native populations of *Anopheles* and *Mansonia dyari* to indoor applied residual DDT insecticide. Field trials were conducted in Caledonia, northern Belize, from September 1995 to May 1996. Two identically constructed huts were used for comparing mosquito behavior during pre- and post-DDT treatment periods, one hut sprayed with 4% DDT ($2gm/m^2$), the other hut serving as an unsprayed control. This represents the first use of experimental huts in Belize for vector studies.

The principal method of mosquito data collection was human-landing catches (HLC). Pre-spray evaluations were made on both huts. Despite the fenestrated walls and open eaves, mosquito collections indicated walls and eaves generally contributed far less to hut access; whereas, open windows appeared to offer a much greater opportunity or preference for mosquitoes to gain entry indoors. Overall, paired hut collections, under identical conditions for indoor access, indicated pre-treatment huts were comparable in attracting indoor mosquito populations. Thirty-one series of pre- and post-treatment HLCs, using one or both huts, clearly showed the *Anopheles albimanus* population to be predominantly exophagic (indoor/outdoor ratio). *Anopheles vestitipennis* appeared much more endophagic, more readily entering the huts to feed on humans. Although a few *An. albimanus* were found in advanced ovarian development during daytime resting collections within the pre-treated huts, this species was predominantly exophilic. *An. vestitipennis* was exclusively exophilic. In general, for all species, outdoor activity was greatest during the first half of the evening. Depending on the species, host-seeking

activity continually declined at various rates to a low between 0300-0500 hr. Only *An. albimanus* showed a consistent bimodal distribution pattern, with a second, lesser peak of activity near the dawn hours. Fluctuating periods of wet and dry season precipitation and natural changing length of photoperiods appeared to influence population densities and activity patterns.

Anopheles albimanus usually presented a distinct bimodal biting pattern during most collection trials, with peak activity during the first 2-3 hours after sunset and another smaller peak 1-hr to sunrise. *An. vestitipennis* generally began increased feeding activity around 2-3 hours after sunset and continued host-seeking throughout the night. Unlike *An. albimanus*, most *An. vestitipennis* had a definite pre-feeding rest period, varying from outdoor walls, nearby low trees and bushes, and indoor wall surfaces before attacking humans. Most anophelines, found resting before or after feeding, preferred to rest on indoor walls at heights below 1 meter. Rarely were specimens seen above this height until after blood feeding. Engorged females would primarily rest either on the lower walls (e.g., *An. vestitipennis*, *An. crucians*) or walls and the lower half of the thatch ceiling *(An. albimanus)* for periods 15 min to all night. Virtually all anophelines departed the huts at sunrise, only a few remaining up to 1000 hr in the morning. Most daytime indoor resting anophelines and culicines were located on the ceiling thatch at all levels.

Comparison of DDT-treated and unsprayed (control) huts were made using both HLCs and window exit collection boxes. Observations were carried out over 3 periods, at approximately 1, 6, and 12 weeks. Although, 6-hr collection periods did detect differences in mosquito behavior, 12 and 13-hr collections provided stronger, more insightful patterns of activity. Entry/exit window traps and modified interception boxes

were found to be insensitive methods of collecting house-frequenting *Anopheles* adults in Caledonia. Patterns of biting activity in the control hut remained reasonably consistent with pre-spray observations. Early evening (1800-2100 hr) feeding patterns within the sprayed hut remained similar to the control. However, normal indoor resting behavior on wall surfaces was greatly diminished in the sprayed hut, while apparently unaffected in the control. Females entering the treated hut had greatly reduced pre-feeding resting times on walls. Biting in the sprayed hut declined markedly after 2300 hr resulting in lower biting collections compared to the control. Overall, the number of entering and attacking *Anopheles* mosquitoes over the entire evening was significantly less than that seen in the control hut. No adult anophelines were found resting during the daytime in the sprayed hut. This investigation supports the historically documented importance of irritant and repellent properties of DDT that provides important and effective suppressive components in defense against malaria. Epidemiologically, the combination of HLCs, sporozoite ELISA, and blood-meal analysis indicate *An. albimanus* and *An. vestitipennis* should be regarded as important vectors in Caledonia, especially during periods of higher biting densities. Additional data and information is provided on various aspects of adult *Anopheles* bionomics and behavior in relation to DDT exposure, malaria epidemiology and vector biology.

INTRODUCTION

"The effective application of any control measure must be based on an adequate understanding of the ecology, biology and behaviour of the target species" **WHO, 1992.**

*"The region [*Americas*] as a whole stands in need of systematic observations on the times and places of man-mosquito contact and their modification in presence of insecticides"* **R. Elliott, 1969.**

"The truly objective interpretation of behaviour is a matter of great difficulty, demanding insight and scientific detachment of a high order or, at best, a gradual stepwise approach unlikely to commend itself to those in search of quick results." **P.F. Mattingly, 1962.**

Malaria remains the most important and pervasive vector-borne disease in the tropical and subtropical world, and the expended resources and efforts to fight it have been waning for decades (Krogstad, 1996; Olliaro et al.1996; Campbell, 1997). For over a century, control programs, sometimes on a grand scale, have been waged against the disease and its destructive power on human health and spirit (Bruce-Chwatt, 1970; Harrison, 1978). Historically, the most successful malaria control interventions have been directed against the vector (Russell, et al. 1963). In particular, the application of DDT residues to interior house walls has been cited as the major reason for the overall success of malaria control in the 1950s and 1960s (WHO, 1995). In general, wherever DDT has been used, malaria has subsided. The once highly organized, well-funded

efforts effectively pushed malaria back into warmer climes where it remains deeply entrenched in many subtropical and tropical countries today. Despite the alarming trends of resurgent malaria that accompany declining house spray rates (WHO, 1997), indoor residual treatment in homes with insecticides continues to play an important and successful role in malaria control worldwide (Roberts, et al. 1997a).

Many aspects of vector biology, including mosquito behavior, are significant components of disease transmission (Mattingly, 1962; Klowden, 1996). Unfortunately, relative to many regions of the world, the literature on the bionomics of malaria vectors in the Neotropics remains scanty and geographically fragmented (Elliott, 1969; Zimmerman, 1992). Mosquito behavior, in particular, is often overlooked for study, or considered an impediment (e.g., exophily) to control. Understanding behavior and habits of disease vectors are essential components to the malaria transmission equation and for providing estimates of risk to human populations (Macdonald, 1957). The potential effects of insecticides on altered behavioral responses of mosquitoes are also critical in the understanding and control of vector-borne diseases (Mattingly, 1962; de Zulueta & Cullen, 1963; de Zulueta, 1964, Hamon, et al. 1970; Elliott, 1972; Elliott & de Zulueta, 1975; Gillies, 1988). The various altered responses of mosquitoes to certain chemicals have been recognized for decades (Ribbands, 1946; Kennedy, 1946; Tarzwell & Fisk, 1947; Muirhead-Thomson, 1950; Downs & Bordas, 1951; Muirhead-Thomson, 1960). The phenomenon of excito-repellency, especially irritancy after contact with a chemical, has generated both interest and controversy in terms of measurement, mechanisms of genetic expression, interpretation and significance in effective control of vectors and/or disease (Gabaldon, 1953; Muirhead-Thomson, 1960; de Zulueta, 1962; Coluzzi, 1963;

Busvine, 1964; Roberts, 1993; Roberts & Andre, 1994). Because of the strong excito-repellency effect of DDT residues, some specialists had considered this property to be an important, or at least probable, obstacle to vector and malaria control (Muirhead-Thomson, 1951; Bruce-Chwatt, 1970). Allegations have been made suggesting irritability may have been responsible for continued transmission in some areas (de Zulueta & Garrett-Jones, 1965; Elliott, 1968). However, others have advocated a serious reexamination of this premise in light of the facts (Roberts & Andre, 1994; Roberts, et al. 1997a). Contrary to conventional thought, in areas that have documented resistance and excito-repellency behavior in the local vector populations, effective control might still being achieved by the regular use of DDT. A stochastic model of vector behavior, supported by analyses of selected field data, suggests that irritancy and repellency (avoidance behavior) predominate, quantitatively, over the toxic properties of DDT as the primary means of reducing indoor human-vector contact (Roberts, et al. unpub. doc.). The behavioral impact of DDT residues by deterring indoor resting and feeding activities of vectors may help explain the continued effectiveness of some spraying programs, despite the presence of physiological resistance in the anopheline populations.

The resurgence of malaria worldwide, including the Americas, has renewed interest in *Anopheles* bionomics (Service, 1989; Zimmerman, 1992). There are considerable variations in malaria epidemiology, even within small areas, due to differences in topography, ecology and human activities (Russell, et al. 1963). The biology and behavior of *Anopheles* vectors (e.g., feeding activity, host preference, longevity, resting habits, flight range, malaria susceptibility, etc.) can vary significantly among and within particular species in response to specific ecological, seasonal and

meteorological conditions. (Gillies, 1988; Elliott, 1969). Evidence also suggests that intraspecific genetic differences over a species' geographical range may account for some of the observed biological variations (Smits, et al. 1996). As malaria is strongly linked to local ecological conditions and climate, detailed studies on vector biology and vector responses to changing 'seasonal' conditions must play a major role in understanding the epidemiology and site-specific risk of malaria transmission (WHO, 1975a). As such, no single, universal control tool or approach is available for all the diverse epidemiological situations encountered (WHO, 1995). Rather than planning malaria control for broad geographic areas, it has been recommended that efforts concentrate on the transmission characteristics within defined ecological zones, as well as seasonal variations that occur at the district and village level (Service, 1989; 1993a).

The primary aim of this study was to evaluate the impact of DDT residual deposits on the indoor behavior of anopheline vectors within a small area in the lowlands of northern Belize, Central America. Coupled with simultaneous studies on physiological susceptibility and excito-repellency behaviors, experimental huts were used to bring together these related components of field biology to define the role of DDT on indoor mosquito activities. The selection and development of the study site, including recruitment and training of mosquito collectors, design and construction of the huts, and the collection of baseline (pre-spray) indoor/outdoor mosquito population data were necessary before beginning the actual post-treatment assessment period.

The use of specially constructed experimental huts has held considerable importance in the evaluation of the efficiency of spraying normal houses with residual insecticides or using insecticide-impregnated bed nets (Smith, 1964; Muirhead-Thomson,

1968; WHO, 1975b; Service, 1993b). Experimental huts have also been instrumental in the study of normal movements of mosquitoes in and out of occupied houses (Roberts, et al. 1987). Although laboratory studies of irritancy behaviors may provide useful information on the actions of insecticides, data from field studies are required to determine if these observed effects are important operationally. Using two identical, experimental huts, one serving as an untreated control, the other as treated with a standard dosage of DDT, observations measured the behavioral response of mosquitoes, before and after indoor walls were sprayed. Each hut was equipped with 4 entry or exit window interception boxes to measure, as near as possible, the natural house-frequenting and host-seeking response of the local vectors. Observations in experimental huts included the use of 6-hr and 12-hr series of human-landing collections (HLC), mark-release-recapture studies to measure the influence of DDT and physiological status (blood-fed and unfed) on behavioral responses, and determination of preferred indoor resting sites between treated and untreated huts. Observations were also made on host-seeking activity outdoors and resting patterns of anophelines during the evening hours. This represents the first use of experimental huts in Belize for vector studies.

Study objectives were to identify the anopheline species in the study area, evaluate the epidemiological importance of different species as vectors of malaria, and quantify behavioral responses of vector species to DDT-sprayed surfaces in experimental huts. I also made observations on seasonal variations in anopheline densities, and bioassayed DDT residues on sprayed wall surfaces in experimental huts. In the course of work described in this section, I was able to briefly investigate daytime outside resting sites of adult *Anopheles* around the immediate study site. Using an ELISA, blooded

mosquitoes captured from outside daytime resting sites were assayed for host preference (Chow, et al. 1993). Additionally, most anophelines captured by HLC were tested with an ELISA to detect circumsporozoite antigen for evidence of sporozoite infection and vector potential (Beach, et al. 1992).

Information on the vectorial roles of *Anopheles* mosquitoes in Belize remains surprisingly sparse considering the historical importance of this disease in the country (Komp, 1940; Kumm & Ram, 1941; Bertram, 1971; Roberts, et al. 1993). Consequently, I attempted to quantify some aspects of malaria transmission in Caledonia. The nature of my field activities provided important opportunities to test specimens for evidence of malaria parasites (i.e., sporozoites) and investigate host preferences.

Population densities were low and in this study only 2 of the 5 *Anopheles* species in Caledonia- *An. albimanus* (Weidemann, 1820) and *An. vestitipennis* (Dyar & Knab, 1906) were abundant enough to analyze. Relative to other anophelines in Central America, an impressive amount of published research has been generated on *An. albimanus* (Frederickson, 1993; PAHO, 1996). The malaria vector status, wide geographical distribution, and normally high population numbers has made this species an easier target of past research. On the other hand, relatively little is known about the bionomics and vector status of *An. vestitipennis*. This species deserves much more attention given its anthropophagic behavior, endophagy, and incrimination as a malaria vector in the region. Background information on the other 3 species, *Anopheles crucians* (Weidemann, 1828), *Anopheles gabaldoni* (Vargas, 1941), and *Anopheles punctimacula* (Dyar & Knab, 1906), as they occur in Belize, is virtually nonexistent, and little is known about their local biology and possible medical importance. Ultimately, more detailed

knowledge of the influence of insecticides on vectors and a clearer understanding of local vector ecology and malaria epidemiology, shculd enable vector control efforts to be more selective and cost-effective.

MATERIALS AND METHODS

General study site: The country of Belize sits at 15-19° N latitude and is located on the southeastern part of the Yucatan peninsula of Central America. Nearly half of the country is low-lying coastal plain, the remainder is hilly or mountainous. Climate in Belize is subtropical with relatively constant maximum and minimum temperatures throughout the year. Average temperatures in the coastal regions range from 24°C (75.2 ° F) in January to 27°C (80.6° F) in July. Annual rainfall averages 1,347 mm (53") at Libertad, a village close to the immediate study location in the Corozal District. The wet season typically lasts from May/June through November. The coastal plain regions consist of lowlands with elevations generally less than 20 m above sea level. A substantial part of the lowlands is characterized by relatively undisturbed herbaceous wetlands providing important anopheline larval habitats. A detailed description of the northern coastal plain and the wetland ecosystems has been provided by Rejmankova et al. (1993, 1995, 1996, 1998).

Caledonia Village (18°13'78"N, 88°28'38"W) is centrally located in the northern Belizean coastal zone adjacent the New River floodplain, in the heart of the Corozal District. (Fig. 1). Caledonia was chosen based on several key criteria during the site selection phase in Belize. These included the presence of suitable larval habitats and base-line adult mosquito densities, relative ease of year-round vehicular access to the village, the availability of human volunteers for evening mosquito collections, and a site with a recent history of relatively high malaria incidence. *Anopheles* mosquito densities were relatively high in the village during pre-study surveys in July-August 1995.

Domesticated animals were common in and around the village, including (in order of relative abundance): cattle, chickens (turkeys), dogs, cats, pigs, horses, and goats.

The general environment of the village proper is one of well-established trees, shrubs and small garden plots. Soil type, with the exception of areas subject to periodic flooding from the New River, is high in limestone. At the time of the study (1995-96), Caledonia had an official population of approximately 1,227, comprising 226 families and 395 houses (VCP, unpub.report). The native population is predominantly Spanish-speaking Mestizo (primarily Honduran and Salvadoran extraction), with most income derived from seasonal sugarcane production. Housing quality in Caledonia proper is generally good, walls made from of wooden planks, cohune palm, or cement block, with metal roofs and screened windows. Access to the village was available year-round by an all-weather road.

Malaria and Control: Historically, the vast majority of the malaria had been *Plasmodium vivax*, only occasionally was *Plasmodium falciparum* reported in the northern districts of Belize. Malaria transmission in Caledonia is generally year round and had been among the highest in the northern health sector of Belize since 1988 (VCP, unpub. report). In 1994, 100 slide-positive cases (91/1,000 population) were recorded from the village, representing the second highest incidence rate in the district. In 1995, Caledonia ranked first in malaria cases among the 47 villages and towns in Corozal District. Most malaria cases had been recorded by 3 local volunteer collaborators through passive case detection.

Vector control has been the backbone of malaria control activities in Belize since the early 1960's. Only DDT has been used for routine indoor residual spraying (IRS). Regular IRS, 1 or 2-cycles/yr, using either 75% DDT wettable dispersible powder (wdp) as a suspension, or technical grade product mixed in kerosene, had been conducted in Caledonia on a near continuous basis for nearly 40 years at the time of this investigation. Structures were sprayed with either a 4% suspension in water on unpainted and split wood and palm pole walls or technical concentrate mixed in kerosene was used for painted surfaces. Spray procedures included application to interior walls, and outdoor eaves and parts of the lower ceiling. In response to the high disease incidence in 1994, Caledonia had village-wide IRS on 05-06 March 1995. The March 1995 spray operation was the last organized effort of IRS in Caledonia before the beginning of this investigation.

Immediate study site: Figure 2 provides a generalized diagram of experimental huts placement, laboratory location, and general distribution of vegetation in the study area. The site was immediately adjacent to the New River. The property was owned by neighboring local inhabitants, whom provided full support and permission to use this area for my studies. A vacant house on site was kindly provided, serving as both temporary living quarters and field laboratory. The experimental huts were constructed less than 20 meters from the river's edge. The land across the river was a large expanse of uninhabited "savanna" and marsh area, extending many kilometers eastward before reaching the town of Progresso.

The specific study locality was represented by a small depression along the New River floodplain. Unlike the well-drained, slightly elevated limestone soil of the village proper, a high water table, with permanent marsh areas and well-established emergent vegetation characterized this site. The available 'dry' areas were composed of alluvial/colloidal soils that would produce indescribably sticky mud when wet. A general botanical assessment of the study site was conducted in October 1995 (Eliska Rejmankova). The habitat was characterized as principally composed of 'graminoids' (aquatic grasses and sedges), with sparse trees. The total cover of emergent vegetation, approximating 20%, included Gramineae spp. (10%), *Sagittaria lancifolia* –arrowleaf (5%), *Eichhornia crassipes*- water hyacinth (1%), *Crinum* sp. (1%), *Mimosa priga* (1%), *Ludwigia octovalis* (1%), *Cypers* cf. *odoratus* (1%), and *Typha domingensis*- cattail (1%).

Climate/weather parameters: During the entire course of this study, daily measurements were recorded for total precipitation (Taylor® 11" Clear-Vu Rain Gauge, Fletcher, NC) and maximum/minimum ambient air temperatures (Taylor®, Model 5458, Fletcher, NC) over a 24-hr period (from 0700-0700 hr), providing monthly climate summary statistics. All measuring devices were read by eye and checked daily. A rain gauge and thermometer were appropriately placed (e.g., out of direct sunlight, human/animal distrubance) for accurate measurement at a sentinel site, close to the immediate study site

During all mosquito collection periods, outdoor ambient air temperature and relative humidity was recorded at the beginning of each collection hour using a hand-

operated sling pyschometer (Bacharach Inc., Pittsburgh, PA). Wind speed was measured using an automated anenometer (DIC Sims® anemometer, Simerl Inst., Annapolis, MD.). During each collection hour, notes were made on relative rainfall activity (none, light or heavy), and cloud cover and moon phase. The nebulosity index was not measured.

Human-landing catches (HLC): The majority of adult mosquito measures were based on human-baited landing captures. During the initial phases of the investigation, volunteer recruitment from the local population was made, followed by several evenings of training on the proper methods of mosquito collecting. All volunteers (total 8) were male and above 16 years of age. All volunteers had understood and agreed to participate by signing a human-use agreement form (Appendix III) before beginning the training. Mosquito collectors were provided an acceptable compensation for their activities. Depending on the needs of the study, various numbers of volunteers (3-6) were used during collection periods.

The standard collection method involved the complete exposure of both legs from just below the knee to lower ankles (WHO, 1975b). Using a hand-held mouth aspirator and a flashlight, each collector was tasked to capture all mosquitoes alighting (landing-biting) on their exposed lower legs only. Mosquitoes were placed in cups labeled by hour and location. Mosquitoes were processed immediately after each hour, killed by chloroform vapor and placed in sealed Petri dishes to protect from potential scavengers (e.g., ants) until species identification the following morning. Collection data were recorded by hour, each hour reflecting the beginning point of collection and the subsequent 45 min.

Collection periods occurred from dusk to midnight (~6-hr) or dusk to dawn (~12-13 hr), inside huts and one or more outdoor locations. Each collection 'hour' included 45 min of uninterrupted collecting and 15 min of rest before the next collection hour began. Collectors were routinely rotated between first half (dusk to ~midnight) and last half (~midnight to dawn), working only 6 consecutive hours per evening shift. Collectors were not allowed to apply repellent to any part of their body or allowed to smoke during the actual collecting activity. After each hour, collectors rotated places (e.g., between huts and outdoor sites) to reduce inherent capture bias (predicated on adeptness, attentiveness and 'attractiveness' of collector to mosquitoes). Special HLCs were conducted for the insecticide susceptibility assays, excito-repellency tests (Chapters 2 & 3), wall bioassays, and the mark-release-recapture trials from a nearby location, far enough away from the routine pre- and post-spray hut and outdoor collections to minimize any possible untoward effects on study site mosquito densities.

Appropriate technology: The bulk of activities during this investigation involved the tedious, sometimes agonizing, activity of evening human-landing collections. The primary goal of this effort was to diligently watch for and swiftly capture mosquitoes attracted to and landing on one's own exposed legs. Depending on skill and quickness, mosquitoes would be deftly aspirated off the skin before biting. Although explicit on protocol, 'landing' collections could often become 'biting' collections depending on the alertness of the collector. Human-landing collections involved the use of 6 simple items: an oral aspirator, collection holding containers, a working flashlight, a timer/alarm, a warm body, and strong coffee.

Aspirator and collection cups were invaluable in the execution of this study. The first 2 items were hand-made (at USUHS), and given their importance, deserve description. The mouth aspirator was a simple device composed of 4 main parts: a rigid clear plastic 0.5" diameter tube or wand, 14" in length, connected to a plastic or rubber tube (0.5" diameter), 21" in length. [Note: the tube should have walls rigid enough so it does not collapse on itself when being moderately bent, yet flexible enough to easily manipulate the position of the wand during mosquito capture.] Wand and tubing were connected using a modified, hard plastic syringe needle cap, cut to 1" length and opened at both ends, by inserting this 'connector' into the proximal and distal end of the wand and flexible tubing, respectively. Before joining, a small (0.5 in^2) fine mesh cloth screen was placed over the open end of the connector before inserting into the leading (wand) end. [Note: screen is critically important- aspiration of small flying insects is disconcerting and needs to be precluded.] After wand and tubing is attached, nylon strapping tape, followed by 1" width masking tape, effectively secures the 2 pieces in place. The free end of the flexible tube was fitted with a plastic mouthpiece by inserting a 1 ml plastic pipette, cut to 1" length, with near equal diameter open ends. Periodically, the entire device was disassembled and cleaned or items replaced (e.g., mesh screen). Collectors in this study were provided with one aspirator each.

Collection cups were made from standard paper (lightly waxed) 8 oz. drinking cups. A 1" square wide hole was cut into the side of the cup at mid-level. Two pieces of rubber latex (Dental Dam®, The Hygienic Corp., Akron, OH), each cut to 1.5" squares, with a 1" slit or cut in the center. Both pieces of latex were placed over the hole on the side of the cup, with the slits opposing one another (forming a cross). The opposing latex

pieces were held in place with masking tape. The result was a convenient, self-sealing 'window' that was used in combination with the aspirator for inserting and removing mosquitoes from the collection cups. The latex was checked daily and replaced when torn or otherwise non-functional. A small amount of dry talc powder periodically applied to the exterior latex seal helped prevent tearing as the aspirator was inserted and removed from the cup. Finally, the open top of the cup was covered with a fine mesh cloth screen secured with a rubber band and masking tape. During use, the side of the cup was labeled with collection (site and hour) information.

Species identification and processing: All adult mosquitoes (Anophelinae and Culicinae) were identified to species by standard morphological criteria (Wilkerson, et al., 1990; Clark-Gil and Darsie, 1983) using a dissecting zoom stereoscope (AO® Scientific Instruments, 1x-6x 580) and standard lighting. Species identifications were recorded by date, hour and collection site. General observations were noted on different body size, coloration and obvious aberrant diagnostic characters, as well presence of aquatic arrenuroid larval mites (Hydrachnidia) attached to adults. *Anopheles* species were placed (pooled < 8/tube) in labeled (date, hour, site, species) 1.5 ml polypropylene microcentrifuge tubes (IMEC, Odenton, MD) and stored over desiccant (silica gel) until sporozoite enzyme-linked immunosorbent assay (ELISA) testing. A representative sample of adult species were re-examined by the Walter Reed Biosystematics Unit (Mr. J. Pecor) for species confirmation.

Experimental huts: With the help of a local carpenter, 2 identically sized experimental huts were designed and built on the dimensions presented in figure 3. Huts were built ~10 m apart and separated in construction time by approximately 1.5 months. Huts were built in conformity with low-income housing, using local materials when appropriate. Both huts were single room structures, raised approximately 0.9 m from terra firma on 6 sturdy wooden posts supporting the 2.4 m by 3 m frame and solid 3.8 cm thick plywood floor. The center frame was made of local tree posts, augmented with standard 5.1 x 10.3 cm and 5.1 x 20.5 cm lumber. Walls were made from palm poles (~ 8 cm in diameter) from the common cohune palm, *Orbignya cohune*, lash-wired together and nailed to the main frame. Walls facing east and west were 3 x 2.13 m, and north and south 2.44 x 2.13 m. Total surface area of all 4 walls (including closed door and windows) was ~ 23.4 m^2. The walls remained unfinished (with bark) and fenestrated between most poles from the inside and out by less than 1.28 cm. Walls were not plastered with clay or cement to fill in the gaps between adjoining poles. Ceilings were constructed using 3 large branch cross beams connecting north and south walls. The roof was made of long flexible tree branches attached to the outer walls with several overlapping layers of palm thatch. Open eaves, gapped approximately 15 cm between upper wall frame and roof, encircled the hut. Only 1 door (1.8 x 0.6 m plywood with 2.56 x 10.25 cm board frame), with wooden steps facing east towards the river, provided access for collectors. Each hut had 4 windows (0.5 x 0.5 m), roughly equal height from the hut floor level (0.9 m), and more or less centrally located on each wall. Windows were equipped with a side hinged solid plywood shutter that could be secured from the inside. The measurement and construction of the window frames were exacting to

accommodate closely the intercept traps. Except for the collector's chair (stool), huts were devoid of furniture during all collection periods.

Preliminary observations identified at least 5 different types of house-frequenting ants in and immediately outside the huts. Most were diurnal foragers, while others appeared active only at night. Attempts to 'ant-proof' both huts in order to reduce the predator/scavenging activities of these industrious creatures were made. Poison baits and exclusionary methods were employed in both huts. Four small bait stations (Maxforce®, Oakland, CA) using 1% hydromethylnon, a non-repellent stomach ant poison, were placed inside each hut, vertically on walls, 2 at floor level, and 2 along the eaves. Another product (Siege® American Cyanamid, Wayne, NJ), a gel formulation of 2% hydromethylnon, was applied along the corners of the baseboards and underneath huts at the connection points between base pillars and floor. Lastly, as an exclusion attempt, multipurpose wheel bearing and chassis grease (NAPA 75-602) was liberally applied in 2" bands around the support pillars one ft from ground level. Other nocturnal predators/scavengers, in particular, scurrying cockroaches, menacing scorpions, (*Centruroides gracilis* (Latreille) 1804), and large, spirited crab spiders cavorting in the thatch roofing were beyond any means of effective control. Shortly after construction, both huts were beset with subterranean (*Reticulitermes* sp.) termites. Effective control of these insects would have involved use of contact insecticides, so no control was attempted.

Intercept boxes: Each hut was equipped with 4 identical intercept boxes. All devices (8 total) were of equal size and could be exchanged and placed in any of the hut

windows to function as either egress or ingress collection units. The original design (Fig. 4) was made of 0.5 x 0.5 x 0.66 m hardwood (2.56 x 2.56 cm) frame, fitting precisely the opening of the hut windows. Five open sides of the frame (top, bottom, back and 2 sides) were covered in a strong plastic mesh (1,024 holes/2.56 cm^2) (PeCap®, Switzerland) and secured using heavy duty staples. The front opening was fitted with an angled frame from bottom and top, connected to a mid-interior 0.5 m support, forming a 2.56 cm gap leading into the interior chamber of the window trap. The baffle portion was fitted with a lighter fabric nylon mesh (military green, standard NSN issue). The fabric at the gap point was kept taunt using length of small gauge wire secured to the frame. One side of the box trap was provided a 0.2 x 0.21 m access hole to the interior chamber for extraction of captured mosquitoes. The access area was covered with 0.25 m length orthopedic stocking as a barrier to mosquito escape.

Through a trial and error process, it was determined that the window trap using the 2.56 cm gap was not collecting enough mosquitoes in either the ingress or escape positions. Boxes were initially modified by enlarging the gap of the funnel, in increments, up to 6.4 cm. These changes were unsatisfactory. Finally, the boxes were simplified by removing the inserted funnel to produce a simple open box, wherein mosquitoes could 'rest' and be periodically collected using an aspirator. Unlike the original window trap design, the window boxes merely functioned to intercept ingress or egress movement of the mosquito (depending on the orientation of the opened end), allowing continued free movement in and out of the box at will. These boxes were useful in the mark-release-recapture studies.

Pre-spray hut evaluations: To gain information on which points of access into a hut were relatively more important or preferred, a small series of HLC experiments were conducted placing each hut under variable (unmatched) conditions. Conditions included variations on open or closed windows (2 or 4 windows), eaves and walls. By convenience, all scenarios (trials 1-7) placed Hut-1 with more restrictive access compared to Hut-2. In all trials, the wall was closed for Hut-1, open for Hut-2. Both huts always maintained a closed door, excepting collector movement in and out. Huts were paired temporally in each trial. Additionally, another small set of trials compared the 2 huts under matching conditions to assess similarity in attracting mosquitoes before DDT treatment. Trials 8-12 were paired 6-hr collections and access conditions were identical (matched) between huts. One collector per hut preformed standard hourly indoor HLC for 6- or 13-hr time periods.

DDT application: After both huts and intercept (window) boxes had been constructed, the inner walls of both huts were covered with water-resistant white plastic woven material that is commonly used in commercial bag production in Belize. All material had previously held dry processed rice. This bag material was attached to walls directly with 2.56 cm length wide-headed tacks. Contact bioassays (24-hr) were performed with using locally wild-caught *An. albimanus* to assess any pre-spray toxic effects of the bag material. WHO insecticide susceptibility tubes and standard testing procedures were used (WHO, 1981a). The only modification was to allow the mosquitoes to have uninterrupted 24-hr contact (vice 1-hr) with the material or control paper.

Hut-1 was selected for DDT treatment before hut comparability studies were conducted to avoid possible selection bias. DDT was provided by the Belize Vector Control Programme. The product was well packaged, dry and within marked expiration date. On 24 January 1996, DDT powder (75% wdp) was suspended in clean rainwater to make a 4% (technical grade) formulation [28 oz. 75% wdp DDT + 3 gallons water or equivalent 70.3 gms/L]. The suspension was placed in a clean standard hand compression pump sprayer (Hudson X-Pert ® 3-gal sprayer, H.D.Hudson Mfg. Co., Chicago) equipped with a new 8002E Teejet® nozzle for flat-fan application to wall surfaces. The discharge rate was calibrated to ensure a velocity of near 757ml/min (WHO, 1996). At 0800 hr, on a clear calm day, the inner walls of Hut-1 were sprayed at the prescribed rate of 2 gm DDT/m^2 of wall surface. The sprayer was pressurized at 40 psi and walls treated with a single controlled pass by a trained local Ministry of Health malaria sprayman. All other surfaces (ceilings, eaves, outside surfaces) were not treated. The control hut (Hut-2) was not sprayed.

Bioassays of insecticide sprayed surfaces: To estimate the contact potency (toxicity) of residual DDT on indoor walls in Hut-1 compared to Hut-2 (control), a series of bioassays were conducted approximately 1, 6, and 12 weeks post-DDT treatment. Special HLCs were conducted for these tests. Wild-caught female mosquitoes were collected outdoors adjacent the study site, near a local house which had provided consistently high outdoor collections of anophelines, in particular *An. albimanus*. Anophelines were placed into a clean, 3.8 L cardboard ice cream container and allowed to randomly mix. Mosquitoes were kept overnight and supplied with water-saturated

cotton pads only. Bioassay tests took place the following morning. Mosquitoes were held less than 12 hr before testing. Because the tests required a sufficient number of replicates and controls, a relatively high number of mosquitoes were needed. To reduce potential damage by excessive handling, mosquitoes were not sorted to species before testing. Only *An. albimanus*, *An. crucians* and *An. vestitipennis* were present in sufficient numbers to allow any reasonable analysis.

All tests were conducted during the cooler morning hours under similar conditions. Ambient temperature and relative humidity was recorded during the exposure period and maximum-minimum temperature recorded over the 24-hr holding period. Assay procedures were as prescribed by WHO (1981b), using conical chambers of transparent plastic (8.5 cm diameter at the base, 5.5 cm high). Ten mosquitoes each (mixed species) were used per test. Mosquitoes were carefully loaded into the chamber, which was firmly attached with adhesive tape to either treated or control walls, respectively. All four walls in each hut were assayed along the midline from floor to ceiling. Mosquitoes were exposed to the wall surface for 60 min, then carefully transferred into clean individual holding cups and provided a 10% sucrose solution on cotton pads as a carbohydrate source. The number of mosquitoes found dead or having experienced knockdown immediately after the contact period were recorded. Knockdown (KD) was defined as the condition when a specimen was unable to fly or effectively walk following exposure. Holding cups were placed in a well-ventilated, vacant house that had not been sprayed with DDT for over 6 years. Cups were placed upright in a large plastic ice chest on floating plastic pans atop a thin layer of water to thwart scavenging ants. At 24-hr post-exposure, mortality was recorded, and mosquitoes

counted and identified to species. Test results were combined for proportion mortality analysis.

Mark-release-recapture trials: A series of paired hut trials using wild-caught marked female mosquitoes released into unbaited treated and control huts were conducted to evaluate the behavioral response of 2 different physiological conditions, freshly blood-fed and unfed, *Anopheles* to DDT deposits. All trials were made approximately 2 mo after indoor application of insecticide. Mosquitoes were collected and handled in a similar manner as described for the wall bioassays in huts. However, all mosquitoes were identified to species before being blood-fed and/or marked with fluorescent powder. Only *An. albimanus* and *An. vestitipennis* were in sufficient number to perform repeated trials.

A random assortment of the female mosquitoes were allowed to feed on the exposed arm of the author approximately 2 hr before marking. Mosquitoes not feeding, were segregated into a separate screened holding cage with the remaining unfed specimens. No more than 25 blood-fed and unfed mosquitoes each (50 total) were selected to be marked and released into each hut per trial. Four fluorescent colors were selected for marking, yellow or blue for blood-fed, and red or white for unfed mosquitoes depending on species. The desired number of mosquitoes (~25) were selected by feeding status and placed in cups containing a very small amount of extremely fine, grade A fluorescent powder (DayGlo®, BioQuip Products, Gardena, CA) on the bottom of the cup. Using a minute amount of chloroform on cotton wool placed on top of the cup, sufficient vapor was allowed to 'knockdown' the mosquitoes placing them in direct

contact with the powder. Within a few minutes, most specimens began to recover, the gradual movement of which increased the impinging of powder on the setae, scales and body surface of the mosquito. Within 10 minutes most, if not all, marked mosquitoes had fully recovered. Marked individuals were carefully removed using a color designated mouth aspirator, to avoid any possible color contamination, and placed in a clean holding cup awaiting release into huts. Blood-fed and unfed mosquitoes were placed in separate containers before release.

Hut trials took place with all 4 window boxes set in the 'escape' position (facing out), with door, walls and eaves closed for both treated and control huts. Huts were not provided bait (human or animal). Simultaneous release of mosquitoes into both huts occurred at approximately 1900 hr. Cups were placed in the center of the hut floor, with the top netting then carefully removed. Mosquitoes were allowed to escape from the cups at will. Huts were visited every 2 hr beginning at 2000 hr (1-hr post-release) throughout the evening until late morning hrs (1000 hr). The observer entered the hut through the door, and collected all mosquitoes found resting in the egress window boxes. Using a handheld, long-wave ultraviolet (UV-366 nm) light source (Blak-ray Lamp®, model UVL-56, UVP, San Gabriel, CA), floor, walls and ceiling of huts were carefully inspected for position and numbers of marked mosquitoes, dead or alive, remaining in the hut. While avoiding unnecessary disturbance in the huts, the entire inspection process took approximately 15 minutes per period. Mosquitoes found in the exit boxes, dead or alive, were recorded as having 'escaped', while dead mosquitoes recovered from floors were not included among the final percentage escape. Each trial was designed to monitor mosquito behavior up to 48 hr post-release. On only 2 occasions mosquitoes were

marked (alternating colors) and released one day apart. Captured ('escaped') mosquitoes were held for 24-hr under the same conditions as the bioassay tests to determine proportion mortality. Specimens were later identified by physiological condition and species.

Analysis and presentation of human-landing and mark-release data: All pre- and post-treatment HLC data from huts and outdoor collections were entered into a relational database (Helix Express® 3.0, Prospect Heights, IL). Collated data were placed on a spreadsheet (Excel®, Microsoft Inc., Seattle, WA) for simple computations and organizing imported data to statistical programs for more advanced data analysis (JMP® 3.1.5, SAS Inst., Cary, NC; DataDesk® 5.0.1, Data Description, Inc., Ithaca, NY; Microstat® 4.1.06, Ecosoft, Inc.). Figures (data graphs) were developed with Excel® or DeltaGraph ® Pro 4.0.1 (DeltaPoint, Inc. Monterey, CA).

. Rainfall and raindays were combined to produce a "degree of wetness index" as described by Russell et al. (1963). In most instances, normal population mean and population standard deviation (standard error) statistics were computed. Simple ratios comparing indoor hut and outdoor HLC were calculated. Natural log transformed HLC (per person/hr) data provided a leveling effect for the more skewed evening distributions, and allowed extreme high and low numbers to be effectively placed on the same figure. Mean hourly proportions from HLC data comparing outdoor and hut collections were also computed. Probability computations of HLC and mark-release-recapture data were compared using proportional analysis. Individual species response to DDT in experimental huts was analyzed several ways. Proportion analysis (z-statistic) compared

post-spray treated and control huts hourly for each respective species population. The test statistic was based on a standard normal approximation (binomial distribution). *P*-values were based on the mean HLC per collection hour. The minimum level of significance was $p<0.05$ (α level=0.05) for 2-sided tests to 'reject' (mathematically) the tested hypothesis. Percent reduction calculations using selected 6-hr collection data from pre- and post-treated and control mean HLC per person/hr were computed using the formula $1-[\,(Tn \times Co) / (To \times Cn)\,]$, where *T*= treated; *C*= control; *o*=pre-treatment; *n*=post-treatment (Hudson, 1984; Roberts & Alecrim, 1991). Calculations were also made for the hourly proportion decline and cumulative rate of decline for all-night post-treatment hut data.

Circumsporozite protein (CSP) ELISA: An ELISA was used to detect the presence of circumsporozoite protein (CSP) in mosquitoes as a means to estimate the malaria inoculation rates during the course of the study. Dried female *Anopheles* were processed and assayed at the Uniformed Services University of the Health Sciences to detect species-specific CSP of *P. falciparum*, and 2 polymorphs of *P. vivax*. Most reagents, including purified monoclonal antibodies (Mabs) and peroxoidase-conjugated Mabs were kindly provided by R.A. Wirtz (WRAIR, Washington, D.C.). Before testing, mosquitoes were carefully re-examined to confirm species identification. The abdomen was removed from each mosquito and the combined head-thorax was individually assayed or pooled by species and collection period (8 or less/pool) in a sandwich ELISA (Wirtz, et al. 1987; Beach, et al., 1992) using U-bottom, 96-well polyvinyl microtiter plates. Laboratory-reared *An. albimanus* served as negative controls. Positive controls of

P. falciparum or *P. vivax*- 210 recombinant proteins and *P. vivax*-247 synthetic peptides were run concurrently.

Results were read visually and at 414 nm using an ELISA plate reader (Titertek Multiskan plus MKII®, Labsystems, Finland) 15, 30, and 45 min after the addition of 2,2'-azino-di [3-ethyl-benzthiazoline sulphonate (6)] (ATBS) peroxidase substrate (Kirkegaard and Perry, Gaithersberg, MD). All wells containing test mosquitoes giving absorbance values greater than 2 times the average of 5 concurrent negative controls were considered positive for the corresponding CSP and were later re-tested by ELISA for confirmation.

Blood-meal ELISA: Mosquitoes were collected from outdoor resting sites using a modified CDC backpack aspirator (Model 1412, J.W. Hock Co., Gainesville, FL). *Anopheles* containing fresh or recent (half-filled abdomen or greater) blood were identified to species, processed and stored over desiccant as described for the CSP ELISA until testing. Most reagents, purified and peroxidase-conjugated anti-host IgG (H+L chains) antibodies for human, bovine, pig, fowl, canine, and positive and negative control sera were kindly provided by R.A.Wirtz. Mosquitoes were re-examined for species identification before testing. Abdomens were carefully removed and tested individually against all 5 anti-host IgG antibodies in coated host antibody-specific U-bottom 96-well polyvinyl microtiter plates. Basic methods are nearly identical to those described for the CSP ELISA technigue and Chow, et al.(1993), except that blocking buffer was not used during the mosquito grinding process. Respective serum (1:500 dilution) was added to absorb out cross-reacting antibody to reduce false positive reactions. Results were read

visually and at 415 nm using an ELISA plate reader (BioTek®, EL311 SL) 30 min after the addition of ATBS substrate. All mosquitoes having absorbency values greater than 2 times the average of 5 concurrent negative controls were considered positive for the corresponding blood-meal source and were re-tested for confirmation. All testing took place at the U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.

RESULTS

Weather parameters: Table 1 presents a monthly summary of mean maximum and minimum ambient air temperatures and range of extremes, total rainfall, number and percentage raindays and a 'wetness index' (WI) for comparison between months. The WI provides a convenient measure of overall moisture over a period of time by combining total amount of rainfall and number of actual raindays. During the approximately 9 months of observation, 1,044.4 mm (42") of rain fell in Caledonia. Following normal climatic patterns, rainfall was greatest during the months of September and October (measured rainfall 452.4 mm) with a WI of 135 and 221, respectively, dropping quickly during the subsequent months to a low period during February and March (62.4 mm), with a combined WI of only 12. April also had low amounts of rainfall (64 mm) and a WI of 19. Between high and low precipitation periods during the 9 months, there was a nearly 30-fold decrease in the wetness index (Fig. 5).

High daytime air temperatures during the corresponding months averaged between 81-92 °F (27.2- 33.3 °C), with minimum (early morning temperatures) from 64-77 °F (17.8-25 °C). The most striking weather events for the study were periodic occurrences of decreased minimum temperatures seen during brief periods from January through early March (Fig. 6). Temperature fluctuations were reflected in a greater high-low temperature range and variations (SD) about the monthly means. Minimum temperatures for these months were normally in the mid-60's, with occasional drops to the low 50's. January 12-15, 1996, experienced a dramatic drop in temperature with periods of prolonged, gusty winds. In some instances, mid-daytime temperatures

dropped to 49°F (9.4°C). Average maximum daytime temperatures in January reached only 81°F (27.2°C). Sustained and prolonged day and nighttime low temperatures adversely affected mosquito collection activities. The months of February and March also experienced periods of cold weather. Local residents commented that these long 'cold' spells were very unusual. During these days of cold weather, the water level in the New River would drop down 0.5-1.0 meter or greater in depth and the surrounding wetland in the study site would rapidly dry. April and May were characterized with more normal temperatures and rainfall.

Excluding periods of unusual extremes, temperatures throughout the evening could still vary dramatically from one collection period to the next. Generally, on calm nights, temperatures would range from early evening highs of 74-80 °F to morning lows of between 68-77 °F. Relative humidity changes were more uniform and predictable, with a steady increase from early evening (83-92%), until reaching near saturation point (98-99%) during the early morning hours. During the drier, cooler months, evening temperatures tended to be lower, whereas relative humidity would begin lower (usually associated with late afternoon gusty breezes) and eventually reach near saturation by morning.

Sampling of resident mosquito species: From June 1995 to May 1996, 29 different mosquito (Diptera: Culicidae) species, representing 9 genera, were collected from human-bait and experimental hut intercept boxes in Caledonia (Table 2). All species had been previously described as resident in Belize (Belkin, et al. 1965; Knight & Stone, 1977). The most common mosquito encountered throughout the study was

Mansonia dyari Belkin, Heinemann & Page, 1970. This culicine species was a particularly tenacious and pestiferous creature both indoors and out. Members of the *Culex* subgenus *Melanoconion* (*Culex erraticus, Cx. pilosus, Cx. taeniopus*, and probably several others not identified) were also common in HLCs. With the exception of *Cx. taeniopus* (with banded hind tarsi), many members of this subgenus are small, dark culicines lacking many distinguishing characters and are extremely difficult to identify to species in the adult stage. With the exception of some of the *Anopheles*, most other species were found at much lower densities. Other *Culex* species (e.g., *Culex coronator* and *Culex nigripalpus*) were far less common. *Aedes* were most represented by *Aedes taeniorhychus* and *Aedes scapularis*. An occasional influx of *Coquillettidia nigricans* and *Mansonia titillans*, persistent and feisty beasts, were seen in outdoor and indoor collections. Thankfully, infamous tormentors, such as large, life-sucking *Psorophora* species were infrequent diners during evening hours in Caledonia. As expected, human-baited collections resulted in very few *Uranotaenia* specimens. Rarer still, only 3 *Limatus durhamii* and 1 *Aedeomyia squamipennis* (from an intercept box) were collected over the entire investigation. As an aside, sand flies (*Lutzomyia* (*Psathyromyia*) sp.) and biting midges (*Culiciodes* sp.) were also uncommon in HLCs. The highest densities of both (only several per total evening collection) were observed in outdoor collections during the December-January time frame.

Five different species of *Anopheles* were collected indoors and out. The 2 most common were *An. albimanus* and *An. vestitipennis*, followed in relatively lower abundance by *An. crucians, An. gabaldoni*, and *An. punctimacula*. *Anopheles darlingi* Root was not captured at any point during the study and attests to its relatively

uncommon and focal occurrence along the northern stretch of the New River (Manguin, et al. 1996). Ecological conditions in the riverine areas between San Estevan and Libertad do not seem conducive to maintaining sufficiently large populations of this important mosquito species. In all, during the 31 evenings, 15 (6-hr) and 16 (12 or13-hr) HLCs, conducted from 09 September 1995 to 16 May 1996, 15,795 *Anopheles* were captured from human-bait and identified to species. Total numbers and relative proportions were: *An. albimanus*, 7,236 (0.46); *An. vestitipennis*, 6,948 (0.45); *An. crucians*, 1,419 (0.09); *An. gabaldoni*, 113 (0.006); and *An. punctimacula*, 79 (0.004). Combined with *Ma. dyari* (n=9,033), 24,828 mosquitoes made up the bulk the HLC data that is presented forthwith.

Human-landing collections: To present HLC data, each designated hour is represented by the beginning point of a 45-min collection. Unless stated otherwise, data from only 4 species of mosquitoes are presented and in the following order, *An. albimanus, An. vestitipennis, An. crucians,* and *Ma. dyari*. In the context of the results and discussion, mosquito "activity" was defined as human-landing and biting (host-seeking behavior), unless stated otherwise. All attempts are made to present the results chronologically and in detail. The figures were selected to provide an assortment of perspectives and information.

Seasonal outdoor HLC: Figures (7-10) depict the distributions of outdoor nighttime biting activity observed in Caledonia for *An. albimanus, An. vestitipennis, An. crucians, An. gabaldoni,* and *Ma. dyari*, respectively. Variations in patterns of activity by time of year (1995-1996) are also depicted. The top figure, expressed as natural log

values, represents the hourly means of all-night HLC (per person/hour). The bottom figure is the averaged proportional distribution of all specimens captured by period and collection hour. September-October and April-May collections covered 12-hr periods starting from 1800-1845 to 0500-0545 hr. December-January included 13 collection hours per evening, 1730-1815 to 0530-0615. In some cases, the first 2 hours (1730-1915 hr) were combined and averaged for hour "1" for comparison and analysis. *An. crucians* was encountered in far lower numbers compared to *An. albimanus* and *An. vestitipennis*. Unfortunately, smaller sample sizes allowed for greater fluctuations (variation) in the figured data and precluded some forms of statistical analysis. *An. gabaldoni* was relatively uncommon in Caledonia HLCs compared to the other 3 *Anopheles*, and was not present in the April-May collections.

Figures 11-13 compare all 4 species and patterns of activity during time of year using natural log values of all-night outdoor HLC data. Total number of mosquitoes collected by period of year is provided in the figures. September-October data , based on 6 (12-hr) sampling nights, showed *An. albimanus* was the dominant anopheline during the wet/warm period of the study, followed by *An. vestitipennis*. Based on 4 (13-hr) sampling nights, *An. vestitipennis* was the most abundant outdoor anopheline during the comparatively drier/cooler months of December-January, followed by near equal numbers between *An. albimanus* and *Ma. dyari*. April-May, based on 5 (12-hr) sampling nights, was dominated by *An. albimanus*, as precipitation and temperatures increased. *Ma. dyari* maintained HLC populations near Dec.-Jan. levels, while *An. vestitipennis* populations decreased nearly 4.5-fold.

Generally, for all species, outdoor activity was greatest during the first half of the evening, and depending on the species, declined at various rates to a low point between 0300-0500 hr. Only *An. albimanus* consistently showed a second peak in activity around dawn. *Anopheles albimanus*, overall the most abundant mosquito attracted to humans outdoors, had a bimodal distribution over the 3 seasonal periods. Starting off swiftly with a strong peak in biting frequency in the first hours of the evening, *An. albimanus* activity would taper off quickly during the night. After reaching lowest HLC rates during the early morning hours (0200-0400 hr), there was a notable rise in activity from 0500 hr to shortly before sunrise. By proportional distribution (Fig. 7), Dec.-Jan. showed the strongest early evening and morning peaks. *Anopheles vestitipennis*, second in overall outdoor mosquito abundance, showed a more delayed activity pattern in the early evening, peaking and leveling out between 2000-2200 hr, and continuing with gradual hourly declines in biting rates until sunrise (Fig. 8). The proportional distribution indicated more activity before 2400 hr. *Anopheles crucians* started the evening off quickly and was active throughout the night, generally peaking before midnight. No discernable increase in dawn activity was seen. The few *Anopheles gabaldoni* collected suggested an early evening peak and continuing activity throughout the night with a slight activity surge at dawn in Dec.-Jan; however, low total capture numbers preclude any meaningfully assessments. *Mansonia dyari* had an outdoor activity pattern similar to *An. crucians*, starting the attack early and continuing activity throughout the night. Proportional distribution found outdoor activity much stronger in the early first half of the evening, with a smaller peak of activity between 0400-0600 hr, depending on the time of sunrise.

Pre-treatment indoor/outdoor collections: Before assessment of DDT treatment on mosquito behavior, 4 collection all-night (13-hr) trials were conducted in December-January to determine (1) species population density and indoor v. outdoor distributions of biting activities, (2) evaluate preferred points of hut entry by mosquitoes, and (3) compare the 2 experimental huts under identical conditions for similarity in attracting mosquitoes. Sample sizes restricted data presentation and analysis to only 3 species, *An. albimanus*, *An. vestitipennis*, and *Ma. dyari*. Figures 14-16 illustrate individual HLC outdoors, and inside Hut-1 and Hut-2. Indoor collections were paired with an outdoor collection at a common site between both houses (referred to as a single outdoor HLC) starting at 1730-1815 and ending at 0530-0615 hr. The single outdoor collection functioned as a 'standard' by which huts could be compared with one another. The data in the top figure is expressed as hourly natural log mean HLC per person/hr, and the lower figure as mean proportional distribution of mosquitoes per person/hr. Total sample size over 4 collection periods for *An. albimanus* (Fig. 15): Out (644), Hut-1 (19), Hut-2 (48); *An. vestitipennis* (Fig. 16): Out (1410), Hut-1 (357), Hut-2 (524); and *Ma. dyari* (Fig. 18): Out (665), Hut-1 (223), Hut-2 (534).

An. albimanus showed the strongest predilection for outdoor activity compared to indoor HLC for either hut. Indoor:Outdoor proportions ranged by hour from 0.04-0.23 throughout the evening. The combined all-night indoor v. outdoor proportion was 0.094 (<10%). [admittedly, this is a biased overestimate as indoor collections were based on 2 collectors and the outdoor effort only 1 collector per hour. Averaging the 2 combined indoor collections would have given a lower proportion (0.047) entering huts compared to outdoor activity]. Overall Hut-2 collected 2.5x more *An. albimanus* than Hut-1. As

will become apparent in the next section of results ('Pre-spray matched and unmatched hut trials'), these 2 huts were not entirely comparable during these 4 trials, as both had variable (unmatched) portals of entry into the hut. In all cases, Hut-1 was the more restricted, with a greater number of entry points closed off compared to the more 'open' Hut-2. Comparing log mean HLC (Fig. 14), outdoor activity was noticeably greater for *An. albimanus*. The proportion distribution by collection site (outdoor and 2 huts) also shows greater activity in the early first half of the evening for all 3 collection intervals. On average, the log means and proportion distributions showed clear early morning increases of activity for all collections beginning around 0430 hr until sunrise.

Anopheles vestitipennis showed much stronger indoor activity compared to *An. albimanus* (Fig. 16). Combined (H1+H2) hut proportions ranged by hour from 0.24-0.54 compared to the single paired outdoor catch. The combined hut all-night proportion was 0.384. Proportionally, indoor activity increased relative to outdoor collections as the evening progressed, including an early morning rise in I:O ratio beginning 0330 hr. Again, under the different conditions imposed on both huts, Hut-2 collected 1.5x more mosquitoes than Hut-1. The log means data for *An. vestitipennis* (Fig. 15) showed the more gradual increase in activity in the early evening as well as the increasing number of indoor mosquitoes captured relative to the outdoor collections. The individual proportion distribution for all 3 locations show close similarity, with much stronger activity in the first half of the evening and a more gradual decline in biting as the evening progressed.

Mansonia dyari provided the highest overall proportion indoor v. outdoor activity of the 3 species, varying from an early evening low of 0.43 to a 2130 hr peak of 0.82. This species remained active throughout the evening and showed an overall all-night near

equal proportion (0.54) of endophagic/exophagic behavior, despite obstacles to entry into the huts. Hut-2 captured 2.4 x more mosquitoes than Hut-1 under the varying access conditions between huts. Both log means and proportional distributions HLCs showed an unmistakable rise in activity in the early evening, with a noticeable plunge in catch during the first half of the evening, gradually tapering off throughout the nocturnal hours (Fig. 16). While the log means catch of Hut-1 fell away from Hut-2 in the later half of the evening, the proportional distribution within both huts and outdoor collections remained similar. Outdoor and indoor HLCs showed a clear bimodal pattern, with a definitive early morning (dawn) increase in activity (Fig. 16).

Pre-spray unmatched and matched hut trials: To gain information on preferred points of house entering (access points) by mosquitoes, a series of experiments were conducted in December 1995 and January 1996 before house spraying. Six experiments (trials) using HLCs were conducted by placing each hut under unmatched conditions with variable open and closed points of entry into the respective huts. Conversely, five separate trials were conducted to compare the 2 huts under matching conditions (same open and closed points of access) to determine similarity in paired huts for attracting mosquitoes indoors. Table 3 lists the principal hut conditions per trial. Huts were paired temporally in each collection. In all unmatched trials (1-7), timing and logistics necessitated that Hut-1 was given the more restrictive access compared to Hut-2. Trials 1, 2, 4, 5, and 7 were 13-hr collections; all others were 6-hr collection efforts. Trials 8-12 were all 6-hr paired collections, with all access conditions identical between huts. The

data are presented in several ways. Table 3 provides a standard ratio between Hut-1/Hut-2 (H2=1), by species, based on total collection hours per trial.

Proportion analysis for each collection is given in Tables 4 and 5 for unmatched and matched trials, respectively. Under unmatched conditions, statistical tests were only possible for trials having sufficient sample sizes. Between huts, a significance difference was seen in trial 4 for *An. albimanus*; trials 1, 2, 4, 5, and 7 for *An. vestitipennis*; and trials 1, 2, 5, 6, 7 for *Ma. dyari*. Overall (all trials combined) sample sizes were considered sufficiently large for *An. albimanus* and *An. vestitipennis*, but not necessarily for each individual trial. In most cases, differences were found highly significant ($p<0.001$). Differences were generally in favor of Hut-2, which had the greater open access.

Conditions providing the least access into Hut-1 (trials 3 & 5), with all general points of entry closed, produced the smallest capture for all species compared to Hut-2. Hut-1 in trials 2 and 6 had only open eaves, produced capture proportions of between 0.14-0.29 in trial 2 and 0.125-0.28 in trial 6 compared to Hut-2. Trials 1 and 7 were conducted under the same variable conditions and produced nearly identical results proportionally and statistically, indicating open walls had contributed to increased mosquito entry. Trials 1, 4 and 7, which allowed 2 or 4 open windows in Hut-1, substantially increased the proportion catch for all species in relation to Hut-2.

Despite the fenestrated condition of the palm wood walls and the generous gap in the surrounding eaves, results indicated walls and eaves generally contributed less to hut access; whereas, windows appeared to offer a much greater opportunity or less restriction for mosquitoes to gain entry. In fact, in trial 4 which allowed 4 open windows in Hut-1

and 4 open windows, eaves and walls in Hut-2, actually produced significantly ($p<0.001$) more *An. vestitipennis* entering Hut-1 during the evening by a ratio of 1.5:1. Conversely, trials 1 and 7 indicated *An. vestitipennis* and *Ma. dyari* would use 'open' walls as an important point of entry, despite 2 open windows and eaves in both huts.

Five trials comparing huts under identical conditions of access produced variable results from one trial to the next. *Anopheles albimanus* showed the greatest variation, which was influenced by the smaller sample size (n=85) compared to *An. vestitipennis* (421) and *Ma. dyari* (660). *Anopheles crucians* were excluded from analysis because of small sample size. Although some statistical differences were seen between huts in individual trials and species, in many cases trial sample sizes were too small for meaningful analysis. Despite the wide range of ratio values within species and between trials (Table 3), when all 5 collections were combined by species, the resulting Hut-1/Hut-2 ratios showed reasonable similarity: *An. albimanus* (0.6:1 for a $p<0.02$), *An. vestitipennis* (1.27:1 for a $p<0.02$), and *Ma. dyari* (1.13:1 for a $p<0.15$). As shown, when all 5 tests are combined, only weak differences emerge between huts for *An. albimanus* and *An. vestitipennis*, and no statistical difference for *Ma. dyari*.

Bioassays of insecticide sprayed surfaces: Before spraying DDT, 24-hr contact bioassays were conducted using wild-caught *An. albimanus* to assess any possible pre-spray toxic effects of untreated plastic sheet material used as wall covering in both huts. After 3 replicate tests, no ill effects (i.e., mortality, knockdown) were noted that differed from controls. After treatment, Hut-1 (DDT) and Hut-2 (control), were compared for (1) mosquito mortality after contact with wall surfaces, and (2) degree of DDT toxicity to

wild-caught populations of *Anopheles* from time of post-spray. Table 6 compares 24-hr combined proportion mortality after 1-hr contact with DDT sprayed wall surfaces and control hut. Tests were conducted at periods approximately 1, 6, and 12 wks post-spray. DDT provided 100% mortality for *An. albimanus, An. vestitipennis* and *An. crucians*, at 1 and 6 weeks post-spray. By week 12, DDT still provided acceptable kill on 1-hr contact (>90%). Control mortality in the untreated hut was under 10%. Knockdown after 1-hr contact was absolute for all 3 species at 1 wk post-spray. Knockdown response decreased slightly at 6 wks and more substantially after 12 wks.

Post-treatment indoor/outdoor collections: After application of DDT to the interior walls of Hut-1, five all-night (12-hr) HLCs were conducted to assess the impact of insecticide on mosquito host-seeking and feeding behavior. Collections were conducted between 29April and 16 May, 1996, approximately 2 months after hut treatment until 15 wks post-spray. Several 6-hr collection trials were also made shortly before and after spraying in January. All night and 6-hr collections were not combined in the analysis. The HLC procedures were identical to pre-treatment assessment, (1) observe species population density and all night patterns of behavior with indoor and outdoor HLC, and (2) compare the 2 experimental huts under identical access conditions for mosquito response to insecticide and non-insecticide conditions. Sample size over 5 collection periods for *An. albimanus* (Fig. 17): Out (1,480), Hut-1 (73), Hut-2 (110); *An. vestitipennis* (Fig. 18): Out (371), Hut-1 (74), Hut-2 (183); *An. crucians*: Out (138), Hut-1 (09), Hut-2 (42); and *Ma. dyari* (Fig. 19): Out (611), Hut-1 (460), Hut-2 (641). Data for *An. crucians* and the 2 other *Anopheles* species was excluded from analysis on the

impact of DDT spraying as sample sizes were too low [*An. gabaldoni* was not detected in collections in Apr.-May and *An. punctimacula* was found in very low (n=15) numbers].

Figures 17-19 illustrate the combined 5 night HLC outdoors, and inside Hut-1 and Hut-2 for *An. albimanus*, *An. vestitipennis*, and *Ma. dyari*, respectively. Data in top figure are expressed as hourly natural log mean HLC per person/hr inside Hut-1 and Hut-2, with a matched single outdoor HLC starting at 1800-1845 to 0500-0545 hr. The lower figure is the all-night mean proportional distribution of mosquitoes per person/hr. During the 5 (12-hr) evening post-treatment periods, the combined 4 species accounted for 4,192 mosquitoes from all locations. Outdoor catches (2,600) made up 62 % of the total, followed by Hut-2 (23.3%), and Hut-1 (14.7 %). *Anopheles* collected by site summed to 2,480 mosquitoes, proportionally with 0.80 outside, 0.14 in Hut-2, and 0.06 in Hut-1. Combined, *An. albimanus* accounted for 67% of all *Anopheles* [excluding *An. punctimacula*), followed by *An. vestitipennis* (25.4%), and *An. crucians* (7.6 %). The combined hourly I:O ratio for *Anopheles* mosquitoes in Hut-1 was 0.07:1, compared with 0.17:1 for Hut-2. Less than half the number of *Anopheles* captured in Hut-2 were caught in Hut-1, for a Hut-1:Hut-2 ratio of 0.46:1.

Similar to pre-spray distribution, *An. albimanus* continued to show a strong propensity for outdoor activity compared to indoors for either hut (Fig. 17). The combined all-night indoor (H1+H2) v. outdoor proportion was 0.11, which was very near the pre-spray figure 0.094. The respective overall I:O ratios for Hut-1 (0.05:1) and Hut-2 (0.07:1) illustrate the relative small difference between sprayed and control huts and the strong exophagic component of the population. Comparing log mean HLC (Fig. 17), outdoor activity was distinctly higher than both indoor collections. The proportion

distribution for each collection site (outdoor and 2 huts) showed greater host-seeking activity in the early first half of the evening. With the exception of the sprayed hut, both the log means and proportion distributions demonstrated a clear early morning rise in activity beginning around 0300 hr until sunrise. Based on individual hour, proportion distribution and log mean catches, indoor activity for both huts peaked around 1900 hr, with a subsequent decline throughout the remainder of the evening. At 2 points (1900 and 2100 hr) Hut-1 had slightly more mosquitoes than Hut-2; however, over the entire evening, Hut-2 collected 1.5x more *An. albimanus*. Both figures illustrate the precipitous drop of activity in Hut-1 beginning around midnight, which plummets to zero after 0200 hr. Comparing huts, (H1/H2) relative proportions ranged by hour from 0.083-0.56 for Hut-1 during the first half of the evening, but spiraled down to 0 during the last 4 collection periods of the early morning hours.

The *Anopheles vestitipennis* population in April-May was considerably lower than pre-spray collections in December-January, a 3.65-fold decrease (2,291 v. 628) in overall average indoor and outdoor HLC. As seen during pre-treatment periods, *An. vestitipennis* continued to show much stronger affinity for indoor activity compared to *An. albimanus* (Fig. 18). In a few cases, hourly indoor catches in Hut-2 (unsprayed) exceeded the paired outdoor collection. Hourly I:O ratios varied from 0.048-0.435 for Hut-1 and 0.294-1.28 for Hut-2, further illustrating greater general endophagy for *An. vestitipennis* compared to *An. albimanus*. Hut-2 (unsprayed) indoor activity increased during the first 2-3 hours, remaining relatively high throughout most of the evening and declining at sunrise. Hut-2 collected 2.5x more mosquitoes than the sprayed hut, the differences becoming more pronounced as the evening progressed. The log means data (Fig. 18) showed a more

gradual increase in activity in the early evening and the leveling out for most of the remaining evening for outdoor and control hut collections. Conversely, Hut-1 showed a sharp fall in HLC by all measures between 2200 hr and midnight. Similar to *An. albimanus*, Hut-1 compared reasonably well with Hut-2 during the first half of the evening, capturing proportionally between 0.29-0.5 of the overall indoor mosquitoes, followed by a steep decline in activity beginning before midnight.

Mansonia dyari again provided the highest overall ratio of indoor v. outdoor activity of all species during the post-treatment collections. Total collection numbers were similar between pre- (1,422) and post- (1,712) collections. Both huts showed pronounced early evening highs of 2.8:1 and 4.5:1 I:O ratios for Hut-1 and Hut-2, respectively. This species remained active throughout the night, more or less equal activity inside and outside, showing a combined all-night indoor preference (I/O proportion of 0.64) compared to a near equal endophagic/exophagic proportion (0.54) during the pre-spray collections. Log means and proportional distribution curves (Fig. 19) were very similar for all 3 collection sites, showing a profound peak of activity in the early evening (1900-2000 hr), followed by an obvious decline in catch during the first half of the evening, and a gradual tapering off throughout the night. Individual site HLC hourly proportions produced virtually identical curves, similar to pre-spray data (Fig 16). In all, DDT seemed to have limited effect in reducing house entry and indoor biting activity.

Post-treatment response: Species response to DDT in experimental huts was analyzed several ways. For all 4 species, differences between huts reflected lower HLC

in the treated hut compared to the control. However, sample sizes by hour were only statistically sufficient to accurately analyze *An. vestitipennis* and *Ma. dyari*, and proportion analysis on those periods with small sample sizes should be viewed with extreme caution. Summarized information is presented in Tables 7-8 for proportion analysis, comparing mean hourly HLC per person/hr from post-spray treated and control huts, for *An. vestitipennis* and *Ma. dyari*, respectively. *An. vestitipennis* had comparable HLC numbers in sprayed and control huts during the first half of the evening. However, from midnight on, the difference in hourly proportion catch were, in most cases, highly significant (p=0.001). Despite the apparent similarity in HLC between huts, *Ma. dyari* had 5 hourly periods scattered over the evening where significant differences were detected between huts. When all hours were combined within species, including *An. albimanus*, differences were found highly significant between treated and control huts ($0.005 < p < 0.001$). [p-value, standard error (SE) and 95% Confidence Intervals (CI) about the proportion for Hut-1 were: *An. albimanus* p=0.005, SE 0.0362, CI: 0.435-0.369; *An. vestitipennis* p=<0.001, SE 0.0282, CI: 0317-026; *Ma. dyari* p<0.001, SE 0.0148, CI:0.433-0.403].

Using the same post-treatment data, the proportion decline (column C) and the cumulative rate of decline (column D) for treated vs. control huts is presented for *An. vestitipennis* and *Ma. dyari* in Tables 9-10, respectively. These 2 patterns of change in HLC numbers in sprayed v. control huts by time at night are displayed in figures 20-22 for *An. albimanus, An. vestitipennis,* and *Ma. dyari.* Column C represents the relative contribution for each hour to the total amount of reduced biting in the treated hut. Negative values indicate a greater amount of HLC in treated compared to control for that

hour. The cumulative values (column D) represent the overall reduced biting in the treated hut by hourly intervals over the entire evening. However, these cumulative values are only useful for studying the pattern of reduced biting and are not measures of amount of reduced biting. With a few exceptions, most proportions of reduction in the sprayed hut varied from none to 0.20 each hour. All 3 species showed reductions in host-seeking activity, with consistently greater (steeper slope) declines in HLC during the last half of the evening. However, any conclusion drawn for *An. albimanus* is hampered by the small indoor sample sizes encountered post-spray.

Another possible way of looking at the data would be to compare the pre and post HLC data, by species, for both huts to derive a proportion (or %) reduction in the treated hut. For a number of reasons (see Discussion), this formula was found inappropriate for comparing the pre (Dec.-Jan.) and post (April-May) all night data collections as an accurate, unbiased measure of mosquito activity or assessment of DDT on house-frequenting/biting mosquito populations. However, five (6-hr) HLC (3 pre, 2 post) conducted around the time of spraying (Jan. 24) were conducted and analyzed. Collections occurred between 11-30 January 1996. Pre and post comparisons of treated v. control huts showed significant percent reductions in HLC during the first half of the night in the sprayed hut: 66.2% *An. albimanus* (n=77); 51.6% *An. vestitipennis* (n=395); 72.2% *An. crucians* (n=52); and 50.6% *Ma. dyari* (n=944). All 4 species combined showed a 52% reduction, and by *Anopheles* alone, a 50% reduction in the sprayed hut compared to the control.

Mark-release-recapture trials: A series of mark-release-recapture experiments were conducted inside treated and control huts in an attempt to observe behavioral differences, if any, between freshly blood-fed (BF) and unfed (UF) adult *An. albimanus* and *An. vestitipennis* in response to DDT. Figures 23 and 24 indicate the combined percentage of freshly BF and UF female *An. albimanus* and *An. vestitipennis*, respectively, remaining indoors (dead and alive) in the DDT treated v. control huts over the course of the evening and early morning hours. Percentage remaining indoors per observation period was based on marked mosquitoes present in escape intercept boxes and UV light observations inside both huts at 2-hr intervals.

Most BF and UF mosquitoes eventually escaped or died in the huts over the course of the evening. For both species, there was a faster escape response among UF in the sprayed hut compared to the UF in the control. Differences were less obvious for BF females between huts. Only BF *An. albimanus* remained in the control hut to any significant degree (18% of released) after 1000 hr. Tables 11 and 12 provide breakdowns of proportion analysis for *An. albimanus* and *An. vestitipennis*, respectively, comparing the cumulative proportions from 2-hr periods for treated and control huts and BF v. UF mosquitoes. Statistical comparisons were not made between species. In nearly all cases, significant differences when seen, whether BF or UF, indicating the more rapid escape response on the part of the mosquitoes exposed to the treated hut compared to the control. The lone contrariety was a greater number of BF *An. vestitipennis* remaining in the treated hut v. control at 2400 hr. Differences between treated and control were greater with UF than BF mosquitoes. For most periods, the difference in percentage remaining indoors for UF in treated v. control for both species were highly significant ($p<0.001$).

BF specimens for both species, on the other hand, appeared more content to remain longer periods in either hut during the evening, and only the early morning (0600 hr) exodus showed a more significant retreat from the treated dwelling.

The number of marked dead mosquitoes on the floor was greater in the treated than control hut and greater among BF than UF. Most recorded deaths occurred between 2000-2400 hr; however, the overall number was small. Less than 5% of released mosquitoes in the sprayed hut were found dead on the floor. Foraging ants and spiders were considered limited during the evening and were not believed to have greatly influenced these observations. Likewise, the percentage mortality seen in the exit boxes was not significantly different ($p>0.05$) between huts either at the time of collection or after the 24-hr. holding period. For reasons that are unclear, the percentage mortality among exit box captures from both treated and control huts after 24-hr holding was over 50%.

With the aid of an UV illuminator, indoor resting behavior was observed in both huts. In the unsprayed huts, over 80% of the UF tended to rest on walls (usually below 1 m from floor). BF mosquitoes tended to rest on walls (40%) and lower ceiling (60%) on supporting poles and thatch. Virtually all BF *An. albimanus* remaining in huts after 1000 hr were found on the extreme upper walls or lower ceiling. Marked unfed specimens of either species were not seen resting indoors after 1000 hr. On very rare occasions, 1 or 2 BF marked specimens could be seen resting on the sprayed walls. All indoor mosquitoes in Hut-1 were observed either in the exit boxes, lower ceiling area, or found dead/moribund on the floor. All marked mosquitoes in the treated and control huts were

accounted for and recorded as having died, been captured (exit box, predators), or escaped by some other means of egress between 0600-1000 hr.

ANCILLARY OBSERVATIONS

Circumsporozoite assays: ELISA results from 2,309 pools (n=11,966) of *Anopheles* from Caledonia found 3 reactive 'positive' pools for CSP, 2 *P. vivax* 210 and 1 *P. vivax* 247-variant as follows: *An. albimanus*: (sample #588), a pool of 6 specimens captured 1830 hr. outdoors (19 December 95), positive for *P. vivax* 247, absorbance value 0.767 (negative control= 0.266, positive control= 1.736) at 15 min; *An. vestitipennis*: (sample # 2311), a pool of 2 specimens captured 1930 hr. outdoors (30 January 96), positive for *P. vivax* 210, absorbance value 0.478 (negative control= 0.161, positive control= 0.736) at 15 min; and *An. gabaldoni*: (sample # 2303), 1 specimen captured 1930 hr. outdoors (29 December 95), positive for *P. vivax* 210, absorbance value 0.320 (negative control= 0.161, positive control= 0.736) at 15 min. Total sample size was 929 pools (5,502 specimens) *An. albimanus*; 1,003 pools (n=5,448) *An. vestitipennis*; 282 pools (n=880) *An. crucians*; 32 pools (n=60) *An. gabaldoni*; and 63 pools (n=76) *An. punctimacula.*

Assuming a sporozoite rate below 1% for the area, the presumption would be each positive pool (mean pool size= 5.2 mosquitoes) contained only one reactive mosquito. Overall sporozoite rate (SR) based on total number of mosquitoes tested was low-0.025%. Breakdown by species, found both *An. albimanus* and *An. vestitipennis* with a SR of 0.018%, and *An. gabaldoni* at 1.67%. The relatively high SR for *An. gabaldoni*

was skewed by the lower sample size and probably overestimates true vector potential for this species. All 3 positive mosquitoes were captured outdoors, during early evening hours exclusively, from Dec.-Jan. collection periods. Based on mean outdoor human landing-biting rates (adjusted 6-hr and 13-hr collections), a derived (EIR) entomological inoculation rate (estimated number of infective bites per unit time) was calculated (sporozoite rate x HLC per person/night). During the Dec.-Jan. period, combined (all species) estimated outdoor risk of infection was 0.131 infective bites per person/day. By species, the EIR was *An. albimanus* 0.029; *An. vestitipennis* 0.065; and *An. gabaldoni* 0.1125 per person/day. The inverse (1/EIR), or the number of days between potential infective bites during the 2 month period would have been less than 1 per person/wk (7.65 days), or the equivalent of roughly 1 infection per 100 person-hrs evening exposure (2 mos. = 720 evening hrs of exposure).

Blood meal assays: Dry season (December '95-January '96) outdoor resting collections captured 77 anophelines containing fresh or recent blood (Table 15). In all, 15 collection attempts were made during mid-morning hours (0900-1000) in and around the immediate study site, producing an average of ~5 blooded anophelines per collection. Using the backback aspirator, *Anopheles* were the most commonly collected genus, followed by *Culex* (*Melanoconian*) species. Preferred outdoor resting sites appeared to be amongst the thick grasses and sedges (e.g., arrowleaf) near and within the marshy areas surrounding the huts. Mosquitoes were not captured resting underneath the huts. The degree of adult dispersal from the immediate study site after blood feeding was neither investigated nor known. Overall, there would appear to have been few other

'protected' sites available nearby, excluding the huts and vertically in surrounding trees. Furthermore, there were no domestic animal shelters near the study site.

Although the sample sizes were small, it was found that human and cattle blood were the preferred hosts at the study site. Both cattle and humans were the dominant large mammals in the area at the time of the surveys. Only 1 specimen (*An. albimanus*) tested positive for canine (dog) blood. Although swine and fowl (chicken) were common in the area, especially during daylight hours, feedings on these animals was not detected in collected mosquitoes. No mixed-host reactions were detected using the ELISA, indicating tested blood was most likely taken from a single host or generic group. Based on these results, *An. albimanus* and *An. crucians* appeared to favor bovine blood over human (human:bovine blood ratio: 1:1.8 and 1:2.5, respectively). *An. vestitipennis* appears to have had a stronger preference for human blood (2.2:1).

Discussion

In a series of experiments using paired experimental huts, a significant difference in behavioral response to DDT residual deposits was observed in natural populations of *An. albimanus* and *An. vestitipennis* compared to unsprayed walls in a control hut. Observations for both species provided clear evidence of behavioral avoidance; however, from the amount of data collected, strong support of this excito-repellency effect was evident in *An. vestitipennis* only. *Anopheles albimanus* produced weaker evidence because of low numbers collected inside houses during the post-spray period. In general, mosquitoes would move into the human habitat beginning just after sunset with observed clustering on indoor walls. Without the presence of DDT on the walls, mosquitoes would enter, rest and feed at their accustomed leisure throughout the night. However, with DDT, entering *Anopheles* mosquitoes were compelled to leave much sooner than normal, affecting their normal host-seeking behavioral patterns. Biting activity appeared restricted to those hours of population movement (host seeking), in particular during the beginning and first half of the evening. Entering mosquitoes that would normally rest indoors before attacking were effectively eliminated in the treated hut. Overall indoor feeding activity was significantly reduced over the entire course of the evening in the treated hut compared to the control, especially during the later half of the evening. Together, the results from experimental hut trials and excito-repellency chambers (Chapter 3), using the same local wild-caught mosquito populations, provide strong complimentary findings on the excito-repellent properties and the resultant behavioral avoidance of female *Anopheles* in the presence of DDT residues. The implications from these observations are considered to have profound epidemiological consequences on the

transmission of malaria. A reduction or deterrence in feeding activity, by any measure, is a reduction in risk of acquiring malaria.

The evaluation of the residual lifespan or 'effectiveness' of DDT has been made in several studies. The excito-repellency activity of DDT has been found to linger many months after contact toxicity has significantly declined (Reid & Wharton, 1956; Nutsathapana, et al. 1986). Taylor, et al. (1981) noted continued efficacy of DDT with *An. arabiensis* after 2 years post-spray. Roberts and Alecrim (1991) saw reduce biting of *An. darlingi* in the sprayed hut compared to the control after 1 year, and Rozendaal (1990) saw almost > 95% mortality with DDT for 12 months post-treatment. Mpofu, et al. (1988) demonstrated DDT deposits were effective in killing *An. arabiensis* up to 15 months post-spray in experimental huts. Sharp, et al. (1990) found minimal loss of toxic effectiveness, giving 100% mortality 8-12 months post-spray. In Caledonia, comparisons of 24-hr combined proportion mortality after 1-hr contact with DDT sprayed wall surfaces produced 100% mortality for *An. albimanus, An. vestitipennis* and *An. crucians*, at 1 and 6 weeks post-spray. By week 12, DDT still provided acceptable kill on 1-hr contact (>90%). Knockdown after 1-hr contact was absolute for all 3 species at 1 wk post-spray. Knockdown response decreased slightly at 6 wks and more considerably after 12 wks post-spray. This indicated the residual life of DDT in the sprayed hut was available at sufficient strength to likely provoke a behavioral response in mosquitoes during the 2-3 month post-spray observations.

Historically, vector control using indoor residual insecticides has been the focus of most malaria control efforts (Pant, 1988). Recently, the use of insecticides, specifically DDT, for vector and transmission control have been seriously questioned

regarding utility, acceptance and impact (Curtis, 1994; PAHO, 1994; WHO, 1994). Understandably, innate vector avoidance of houses via exophagy and exophily has been a legitimate consideration limiting the impact of indoor residual spraying (Gillies, 1956). However, other reasons behind the proposed changes from past malaria control strategies is the widely held belief that insecticide resistance (i.e., reduced susceptibility) and behavioral avoidance of residual insecticides would effectively negate any significant reduction in transmission (de Zulueta, 1959; Busvine & Pal, 1969; Hadaway, et al. 1970; Bang, 1985; Sharp, et al. 1990). However, convincing evidence and rationale for the continued use of DDT or other chemicals, as a sound means for vector-borne disease control, has been presented (Brown, 1972; Brown et al., 1976; Service, 1992, Roberts & Andre, 1994; Roberts et al. 1997a). In some countries that have continued or renewed the use DDT, in spite of high levels of physiological resistance present in vector populations, this chemical continues to elicit profound efficacy in reducing disease incidence (WHO, 1986; Haworth, 1988; Roberts & Andre, 1994). One possible explanation would be the altered patterns of host-seeking and feeding activity, including behavioral avoidance, acting as a prime mechanism to reduce human-vector contact. Hudson (1984) concluded residual insecticide (DDT) worked to reduce malaria not just by killing but by reducing the number entering the sprayed house, inhibiting blood feeding by those which did enter, and by driving normally endophilic mosquitoes outdoors after feeding.

Roberts et al. (unpub. doc.) developed an informative stochastic model to evaluate a chemical's toxic and excito-repellent attributes for use in vector control. The model quantitatively partitioned the 3 principal roles of insecticidal residues: toxicity,

repellency, and irritancy. For DDT residues, support from selected field data and experimental hut studies, has shown excito-repellency exerts the dominant action on modifying indoor host-seeking behaviors of certain vector species and that toxicity, although of some importance, provides the least impact suppressing host-vector contact (i.e., malaria transmission). The pre- and post-HLC and mark-release study data from Caledonia suggests that the conceptual model is indeed accurate and that predictions of behavioral impact are correct.

In this same unpublished work, Roberts et al. provided a series of pictorial representations (Figs. 25 & 26) of the various sequences of behavior a particular individual or species population may exhibit during host-seeking activities, with or without the presence of insecticides. The epidemiological descriptors revolved around combinations of movement, endo or exophagic and endo- or exophilic behaviors. In all scenarios, blood-feeding, followed by indoor or outdoor resting, occurs. The pattern of activity during pre and post-spray periods that most accurately described the observed and inferred behaviors seen in the Caledonia anophelines is illustrated in this series of host-seeking scenarios. Post-spray outdoor biting activity (Fig. 25) was undoubtedly less affected than indoor behavior. In general, for all species, the predominate outdoor host-seeking activity sequence before spraying was as depicted in # 1- mosquitoes exclusively resting and feeding outdoors. Even after spraying, certain portions of population may have ventured into the sprayed hut and either immediately moved out (#3), or attempted to rest first before proceeding outdoors to feed and rest (#4). These two scenarios would indicate either normal behavioral patterns at work or mechanisms involving primarily repellency (#3) or irritancy (#4) to DDT, respectively.

Six possible host-seeking scenarios were presented for indoor feeding activity (Fig. 26). Pre-spray activity in Caledonia was a combination of # 3, 5, and 6, all involving indoor blood feeding and eventual outdoor resting. Only on a limited basis did endophily occur with the anophelines in Caledonia, predominately *An. albimanus*. Post-spraying limited acceptable host-seeking choices, predominately to # 2. As witnessed with both *An. albimanus* and *An. vestitipennis*, early evening appetitive movement would start with outdoor resting of unfed females and movement closer to baited huts. Resting occurred on nearby vegetation and occasionally on outdoor walls and roofing thatch. At some point during the first half of the evening, a move would be made indoors (often peaking earlier for *An. albimanus*, followed within a few hours by *An. vestitipennis*). In the unsprayed structure, indoor movement would be followed by immediate blood seeking (#3) or resting (primarily on walls) (#5 & 6). *An. vestitipennis* was far more inclined to rest on walls during the evening before advancing to a host for feeding. Again, at some point, the mosquito would be driven to either rest on the walls or vacate the hut immediately after the blood meal (#2 & 3). Pre-feed indoor resting on walls covered varying periods of time, from as short as 5-10 minutes through to most of the evening. In some unusual instances, an unfed female would rest the entire evening in the hut, only to depart at daybreak without attempting to feed. In the control hut, mosquitoes preferring to rest on walls after a blood meal, would eventually leave the huts, at leisure, over the course of the night.

In the sprayed structure, the predominant sequence was # 2- entry into the hut, normal levels of blood feeding and a quick exit to rest outdoors. Rarely was an unfed or bloodfed specimen seen resting on sprayed walls. It would appear that either a repellency

or irritancy (i.e., induced by very brief tactile contact with DDT) influenced the early movement out of the sprayed indoor environment. In summary, pre-spray feeding behavior was provided with more host-seeking 'options' for entering, resting and indoor/outdoor movement; whereas, sprayed structures appear to significantly limit the course of acceptable events a mosquito is willing or capable to take. As suggested in this study, an overall reduction in indoor feeding (invoking Fig. 39, outdoor scenarios #3 & 4) or quicker exiting response during the first half of the evening (indoor scenario #2) took place in sprayed hut.

Contact irritancy, and secondarily, non-contact repellency can account for a reduction and a change in time of peak indoor biting activity. Kartman and da Silveira (1946) found that very short periods of contact with DDT were sufficient to inhibit most *An. gambiae* from taking a blood meal 1-2 hr after contact. Some experimental hut studies have reported little apparent interference with normal entry behavior in treated huts, and /or biting activity of mosquitoes once inside the hut (Symes & Hadaway, 1947; Muirhead-Thomson, 1947; Hadaway, 1950; Wharton, 1951; Colless, 1953; Thevasagayam, et al. 1979). However, many of these studies have recorded overall reductions in catch of blood-fed mosquitoes in exit-traps from sprayed huts (Muirhead-Thomson, 1950, 1960; Sharp, et al. 1990; Roberts & Alecrim, 1991). The effects of DDT on resting behavior, either reducing or eliminating pre- and post-feeding resting periods, is well documented (Muirhead-Thomson, 1960; Wilson, et al., 1973). Roberts & Alecrim, (1991) observed *An. darlingi*, while in the presence of DDT, would not remain indoors due to the excito-repellency actions of the chemical. Some normal level of early evening biting indoors might still occur, albeit reduced; however, feeding activity later in

the evening was greatly reduced or eliminated compared to the control. Colless (1953) found *An. balabacensis* was "activated" by DDT residues, causing the mosquitoes in treated structures to modify behavior and proceed to the next operation in the host-seeking scenario. However, in Colless' study, DDT afforded only minor protection against attack with only 20% of the indoor vectors leaving the hut unfed. He further speculated that the greater the influence of DDT, the greater the likelihood of complete inhibition of the feeding sequence, resulting in either mosquitoes exiting the hut unfed or deterrence of a post-resting period. This general scenario has also been suggested by studies with *An. albimanus* in Mexico and Colombia (de Zulueta & Garrett-Jones, 1965; Elliott, 1968). Other workers, however, have concluded that behavioral patterns accounting for reduced catches in treated huts were because of a reduced entry (repellency) and not to increased or unaccountable exit of unfed mosquitoes (Wharton, 1951; Wilkinson, 1951; Kuhlow, 1962; Slooff, 1964; Coz, 1965; Soerono, et al., 1965; Smith & Webley, 1969; Suwonkerd, et al. 1990). In other words, for some species, a greater proportion of the population would apparently remain outdoors to feed rather than venture into an unacceptable sprayed environment.

In my study, HLC data indicated that mosquitoes did enter the sprayed hut and that some normal level of biting occurred during the first half of the evening compared to the control. For both huts, the bulk of the nightly ingress occurred during the early evening (dusk-2200 hr). Mosquitoes in the treated hut were irritated/repelled from resting on indoor sprayed walls, disrupting the normal sequence of events in host-seeking. As the evening progressed, far fewer anophelines were available or attempted to take a blood-meal in the treated hut. These sprayed hut populations appeared more

inclined to leave the hut unfed later at night. As the resting population diminished, so did indoor biting densities during the later half of the evening. If we can assume that the same number of anophelines enter each hut, we might expect the same number of cumulative feeding attempts over the entire evening. This was not seen. Instead, succeeding hours after midnight found significantly reduced levels of biting in the sprayed hut, while the control hut continued with normal activity patterns. The result was an overall reduction in feeding (reduced vector-host contact) because of either reduced entry or, more likely, after entry into the hut, departure before attempting a blood meal.

Commonly, mark-release-recapture studies have been used to investigate adult mosquito dispersal patterns, estimate population size, feeding behavior, duration of gonotrophic cycle, and survival rates (Service, 1993b). In this study, marking techniques were used to investigate the influence of physiological conditions (freshly BF or UF) between treated and untreated huts. Significant differences by collection period were seen between BF and UF specimens in the treated and control huts, respectively. In general, BF specimens of *An. albimanus* appeared far more likely to remain for longer periods of time in either hut than UF. The treated hut saw an earlier exodus of UF v. BF for both *An. albimanus* and *An. vestitipennis* females. Control hut trials had a significantly greater percentage escape of UF compared to BF during the later half of the collection period (0200-1000 hr) for *An. albimanus*. The reverse was seen occurring in the control throughout the evening-early morning (2000-0800 hr) for *An. vestitipennis*, wherein the BF departed the control hut earlier and in greater number than UF. In the treated hut, BF *An. vestitipennis* were more likely to remain longer; whereas, UF females made a quick exodus from the hut compared to the control population.

Intrinsic factors concerning the mosquito and environmental conditions can effect irritability. Besides innate species differences, the influence of physiological variability (e.g., age, nutritional and gonotrophic status) in test populations and their response to insecticides has been an important consideration in the interpretation in excito-repellency tests (Cullen & de Zulueta, 1962; Coluzzi, 1963; Roberts, et al. 1984). Blooded mosquitoes have shown less irritability than unfed females (Barrera et al. 1959; Hecht et al. 1960; Roberts et al. 1984) which may cause delayed escape responses. The assumption is that the same effects would be applicable to mosquitoes exposed to treated experimental huts.

Anopheles albimanus is predominantly an exophilic/exophagic vector throughout its geographic range (Frederickson, 1993). The patterns of behavior seen in the series of mark-recapture trials seems reasonable, since freshly fed mosquitoes are more likely to rest for varying periods of time soon after feeding; whereas, UF females would remain in the host-seeking mode in search of blood. Because huts in these mark-release trials were devoid of bait (human or otherwise), UF *An. albimanus*, normally a more exophagic species, would more likely seek out alternative sites (i.e., outside the hut) for blood. Moreover, *An. albimanus* showed a slightly greater preference (neither species was endophilic) to rest indoors after feeding than *An. vestitipennis*. *An. vestitipennis*, on the other hand, appears to have had a much greater predilection for moving from the hut to outdoor resting sites soon after taking a blood meal. UF females were more likely to remain indoors for a time as this species showed a stronger pre-feeding resting behavior and endophagy in pre-spray collections. These series of observations are important, as physiological condition (BF v. UF) appears to significantly influence behavior with or

without the presence of insecticide (Tables 11 & 12). Intuitively, we could conclude that mosquitoes seeking blood meals and entering huts would be more likely to leave a treated structure before feeding than in an untreated structure. It also appears that mosquitoes that successfully acquire a blood meal, are also more likely to depart a treated hut earlier, thereby decreasing the likelihood of lethal contact with residual spray.

DDT has remained the most cost-effective and widely used residual insecticide in the world for the control of malaria (Service, 1992; Roberts, et al. 1997a). Yet, there exists a tremendous amount of conflicting information and perceptions regarding DDT's mode of effectiveness, or lack of, to control vectors and malaria transmission. In particular, the issue of behavioral responses (i.e., excito-repellency), reported over 50 years ago, has continued to spark debate and misunderstanding regarding its true implications for malaria control (Kennedy, 1946; Downs & Bordas, 1951; Gabaldon, 1953; Muirhead-Thomson, 1960; Roberts & Andre, 1994). Moreover, the literature is fraught with apparent contradictory observations and interpretations on avoidance behavior, possibly the result of experimental error or uncontrollable physiological, environmental and other biological factors. Considerable variations in excito-repellency among different natural field populations and within the same population have been reported, even under carefully controlled conditions (Cullen and de Zulueta, 1962; Coluzzi, 1963; Coz, et al. 1965; Quinones & Suarez, 1989). Unfortunately, issues of persistent environmental contamination and possible adverse effects on human health interceded in the late-1960's to nearly stop all research on DDT during the 1970's (Mpofu, et al. 1988). This has continued up to the present time, leaving most issues surrounding DDT unresolved. The intent of this study was not directed to prove or

disprove the various arguments on the use of DDT or debate the current 'global strategies' of control being advocated (Trigg & Kondrachine, 1998), but instead to show what behavioral effects chemicals, like DDT, may actually have on vector populations under natural conditions. Avoiding, to the extent possible, the vocal and political fray revolving around DDT and its continued use, this investigation aimed to provide a clearer understanding of the true impact and importance of certain insecticides, so that rational decisions on their use could be made.

Roberts (1993) has reviewed much of the background and issues surrounding behavioral avoidance of mosquitoes to insecticides, and the terms 'repellent', 'irritant' and 'excito-repellent' as defined by Roberts (1993) have been adopted throughout this study. Herein, 'behavioral avoidance' (excito-repellency) is defined as the ability of an insect to detect and avoid an insecticide-treated surface by contact irritancy (i.e., with physical tarsal contact) and/or non-contact repellency (i.e., without tarsal contact). Irritability is a general property of most insects and is an essential part in any protective behavior mechanism, representing one of the possible chemoreceptive responses to disagreeable external stimuli (Chapman, 1982). It is well known that DDT residues exert a direct irritant effect on most adult mosquitoes (Kennedy, 1946), and DDT contact has been shown to elicit specific effects on insect chemoreceptors and sensory hairs (Smyth & Roys, 1955).

Compared to irritancy, the repellent effect of insecticides has been far more difficult to objectively measure. Shortly after DDT and "Gammexane" (benzene hexachloride) were introduced for house spraying, the observed 'fumigant' effect and repellent properties of both were being advanced (Metcalf, et al. 1945; Gabaldon, 1949,

1952; Field, 1950; van Thiel, 1951; Muirhead-Thomson, 1951). De Zulueta and Cullen (1963) speculated such a deterrent effect must be detected at a distance, either due to a released "vapour effect" or to particles drifting in the air near treated dwellings. Smith and Webley (1969) were the first to demonstrate an outflow of DDT from the house interior after spraying which, in their estimation, resulted in strong repellency ("deterrency") on the part of local anophelines. Singh, et al. (1992) detected DDT residues from indoor air over many months post-spray, further indications that might account for the apparent noncontact responses.

As with physiological/biochemical resistance patterns, behavioral aspects must be evaluated in discrete and separate populations to determine their role in area-specific pathogen transmission and the assessment of control methods. The epidemiological relationship and response of each mosquito varies with the physiological condition, innate response to stimuli and prevailing environmental conditions (Hamon, et al. 1970, Roberts, et al. 1984). Problems in the interpretation of behavioral observations still exist because of the difficulty in measuring and analyzing behavioral patterns in populations and the paucity of quality field studies. Controversies still surround issues of development of so-called behavioral 'resistance' or adaptations (i.e., a population-based change in a species genome) in response to insecticides as opposed to behavioral avoidance as an innate natural response present in a population and not necessarily 'selected' for or against during time of exposure. (Muirhead-Thomson, 1960; Gerold & Laarman, 1964; de Zulueta, 1959, 1964; Taylor, 1975; Lockwood, et al. 1984). Despite the 40 years or more of DDT spraying in Caledonia, vector information prior to routine DDT spraying was not available. The question of behavioral resistance and mechanisms

involved in its purported development, were beyond the design of this study. Whether behavioral avoidance is an innate or selected characteristic, the impact of excito-repellency is significant.

Within panmictic (random mating) populations, behavioral heterogeneity of preferred resting sites has been reported for a number of important vectors (Smits et al. 1996). This intraspecific genetic variability supports the contention that non-uniform (non-random) exposure of a given spatial population, made up of two or more genetically defined subpopulations, is more reasonable under many natural conditions (Molineaux et al. 1979), contrary to uniform (random) exposure implicit in many models of malaria transmission and control (Macdonald, 1957). This may be valid; however, exposure and subsequent toxicity to an insecticide is not an issue when examining excito-repellency and its impact on vectorial capacity. If the assumption stands that mosquitoes must enter a house to obtain a blood meal (and transmit malaria), then regardless if a particular portion of the population is endophilic or exophilic, they must first obtain a blood meal before moving to their preferred resting site. However, in the sequence of events leading to a bloodmeal, many vectors may actually prefer to rest indoors before moving to a host (via a step-by-step attack). DDT and other insecticides clearly can have a marked effect on disrupting normal resting behavior (Tarzwell & Fisk, 1947; Wilson et al. 1973; Roberts & Alecrim, 1991). In this way, excito-repellency serves to interrupt or prevent blood feeding, independent of toxicity (but not necessarily insecticide concentration). Unfortunately, most transmission and control models have been based on the toxic impact of insecticides and have neglected the importance of avoidance behavior in the equation (Roberts & Andre, 1994).

There is a rich literature base on the influence of insecticides, particularly DDT, on mosquitoes inside sprayed houses. Field studies from Africa, Asia and the Americas have clearly demonstrated strong behavioral avoidance of sprayed walls by anophelines when using experimental huts (Muirhead-Thomson, 1960; Roberts, 1993). De Zulueta and Cullen (1963) described the repellent ("deterrency") effect of DDT and dieldrin that effectively prevented some anophelines from entering insecticide-treated houses. Similarly, others have found convincing evidence for a repellent effect on *An. gambiae* and /or *An. funestus* avoiding entering sprayed houses (Muirhead-Thomson, 1950; Wilkerson, 1951; Kuhlow, 1962; de Zulueta et al. 1961; Cullen & de Zulueta, 1962; Smith, 1963). In the Americas, Roberts and Alecrim (1991) demonstrated significant repellency in *An. darlingi* for up to two months post-spray in the Amazon Basin. Reduced house entering by *An. darlingi* after spraying has been observed in Guyana (=British Guiana) and Surinam (Symes & Hadaway, 1947; Rozendaal, et al. 1989). Repellency was a factor in the reduction of house entering and indoor human blood feeding by *An. pseudopunctipennis* in Mexico (Downs & Bordas, 1951; Loyola, et al. 1990; 1991a). In Asia, repellency has been shown or strongly indicated for members of the *An. punctulatus* complex in New Guinea (Slooff, 1964), *An. minimus* and *An. dirus* in Thailand (Nutsathapana, et al. 1986; Suwonkerd, et al. 1990), *An. maculatus* (with γ-isomer BHC) in Peninsular Malaysia (Wharton, 1951), and *An. culicifacies* in India (Shalaby, 1966). Roberts (1993) has provided further background evidence of insecticide excito-repellency in mosquitoes.

The two preferred methods for vector behavior studies have involved specially constructed experimental houses (huts), designed with entrance and exit traps to sample

mosquito populations, and the use of excito-repellency (ER) test chambers (boxes) to quantify direct responses to contact (irritancy) and noncontact (repellency) aspects of avoidance behavior. The 2 approaches used together can provide strong complimentary findings; however, few published studies have combined the two (Rachou, 1966; Roberts, et al. 1984; Roberts & Alecrim, 1991; Rozendaal, 1990). Slooff (1964) had also combined experimental huts and the excito-repellency test method measuring number of take-offs per min method (Coluzzi, 1963). The concept and box design is analogous to a single house where mosquitoes are placed inside and provided behavioral options to either rest, fly or exit the chamber (Roberts, et al. 1997b). ER boxes measure egress responses only, with mosquitoes being deliberately placed inside the chambers to measure escape only.

A more realistic system involves the use of specially constructed experimental huts located in the actual environment of the target mosquitoes, subject to natural environmental conditions (Smith, 1964; Muirhead-Thomson, 1968; Service, 1993b). Experimental huts have the advantage over ER boxes in measuring the free movement of mosquitoes into and out of sprayed dwellings. Certain disadvantages, such as uncontrolled climatic events, fluctuating and seasonal mosquito population sizes, and heterogeneous (e.g., age, physiological conditions) species populations, are generally outweighed by the greater opportunity to observe more natural vector responses compared to the reliance on experimental test chambers alone. Most often, these huts have been equipped with a variety of entry and exit traps fitted to windows, doors or eaves (Worth, 1953; van Thiel & Metselaar, 1955). Other devices have been designed and modified to augment captures in and around huts, such as verandah traps, louver

traps and the "Colombia curtain" (van den Assem, 1959; Smith, 1963, 1965; Elliott, 1972; Bown, et al. 1986). A review of the various hut designs, associated trapping methods, their use and interpretation is given by Service (1993b).

A number of studies investigating mosquito behavior have used experimental huts in Central and South America, either with or without the presence of insecticides (Symes & Hadaway, 1947; Giglioli, 1948; Downs & Bordas, 1951; Muirhead-Thomson & Mercier, 1952; Trapido, 1952; de Zulueta & Garrett-Jones, 1965; Duret, 1958; Rachou, et al. 1965, Rachou & Moura-Lima 1966; Rachou, et al. 1973; Elliott, 1972; Roberts, et al. 1984, 1987; Hudson, 1984; Rozendaal, et al. 1989; Roberts and Alecrim 1991; Loyola, et al. 1991a; Bown, et al. 1986, 1987, 1993; Cases, et al. 1994a,b). Studies using DDT or pyrethroids, found strong evidence of excito-repellency and avoidance behavior in sprayed structures.

This study represents the first use of experimental huts for vector studies in Belize for collecting or evaluating the response of mosquitoes to an indoor residual insecticide. However, prior to this study, Bertram (1971) attempted the use of a portable hut with human bait to catch mosquitoes entering cubical window traps in Belize, but found it "not successful enough for routine use". Both huts in Caledonia were identical in size and construction materials. These simple, single room structures were built along the lines of lower income housing utilizing a predominance of local materials (palm wood, palm thatch, etc.).

The decision to use plastic sheeting to cover interior walls of the huts was because (1) walls appeared to contribute less to entry access by mosquitoes compared to other open sites (i.e., windows), (2) covering the walls in both huts would make them more

comparable and would provide a more uniform coverage of spray in Hut-1, and (3) walls lined with light-colored material allowed mosquitoes to be more easily observed during resting (Duret, 1958). Another consideration was the potential, if necessary, to alter conditions of or between huts during the investigation. Spraying the walls directly would have permanently contaminated the huts for the duration of their functional life.

Because of the shortfalls of the intercept traps in Caledonia, direct measures of time of entry by mosquitoes could not be made. Human-landing collections served as a surrogate to number of mosquitoes entering a hut, but provided no specific information as to when they entered or how long they had rested inside the hut before attempting to feed. However, other published studies have managed to provide information on ingress behavior and measure the impact of indoor house spraying on other species of mosquitoes (Roberts & Alecrim, 1991).

Despite modifications of the entry and exit window traps into simple resting intercept boxes, these devices proved inadequate in obtaining sufficient numbers of mosquitoes to be of analytical use. Several studies have noted window traps as an inefficient means at capturing *An. albimanus* escaping from houses (Trapido, 1952; Muirhead-Thomson & Mercier, 1952; Mekuria, et al., 1990). In Jamaica, Muirhead-Thomson and Mercier (1952) had commented that the strong exophagic behavior of *An. albimanus* was a severe obstacle towards effective use of hut window traps. Only after the onset of the rainy season and baiting the hut with a donkey did collections increase. In Caledonia, behavior and population densities of *An. albimanus* were likely both factors in the efficiency of the intercept boxes and the overall indoor human-landing collections.

Overall, given the amount of effort, the return from the window intercept boxes was disappointing. With the exception of the mark-release study, very low numbers of mosquitoes (of any species) were captured exiting the huts during pre and post-treatment trials making analysis impossible. Far less mosquitoes were captured when boxes were positioned for entry collections. Generally, the only species caught entering the huts were *Culex quinquefasciatus*. The boxes were used as often as possible in the evening and general observations were possible. Whenever possible, boxes were left in place during the daytime to observe any activity during dusk. Interestingly, a few unusual species were captured in the exit boxes (*Aedeomyia squamipennis* and *Uranotaenia socialis*), both considered fortuitous events. With the exception of a few semi-gravid *An. albimanus* escaping huts at dusk, the greatest and most consistent activity was seen with *Culex quinquefasciatus*. On a number of occasions, numerous males and gravid females would depart from the huts. Interestingly, this species was very uncommon in HLCs and is believed this population had been feeding mostly on birds (chickens) common to the area.

Although low mosquito population densities were the most likely cause of window traps and modified interception boxes failing to capture sufficient mosquitoes, especially ingress populations, other factors, including box design, placement on the hut, competing sites of entry and exit, the behavior of the target species, likely influenced the inefficiency of these devices. It is possible that placement of any device in windows may have somehow been 'unattractive' or inhibitory to mosquito movement. Mosquitoes can exhibit visual responses to targets, especially conspicuous ones (Bidlingmayer & Hem 1979). Muirhead-Thomson (1991) has reviewed evidence for visual cues (shapes

and colors) as attractants or deterrents, noting interpretation of capture data must allow for known, and unknown, visual responses by the mosquito.

The attraction of a single human collector may not have been sufficient to entice more mosquitoes to enter huts. Other workers have overcome similar problems by baiting the huts with larger animals (Muirhead-Thomson & Mercier, 1952; Soerono, et al., 1965). Another problem may have been the frequency of inspection of the intercept boxes, 1-hr intervals being too insensitive to detect significant mosquito movement. Rachou, et al. (1965) modified window traps to eliminate as much as possible any obstacles (e.g., slits, cone-type openings) to the natural movement of *An. albimanus*. He used a simple open box, without a funnel or baffle, and inspected these window boxes at shorter intervals (every 30 min). He found this method to be a more acceptable because he thought that mosquitoes which entered the box unimpeded would be collected before they showed any tendency to move unobstructed back into or out of the hut again.

There is no argument that such a measure of ingress is of tremendous value in studies of house-frequenting behavior; however, an accurate, reliable method to assess entry behavior with certain species (e.g., *An. albimanus*) has confounded many investigators. Alternative methods have been used to overcome standard window trap deficiencies in measuring house-haunting mosquitoes. In particular, the Colombia curtain (net) deserves brief mention as an alternative to the more traditional hut designs (Elliott, 1972; Bown, et al. 1986; Loyola, et al., 1991a; Arredondo-Jimenez, et al. 1997). When placed over a house, the curtain serves a similar function as the verandah trap, enabling a sampling of mosquitoes exiting an enclosed structure. One method has been to collect resting mosquitoes off the net and place them inside the house to observe their

response. This method, has been accepted by some as a surrogate measure of ingress behavior. However, this appears to be an unreasonable assumption, that is, mosquitoes found landing on the outside of the curtain constitute some sort of implied or immediate entry behavior. By most designs it would be impossible for the observer to distinguish between normal outdoor resting (or aggregation) behavior prior to entry and actual time of movement inside the house. The obvious advantage that huts equipped with functional window traps have, is the actual measure of entry time and behavior.

There are approximately 14 species of *Anopheles* listed as present in the country of Belize (Wilkerson, et al. 1990). Fortunately, only a few species are believed important primary vectors of malaria in the country and the region as a whole, with *An. albimanus*, *An. darlingi,* and *An. pseudopunctipennis* generally topping the list (Kumm & Ram, 1941; Bertram, 1971; Roberts, et al. 1993). The presence of these 3 species in northern Belize has been documented (Kumm & Ram, 1941; Rejmankova, et al. 1993, 1995, 1998). *Anopheles darlingi* was not detected in Caledonia, despite its presence along the New River north and south of the study site (Manguin et al. 1996; Andre, et al. unpub. data.). Ecological conditions in the riverine areas between San Estevan and Libertad do not seem conducive to maintaining sufficiently large populations of this important mosquito species. Likewise, *Anopheles pseudopunctipennis* Theobald was not encountered, presumably because of unacceptable riverine habitats as well (Rejmankova, et al. 1993). Lastly, *An. vestitipennis*, the second most common biting anopheline in Caledonia encountered during my study, has been strongly implicated as an important vector in Belize (Kumm & Ram, 1941; Roberts, et al. 1993).

Species abundance is a critical factor in the study of mosquitoes and is among the greatest limiting factors in mosquito field research. In this study, only *An. albimanus* and *An. vestitipennis* were in sufficient numbers to merit detailed study. *Anopheles crucians* was captured in moderate to very low numbers, while the last 2 species encountered, *An. gabaldoni* and *An. punctimacula,* were relatively uncommon or very rare during the study. *Mansonia dyari* was the most common human-bait captured mosquito and was included for comparison with the *Anopheles*.

Relative to other anophelines in Central America, an impressive amount of published research has been generated on *An. albimanus* (Breeland, 1980; Elliott, 1969; Frederickson, 1993; PAHO, 1996). *Anopheles albimanus* has long been recognized as a major vector of malaria throughout most of its range (Foote & Cook, 1959), and has been found naturally infected with *P. falciparum* and *P. vivax* in nearly every country in which it is encountered (Frederickson, 1993). It is regarded the most important coastal vector of malaria in Mexico and Central America, and is considered a primary malaria vector in Belize (Kumm & Ram, 1941; Foote & Cook, 1959; Russell, 1963). It is generally the more abundant vector encountered on the northern coastal plain of Belize (Faran, 1980; Rejmankova, et al., 1993; Rodriguez, et al., 1993). This species often occurs in high densities, with periodic, seasonal fluctuations. Periods of maximum abundance generally coincide or immediately follow periods of high rainfall (Kumm & Zuniga, 1944). Throughout its range it has feeding habits considered primarily exophagic and zoophagic (Elliott, 1969; Elliott, 1972; Breeland, 1972; Garrett-Jones, et al. 1980). Much that has been described for this species was consistent with observations in the 1995-1996 Caledonia populations. *Anopheles albimanus* was most common during periods of

increased rainfall. Outdoor collections in Caledonia showed a regular bimodal distribution during the 3 collection periods. HLC were consistently higher in the early evening hours than the activity peak seen in the early morning (dawn) hours. This vector was almost exclusively exophilic. Strong bimodal biting patterns have been described for *An. albimanus* in Central and South America (Elliott, 1968; Frederickson, 1993), in Jamaica (Muirhead-Thomson & Mercier, 1952) and the Dominican Republic (Mekuria, et al. 1990).

The behavior of *An. albimanus* in response to insecticides, particularly DDT, was noted shortly after the introduction of residual house spraying in Panama (Trapido, 1952). Excito-repellency (irritability) to DDT in *An. albimanus* has been reported over most of its geographic range, from Mexico to Colombia (Brown, 1958; de Zulueta, 1964; Frederickson, 1993). Recently, tests carried out in Belize using excito-repellency boxes, have shown a strong excito-repellency escape response by 2 field populations to DDT, permethrin, and deltamethrin (Chareonviriyaphap, et al. 1997), and in Caledonia during my study (Chapter 3). Similarly, in coastal Chiapas (southern Mexico) and northern Guatemala, the pyrethroids, lambdacyhalothrin and permethrin, respectively (impregnated on bednets) produced noticeable excito-repellency against *An. albimanus* (Richards, et al. 1994; Arredondo-Jimenez et al. 1997).

Compared to *An. albimanus*, very little is known about the bionomics and malaria vectorial status of *An. vestitipennis*. Unfortunately, limited studies have reported on *An. vestitipennis*, while others have made little more than cursory comments on this species. Only recently, has the incrimination of *An. vestitipennis* as a malaria vector become stronger. It has been incriminated as a potential, probable, or secondary vector in

northern Guatemala, southern Mexico, Dominican Republic and Belize (Kumm & Ram, 1941; Mekuria et al. 1991; Loyola, et al. 1991b; Padilla et al. 1992; Roberts, et al. 1993; Arredondo-Jimenez, et al. 1998). In most cases, where *An. vestitipennis* has been 'incriminated', it has been a predominant species biting humans and often in association with *An. albimanus*. In Belize, it is thought that the vector efficiency of *An. vestitipennis* (i.e., indoor human biting rate) maybe a greater factor than shear numerical abundance for malaria transmission (Rejmankova, et al. 1998). Although adult *An. vestitipennis* has been described as comparatively uncommon throughout its range, it can be focally abundant as witnessed in Caledonia. It generally is seen at highest biting densities during or immediately following rainy periods (Rejmankova, et al. 1998). In Caledonia, this species was most common after the wet season, during the dryer, cooler months (Dec. '95-Jan '96). Although exceptions exist, this species has been found to be anthropophilic, while showing both exo- and endophagic host-seeking behaviors (Komp, 1942; Foote & Cook, 1959). This species has been reported commonly entering houses and readily feeding on humans in Guatemala (Richards, et al. 1994), Mexico (Vargas & Martinez-Palacios, 1956), and Belize (Foote & Cook, 1959; Roberts, et al. 1993). At one site in southern Mexico, it did not show a particular predilection for either indoor or outdoor feeding (Loyola, et al. 1991b). This species was found more exophagic than *An. albimanus* during collections in the Dominican Republic (Mekuria, et al. 1990). The Caledonia populations showed a greater proclivity to enter and attack humans indoors, especially compared to *An. albimanus*. Evening biting activity has been reported as unimodal (early evening) in the Dominican Republic (Mekuria, et al. 1990) and bimodal in Cuba (Marquetti, 1990). In Caledonia, this species was primarily unimodal, with an

early evening peak of activity but remain active throughout the evening. In Belize, Heinemann and Belkin (1977) recorded information on landing-biting collections from surveys in 1967 in the Cayo District by Bertram (1971). In that work, *An. vestitipennis* was found to be a frequent human biter during late afternoon and early evening collections within deciduous broadleaf forests around Roaring Creek and the Caves Branch area. *Anopheles vestitipennis* is generally regarded as exophilic (Elliott & de Zulueta, 1975); however, Navarro et al. (1986) has reported the species as sometimes endophilic in Cuba. In Caledonia, his species was found exclusively exophilic.

Little has been reported on insecticide resistance and behavioral responses to insecticides *An. vestitipennis* (WHO, 1992). However, Richards, et al. (1994) found a marked reduction in the indoor resting population in houses that had permethrin-impregnated bed nets compared to control households, suggesting to the authors both a repellency and toxic effect. Likewise, excito-repellency tests in Caledonia showed a marked exodus from chambers containing DDT and deltamethrin (see Chapter 3) compared to controls.

Consistent with most published information, all evidence from the Caledonia study, including the sporozoite ELISA, blood-meal analysis, and HLC data suggests that *An. vestitipennis* may be a more important vector in Caledonia than *An. albimanus*, especially during periods of increased indoor biting densities. Given *An. vestitipennis* behavioral response to DDT, data from Caledonia would also indicate that indoor spraying would have a more dramatic effect on this species, compared to the more exophagic *An. albimanus*, thus resulting in a greater reduction in malaria transmission by this species.

Anopheles crucians, *An. gabaldoni*, and *An. punctimacula* made up a much smaller proportion of the human-landing population compared to *An. albimanus* and *An. vestitipennis*. *An. crucians* was the third most common anopheline taken in collections in Caledonia. Numerous studies attest to this species ability to experimentally develop human *Plasmodium*, and it has been found naturally infected in the southern United States (Simmons, 1941). *Anopheles crucians* is considered primarily zoophilic throughout its range; however, its importance as a natural vector remains unresolved (Horsfall, 1955; Foote & Cook, 1959; Floore, et al. 1976). Unfortunately, low capture numbers precluded any meaningful conclusions regarding behavior of this species indoors or out.

Anopheles punctimacula is regarded as a secondary vector of malaria in Central (Panama) and northern South America (Gabaldon, 1949; Wilkerson, 1990). It has wide distribution in the Neotropics, including all Central American counties (Rozeboom, 1941). Vargas and Martinez-Palacios (1956) seldom recorded the species inside houses, whereas in Panama, Simmons et al. (1939) reported this species as commonly taken in large numbers from houses and an avid feeder on humans. Gabaldon (1949) reported this species as highly domestic in Colombia, Venezuela and Brazil. In Caledonia, very few *An. punctimacula* were captured during the HLC activities and its role in malaria transmission remains unknown.

Information concerning *An. gabaldoni* is virtually nonexistent throughout its range and very little is known about its biology and possible medical importance in malaria transmission. White (1984) reported this species as a "putative" vector in Mosquitia, Honduras (Atlantic coast) because of its relatively high density indoor biting

activity during the early evening (1800-2100 hr). Landing catches of this species were low through most of the Caledonia study and completely absent during April-May. Of note, was the one CSP ELISA positive specimen. As far as known, this is the first indication that this species may play a role in malaria transmission in Belize.

The only known medical importance of *Ma. dyari* has been a few natural isolations of VEE virus during epidemic periods (Karabatsos, 1985). During the Caledonia study, this species was generally the most common mosquito in HLCs, and as a result, was included in the analysis. *Mansonia dyari* densities increased during the dryer periods of the study compared to the wet months (Sep.-Oct.). Read and Adames (1980) noted the same response for *Ma. dyari* in Panama, being a more frequent biter during the dry season. Despite the biased unmatched hut conditions in the pre-treatment phase and the post-spray treated v. control conditions, *Ma. dyari* indoor biting numbers appeared relatively unaffected when compared to paired outdoor collections and between huts. Compared to *An. albimanus* and *An. vestitipennis*, this species appeared to display a general disregard to the potential influence of reduced access and insecticide treatment. This species appeared to have no strong preference for feeding site, attacking collectors indiscriminately both indoors and out. Outdoor and indoor pre- and post-spray observations differed the least among all species, indicating that DDT had less of an effect (although still significant) on indoor activity of *Ma. dyari* than on *An. albimanus* and *An. vestitipennis*. Interestingly, in Uganda, de Zulueta and Cullen (1963) observed *Mansonia* species were not reduced in indoor numbers by DDT, whether applied indoors and /or on outdoor ingress surfaces. DDT also had a very low indoor irritant effect on *Mansonia uniformis* compared to anophelines in Tanzania (Smith & Webley, 1969).

The dstributions of various mosquito species throughout the Americas have been reported for many of the species captured in Caledonia. Faran (1980) reviewed literature of *An. albimanus* larval habitats and other mosquito species associations. Species commonly associated with *An. albimanus* in more permanent bodies of water (ponds, lakes and marshes) were *An. crucians, Ma. dyari,* and *Ma. titillans*. In general, other species commonly associated with *An. albimanus* and also found in HLC in Caledonia were *Culex* (*Melanoconion*) species, *Cx. coronator,* and *Cx. nigripalpus*. Both *An. vestitipennis* and *An. crucians* larvae have been found in association with *An. albimanus* in the Dominican Republic and Mexico (Mekuria, et al. 1990; Avila, 1977). Other species captured in Caledonia commonly associated with these 3 anophelines have been *Culex erraticus, Cx. pilosus,* and *Uranotaenia lowi* (Avila, 1977; Carpenter & LaCasse, 1955).

Rejmankova, et al. (1993, 1995, 1996) have described larval habitats of *Anopheles albimanus* and associated species (e.g., *An. crucians*) in northern Belize. *Anopheles vestitipennis* and *An. punctimacula* larval habitats also have been described (Rejmankova, et al. 1998). Although, *An. albimanus* and *An. vestitipennis* can commonly occur together as adult populations, respective larval habitats differ considerably. *Anopheles albimanus* prefers wetlands dominated by sparse emergent macrophytes (*Eleocharis* spp. and sawgrass), areas often developing extensive floating mats of cyanobacteria (blue-green algae) promoted by higher (limestone) water pH values. On the other hand, *An. vestitipennis* has a strong predilection for areas dominated by larger trees or tall dense macrophytes (e.g., swamp forest habitats) that provide the necessary

shade and cool fresh waters this species prefers. Adult aggregation of these 2 species appears influenced by feeding behavior.

Field studies commonly encounter a plethora of issues and obstacles not generally found in laboratory-based work. Controlled experiments investigating aspects of mosquito ecology and behavior are particularly difficult and time consuming (Service, 1993a). Likewise, it is known that mosquito behavior and population dynamics can vary temporally and spatially (Mattingly, 1962; Service, 1989). Studies are at the mercy of meteorological vagaries, terrain features, human activities and other uncontrollable local conditions that can, and often do, greatly influence vector distribution, behavior, and outcome measures (Garrett-Jones, 1970; Bidlingmayer, 1985). It has long been recognized that the local distribution of mosquitoes, such as *An. albimanus*, varies greatly from one period of time to the next (Zetek, 1915). Within broad limits the distribution and activity of insects in an area is probably governed by climate. The number of atmospheric variables that may affect biting activity are considered complex (Read & Adames, 1980). Adult insect human-biting activity is considered largely dependent on the environmental conditions of temperature and vapor pressure (Read, et al. 1978). Wolda & Galindo, (1981) commented that the most likely candidate for an environmental factor involved in the determination of variations in mosquito abundance was some feature in the weather, especially rainfall.

The Corozal District is notably drier than the southern districts in the country (Hartshorn, et al. 1984). Average annual rainfall for this region normally does not exceed 139 cm (54 in), reflecting a 3-fold lower annual rainfall compared to southern Belize (King, et al. 1992). This difference in precipitation has been described as having a more

pronounced seasonal effect on local mosquito larval habitat availability, affecting species distribution and resultant adult densities (Rejmankova, 1998).

Influence of climate on adult mosquito activity would seem intuitive for poikilothermic organisms. As temperatures decrease, so do normal activities (i.e., movement, feeding, etc) because of depressed metabolism. Other factors, such as air movement, can adversely influence activity. Generally the greater the air movement (wind) the less likely directed flight will take place. Increased air movement can also reduce atmospheric relative humidity, another critical element for adult insect survival. The subtropics (latitudes above 13°) have higher extreme and mean temperatures than occur in tropical areas. In Belize, the seasonal climatic effects are greatest in the central and northern (Corozal) regions (Hartshorn, et al. 1984). January through April-May are normally dry (less than 100 mm/month), and temperatures generally cooler (minima range 60.8-62.6°F or 16-17°C) than other months of the year. A meteorological event, a so-called "norther", can greatly alter typical weather patterns. Northers are cold, wet, northeast air occasionally pushed far to the south by arctic air masses during November to February. Local effects are cooler than normal temperatures and heavy rains. During this study, a "norther" occurred with much lower than average minimum temperatures, but without the normal accompanying increased rainfall. Human-landing collections in January were believed to be suppressed significantly by the inclement weather patterns. Unusually cool and dry conditions continued through Feb.-March. Roberts, et al. (1987) reported a similar effect on reduced mosquito host-seeking activity in Brazil during spells of unusually cool evening temperatures. In Caledonia, the prolonged period of unusual

weather may also have had an impact on mosquito collections that immediately followed in April-May.

Mosquito activity patterns (distribution) varied seasonally based on length of daylight. In Belize, time of sunset and sunrise varies about 50 minutes between short and long days during the year (Bertram, 1971). Varied times of sunset during the 3 periods of collections in Caledonia is reflected in the apparent shift in HCL activity. Shorter days (increased scotophase) resulted in 13-hr collections over the evening. April-May saw longer periods of daylight. resulting in a shift of delayed peak early evening biting by 1-2 hours.

Sampling bias and interpretation of biological and behavioral aspects of mosquitoes are influenced by variations in place, time and environment (Mattingly, 1962). Factors that influence mosquito host selection and biting behavior are considered complex and highly variable (Garrett-Jones, 1970; Bidlingmayer, 1985). Each time period (day, hour) represents a different combination of environmental factors and each collecting site a different ecological setting. The use of capture, trapping and other sampling methods play an essential part in all studies on the ecology and behavior of mosquitoes. The influence of trap design, timing, location and personal experience can greatly affect capture efficiency (Gillies, 1970; Bidlingmayer & Hem, 1979, Muirhead-Thomson, 1991). Usually a combination of sampling techniques are helpful to supplement the limitations of individual sampling methods (Muirhead-Thomson, 1968). For example, vector catch at a given time depends not only on the attractiveness of the individual bait and the availability of the mosquito, but also on the efficiency of the collector or catching device in enumerating the vectors present. Human-baited landing

catches possibly introduces the worst biases, as generally only mosquitoes which attack the host persistently are captured, and collection efficiency may not be constant throughout the evening hours. Understandably, some obstructing factors can come into play that may reduce collector efficiency. The density of mosquitoes, when great, can often overwhelm the attending collector's ability to aspirate all mosquitoes in a timely manner. Naturally, as the evening wears on, fatigue, boredom, or sleep deprivation can sometimes intercede, not uncommonly resulting in a higher proportion of blood feeding success during the later hours. Nevertheless, the information presented on the activity patterns or trends of adult mosquitoes is considered reflective of the behavior of these mosquitoes under natural conditions.

The inherent heterogeneity in biological measures and responses of individuals and populations, often requiring sufficiently large numbers of experimental replication of sampling and testing, complicates field studies further. For example, in the pre-spray, paired hut trials, any number of factors could have been at play affecting the comparability of huts under 'identical' conditions. For example, the nearly 2.5-fold difference seen in HLC between paired trials, 10 and 11, were separated in time by only 7 days (Table 3). In the final analysis, the huts were either not equal in their attractiveness to mosquitoes, or issues of inadequate sampling or other environmental or biological factors were involved that are beyond explanation.

The single greatest drawback in this study were issues of sample size and number of replicate trials. Unfortunately, the delay in post-spray assessment, number of collection trials, and the fluctuation in mosquito population densities during Apr.-May post-spray compared to Dec.-Jan. pre-spray made analysis and interpretation

considerably weaker than desired. Comparing periods, *An. vestitipennis* had a 3.65-fold decrease (2,291 v. 628) in overall average HL population in Apr.-May compared to Dec-Jan. *Mansonia dyari* remained relatively stable, with a small increase (1,712 v. 1,422) in HLC numbers in Apr.-May. Small sample sizes, in general, have much more variation about the means, lending to wider confidence intervals and weaker information. Test statistic approximations (sampling of independent observations) improve as sample size increases. In particular, *An. crucians* suffered considerably by low sampling numbers, and *An. gabaldoni* and *An. punctimacula* were beyond redemption in gathering any meaningful information.

The success of hut experiments is generally predicated on a high population of *Anopheles* mosquitoes maintained over several months (Muirhead-Thomson, 1949). The basis and assumption with using experimental huts is that for both pre and post-treatment of huts the data are comparable, with the only real measure of difference being the use of DDT in one of the structures. Unfortunately, this was not entirely the case in this study. The period between pre-hut and post-hut data ran between 2-3 months. Environmental (seasonal) changes alone were different enough over this time span to reasonably assume some sort of temporal influence on mosquito populations, both numerically and possibly physiologically. Overall, HLCs inside both huts were relatively low, a reflection of the overall mosquito population densities and house-entering behavior seen during the study.

Because of time limitations and the desire to answer a variety of technical questions regarding the huts, all of the 13-hr pre-treatment collections had unmatched hut conditions, which prevented statistical comparability with post-spray collections. Matched hut trials, when conducted, were for only 6-hr (half night) intervals. What has

been made clear from the HLC data presented, was the necessity to conduct all-night collections in order to observe the complete influence of DDT on reducing house-frequenting activity/biting behavior throughout the evening. Attempting to compare the half-night collections would provide less than half the possible information available with 12/13-hr collections. For a variety of reasons, all night collections were not always possible. During pre and post collection periods, sporadic periods of employment for the collectors from seasonal sugarcane production interfered and hampered organizing collections. Sugarcane work schedules were often set only 24-hr in advance, placing planned mosquito collection programs in disarray. Collectors, understandably tired from the day-long activities working in fields, were less than amenable to remaining awake all night collecting mosquitoes, only to return again, exhausted, to the field that following morning.

The percent reduction formula (see materials & methods section) using pre- and post-treatment and control data was inappropriate as an accurate measure of influence of DDT on mosquitoes. The data as collected, producing low mosquito densities, and the time span between pre and post collections, unfairly biased any meaningful analysis using this approach. The pre-treatment HLC in Hut-1 produced very few indoor samples, partly because of the more restricted access conditions placed on this hut greatly reducing numbers captured indoors. Reduced access to Hut-1 during pre-treatment trials compared to post-treatment collections reduced the calculated impact of spraying. However, for the sake of argument, if we could assume both Hut-1 and Hut-2 were equal ± a factor of 0.1 (10%) during the pre-treatment trials, the resulting calculations would show an overall

reduction in indoor biting activity. The range of percent values would have appeared as 26-40% (*An. albimanus*), 55-63% (*An. vestitipennis*), and 20-35% (*Ma. dyari*).

Ancillary observations provided some interesting information on the potential malaria vectors in Caledonia. Sporozoite ELISA results found 3 species were involved to some degree in the transmission of *P. vivax* malaria in Caledonia. The finding of positive *An. albimanus* and *An. vestitipennis* were exceptional, yet not unexpected finds, as both have been suspected or implicated in transmission of malaria in Belize (Kumm & Ram, 1941; Roberts, et al. 1993; Roberts, 1997a) and elsewhere in the Americas. The single sporozoite positive *An. gabaldoni* was a new finding, suggesting this species under appropriate conditions may be focally important in malaria transmission (White, 1984). However, during this study in Caledonia, overall HLC population densities were very low, relegating this species to a secondary role, at best.

In general, *An. albimanus* has shown exceptionally low natural sporozoite rates, rarely reaching 2.0%, and generally below 1.0% (Frederickson, 1993). This species is considered to have a low vectorial capacity and generally requires large population numbers for effective malaria transmission (Elliott, 1972; Rodriguez, et al. 1992; Loyola, et al. 1993). However, even high population densities of this species do not necessary translate to increased transmission rates to the human population (Bown, et al. 1991). Much less is known natural malaria infections in *An. vestitipennis*. Arredondo-Jimenez, et al. (1995) reported a sporozoite rate of 0.6/1000 (0.06%) mosquitoes collected in the Lacondon Forest of southern Mexico near Belize.

In Caledonia, all 3 infected mosquitoes were found from early evening outdoor collections. Analysis found sporozoite rates (0.018% or 0.182/1000) were within range

of previous reports for both *An. albimanus* and *An. vestitipennis*. The combined *Anopheles* HLC rate outdoors during Dec.-Jan. was 521 per person/night, and a combined indoors rate 237 per person/night. Although indoor infected mosquitoes were not detected, it may have been influenced by the lower sample size. Based on the results, during December 1995 and January 1996, a person living in Caledonia was nearly 8 times more likely to contract malaria outdoors than inside a house. The infection rates were low in both species, indicating infection risk to human populations in Belize is lower compared to many endemic regions of the world (Arredondo-Jimenez, et al. 1996).

Bloodmeal analysis of outdoor resting collections indicated local anophelines would readily feed on humans and larger mammals (bovines). Other animals (pigs, chickens and dogs) although present, were not represented in blooded mosquitoes. *Anopheles albimanus* has shown a preference for larger animals, and on some occasions would feed on fowl (Faran, 1980). In Jamaica, pigs and goats were not found attractive for *An. albimanus*, while the species was considered an indiscriminate (random) feeder (Muirhead-Thomson & Mercier, 1952); whereas, pigs were found to be acceptable baits for both *An. vestitipennis* and *An. crucians* in the Dominican Republic (Mekuria, et al. 1990).

Partial gravid and fresh fed female mosquitoes were found in daytime resting among dense grass and scrubs. Again, information on host preference appears limited to *An. albimanus*, wherein this species has been observed resting in low and high grasses by day (Zetek, 1915; Frederickson, 1993). In general, most attempts by other investigators to locate outside resting sites have yielded poor results. *An. albimanus* and *An. crucians* appeared to favor bovine blood over human (human:bovine blood ratio: 1:1.8 and 1:2.5,

respectively). *Anopheles vestitipennis* appears to have had a stronger preference for human blood (2.2:1). No attempt was made to determine the pregravid rate in blooded mosquitoes, an indication of multiple blood meals may be required to complete the initial or subsequent gonotrophic (ovarian) cycles. Arredondo-Jimenez, et al. (1998), found a high proportion (> 0.29) of *An. vestitipennis* gravid females with fresh blood in the Lacandon Forest, indicating that multiple contacts occur frequently between mosquito and hosts and that additional blood meals may be needed to complete the ovarian cycle. Ecologically, the Caledonia area is considered an extension of the Lacandon Forest of southern Mexico. Multiple feedings have also been suggested in laboratory studies using *An. albimanus* (Briegel & Horler, 1993). Epidemiologically, additional blood meals taken by pregravid females increases the potential infection rate in the vector population. Although both species appear to play a role in malaria transmssion in Caledonia, the evidence taken together, from sporozoite ELISA, blood-meal analysis, and indoor HLC, suggests that *An. vestitipennis* may be a more important vector in Caledonia than *An. albimanus*, especially during periods of increased indoor biting densities.

Accurate knowledge of the influence of insecticides on vectors and a clearer understanding of the local malaria ecology and epidemiology, should enable vector control efforts to be more selective and cost-effective. This study provided important new information from Belize for comparison with other malaria vector populations in Latin America. Issues over the continuation of use of DDT or other insecticides for the control of malaria will remain a debated subject into the future (Gabaldon, 1978; Davidson, 1989; Farid, 1991; Curtis, 1994; Roberts et al., 1997a). This study does not attempt to answer this broad question. Instead, this study presents results and

interpretations that were temporally and geographically limited. Caution should be exercised extrapolating results beyond the site examined to other regions having the same vector species and seemingly similar epidemiological or ecological characteristics. Nevertheless, the success or failure of any control approach, whether standard (e.g., insecticides, chemotherapy) or emerging technologies (e.g., vaccines, biological/genetic control) will depend ultimately on a better understanding of the natural dynamics of malaria transmission in the field.

REFERENCES CITED

Arredondo-Jimenez, J.I. 1995. Comparative ecology of allopatric populations of *Anopheles (Anopheles) vestitipennis* (Diptera: Culicidae). Ph.D. Dissertation. Department of Entomology, University of California, Davis, CA. 172 pp.

Arredondo-Jimenez, J.I., M.H. Rodriguez and R.K. Washino. 1996. Temporal and spatial dynamics of *Anopheles* vectors in Chiapas, Mexico. VI Symposium. Mosquito Vector Control and Biology in Latin America. 62nd Ann. Mtg. Am. Mosq. Control Assoc. Norfolk, VA. (Abstract).

Arredondo-Jimenez. J.I., M.H. Rodriguez, E.G. Loyola and D.N. Bown. 1997. Behavior of *Anopheles albimanus* in relation to pyrethroid-treated bednets. Med. Vet. Entomol. 11:87-94.

Arredondo-Jimenez. J.I., M.H. Rodriguez and R.K. Washino. 1998. Gonotrophic cycle and survivorship of *Anopheles vestitipennis* (Diptera: Culicidae) in two different ecological areas of southern Mexico. J. Med. Entomol. 35:937-942.

Assem, J. van den. 1959. A window-trap experiment to test the effects of dieldrin under local conditions in the Merauke area (Netherlands New Guinea). Trop. Geog. Med. 11:32-43.

Avila, I.G. 1977. Fauna Cubana de Mosquitos y sus Criaderos Tipicos. Academia de Ciencias de Cuba. Dir. Pub. ACC, Habana. 84 pp.

Bang, Y.H. 1985. Implications in the control of malaria vectors with insecticides in tropical countries of South East Asia. J. Com. Dis. 17:199-218.

Barrera, A., O. Hecht and O. Mancera. 1959. Estimacion cuantitativa de la excitacion producida por el contact con superficies tratades con DDT en tres especies de anofelinos mexicanos. [Quantitative estimation of the irritation produced by contact with surfaces treated with DDT in three species of Mexican anophelines.] In: Trop. Dis. Bull. 1960, 57:788-789.

Beach, R.F., C. Cordon-Rosales, E. Molina and A. Wirtz. 1992. Field evaluation of an enzyme-linked immunosorbent assay for estimating the sporozoite rate in *Anopheles albimanus*. Am. J. Trop. Med. Hyg. 47:478-483.

Belkin, J.N., R.X. Schick and S.J. Heinemann. 1965. Mosquito studies (Diptera:Culicidae). V. Mosquitoes originally described from Middle America. Contr. Am. Entomol. Inst. 1(5):1-95.

Bertram, D.S. 1971. Mosquitoes of British Honduras, with some comments on malaria, and on arbovirus antibodies in man and equines. Trans. Roy. Soc. Trop. Med. Hyg. 65:742-762.

Bidlingmayer, W.L. and D.G. Hem. 1979. Mosquito (Diptera: Culicidae) flight behavior near conspicuous objects. Bull. Entomol. Res. 69:691-700.

Bidlingmayer, W.L. 1985. The measurement of adult mosquito population changes some considerations. J. Am. Mosq. Control Assoc. 1:328-348.

Bown, D.N., J.R. Rios, C. Frederickson, G. Del Angel Cabanas and J.F. Mendez. 1986. Use of an exterior curtain-net to evaluate insecticide/mosquito behavior in houses. J. Am. Mosq. Control Assoc. 2:99-101.

Bown, D.N., E.C. Frederickson, G. del A. Cabanas and J.F. Mendez. 1987. An evaluation of bendiocarb and deltamethrin applications in the same Mexican village and their impact on populations of *Anopheles albimanus*. Bull. Pan Am. Hlth. Organ. 21:121-135.

Bown, D.N., M.H. Rodriguez, J. I. Arredondo-Jimenez, E.G. Loyola and M. del Carmen Rodriguez. 1991. Age structure and abundance levels in the entomological evaluation of an insecticide used in the control of *Anopheles albimanus* in southern Mexico. J. Am. Mosq. Control Assoc. 7:180-187.

Bown, D.N., M.H. Rodriquez, E.G. Loyola, J.L. Arredondo-Jimenez and M.C. Rodriquez. 1993. Intradomiciliary behavior of *Anopheles albimanus* on the coastal plain of southern Mexico. Implications for malaria control. J. Am. Mosq. Control Assoc. 9:321-324.

Breeland, S. 1972. Studies on the ecology of *Anopheles albimanus*. Am J. Trop. Med. Hyg. 21:751-754.

Breeland, S. 1980. A bibliography to the literature of *Anopheles albimanus* (Diptera: Culicidae). Mosq. Syst. 12:50-150.

Briegel, H. and E. Horler. 1993. Multiple blood meals as a reproductive strategy in *Anopheles* (Diptera: Culicidae). J. Med. Entomol. 30:975-985.

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

Brown, A.W.A. 1972. The ecological implications of insecticidal usage in malaria programs. Am. J. Trop. Med. Hyg. 21:829-834.

Brown, A.W.A., J. Haworth and A.R. Zahar. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13:1-25.

Bruce-Chwatt, L.J. 1970. Global review of malaria control and eradication by attack on the vector. *In: Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Amer. 7(1):7-27.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitoes. Bull. Wld. Hlth. Organ. 31:645-656.

Busvine, J.R. and R. Pal. 1969. The impact of insecticide-resistance on control of vectors and vector-borne diseases. Bull. Wld. Hlth. Organ. 40:731-744.

Campbell C.C. 1997. Malaria: an emerging and re-emerging global plague. Imm. Med. Microbio. 18:325-331.

Carpenter, S.J. and W.J. LaCasse. 1955. *Mosquitoes of North America*. U.C. Press, Berkeley. 360 pp. + plates.

Casas, M., D.N. Bown and M.H. Rodriguez. 1994a. Intradomicillary pre- and postfeedingbehavior of *Anopheles pseudopunctipennis* of southern Mexico: Implications for malaria control. J. Am. Mosq. Control Assoc. 10:348-354.

Cases, M., M.H. Rodriguez and D.N. Bown. 1994b. Peri-/intradomicillary behavior in relation to host-seeking of *Anopheles pseudopunctipennis* in southern Mexico. J. Am. Mosq. Control Assoc. 10:355-362.

Chapman, R.F. 1982. *The Insects: Structure and Function*. 3ed. Harvard Univ. Press, Cambridge. Chapter 30: Chemoreception. pp. 736-755.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.H. Harlan, S. Manguin and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus* (Diptera: Culicidae), a malaria vector in the Americas. J. Am. Mosq. Control Assoc. 13:171-183.

Chow, E., R.A. Wirtz and T.W. Scott. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich of enzyme-linked immunosorbent assay. J. Am. Mosq. Control Assoc. 9:196-205.

Clark-Gil, S. and R.F. Darsie. 1983. The mosquitoes of Guatemala. Their identification. distribution and bionomics with keys to adult females and larvae. Mosq. Syst. 1 5:151-284.

Colless, D.H. 1953. The effect of DDT and kerosene upon adult behaviour in *Anopheles balabacensis* Baisas (=*A. leucosphyrus* Auct.). Ann. Trop. Med. Parasitol. 47:261-271.

Coluzzi, M. 1963. Studies on irritability of DDT to anopheline mosquitoes. WHO/VBC 6/25/63, 33: 22 pp.

Coz, J., M. Eyraud, P. Venard, B. Attiou, D. Somba and V. Ouedraogo. 1965. Expériences en Haute-Volta sur l'utilisation de pièges pour la measure de l'activité du DDT contre les moustiques. Bull. Wld. Hlth. Organ. 33:435-452.

Cullen, J.R. and J. De Zulueta. 1962. Observations on the irritability of mosquitos to DDT in Uganda. Bull. Wld. Hlth. Organ. 27:239-250.

Curtis, C.F. 1994. Should DDT continue to be recommended for malaria vector control? Med. Veter. Entomol. 8:107-112.

Davidson, G. 1989. Insecticide usage: an anti-alarmist view of its advantages and disadvantages. Trop. Dis. Bull. 86:R1-6.

De Zulueta, J. 1959. Insecticide resistance in *Anopheles sacharovi*. Bull. Wld. Hlth Organ. 20:797-822.

De Zulueta, J., G.W. Kafuko, J.R. Cullen and C.K. Pedersen. 1961. The results of the first year of a malaria eradication pilot project in northern Kigezi (Uganda). E. Afr. Med. J. 38:1-26.

De Zulueta, J. 1962. The irritability of mosquitos to DDT and its importance in malaria eradication. Riv. Malariol. 41:169-178.

De Zulueta, J. and J.R. Cullen. 1963. Deterrent effect of insecticides on malaria vectors. Nature, Lond. 200(4909):860-861.

De Zulueta, J. 1964. Ethological changes in malaria vectors. A review of the situation in the light of recent findings. Riv. Malariol. 43:29-36.

De Zulueta, J. and C. Garrett-Jones. 1965. An investigation of the persistence of malaria transmission in Mexico. Am J. Trop. Med. Hyg. 14:63-77.

Diehl, S.R. and G.L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. 29:471-504.

Downs, W.G. and E. Bordas. 1951. Control of *Anopheles pseudopunctipennis* in Mexico with DDT residual sprays applied in buildings. Part V: Effectiveness of residual applications of DDT and gammexane up to one year after application under controlled conditions. Am. J. Hyg. 54: 150-156.

Duret, J.P. 1958. Quantitative measurements toward an understanding of behavioristic resistance in Panama (in the field). WHO/PAHO seminar on the susceptibility of insects to insecticides. pp. 318-322.

Elliott, R. 1968. Studies on man-vector contact in some malarious areas in Colombia. Bull Wld. Hlth. Organ. 38:239-253.

Elliott, R. 1969. Ecology and behaviour of malaria vectors in the American region. Cah. ORSTOM., ser. Ent. Med. et Parasitol. 7:29-33.

Elliott, R. 1972. The influence of mosquito behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21:755-763.

Elliott, R. and J. de Zulueta. 1975. Ethological resistance in malaria vectors: behavioural response to intradomestic residual insecticides. WHO/VBC/75.569. 15 pp.

Evans, R.G. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambdacyhalothrine and DDT to *Anopheles gambiae*. J. Am. Mosq. Control Assoc. 9:285-293.

Faran, M. E. 1980. Mosquito studies (Diptera: Culicidae) 34. A revision of the Albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. 15:1-215.

Farid, M.A. 1991. Views and reflections on anti-malaria programmes in the world. Kaohsiung J. Med. Sci. 7:243-255.

Field, J.W. 1950. Fumigant and repellent effects of BHC (Gammexane) and DDT upon *Anopheles*. Trans. Roy. Soc. Trop. Med. Hyg. 43:547-548.

Floore, T.G., B.A. Harrison and B.F. Eldridge. 1976. The *Anopheles* (*Anopheles*) *crucians* subgroup in the United States (Diptera: Culicidae). Mosq. Syst. 8-1-109.

Foote, R. and D. Cook. 1959. *Mosquitoes of Medical Importance.* Agriculture Handbook No. 152. U.S. Dept. of Agriculture. Washington, D.C. 158 pp.

Frederickson, E.C. 1993. Bionomics and control of *Anopheles albimanus*. Tech. Paper 34. Pan Am. Hlth. Organ. Washington, D.C. 76 pp.

Gabaldon, A. 1949. The nation-wide campaign against malaria in Venezuela. Trans. Roy. Soc. Trop. Med. Hyg. 43:113-160.

Gabaldon, A. 1953. Possible effects of residual insecticides on the interruption of malaria transmission. Riv. Malariol. 32:155-171.

Gabaldon, A. 1978. What can and cannot be achieved with conventional anti-malaria measures. Am. J. Trop. Med. Hyg. 27:653-658.

Garrett-Jones, C. 1970. Problems of epidemiological entomology as applied to malariology. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):168-180.

Garrett-Jones, C., P.F.L. Boreham and C.P. Pant. 1980. Feeding habits of anophelines (Diptera: Culicidae) in 1971-78, with references to the human blood index: a review. Bull. Entomol. Res. 70:165-185.

Gerold, J.L. and J.J. Laarman. 1964. Selection of some strains of *Anopheles atroparvus* with different behavioural responses to contacts with DDT. Nature. 204(4957):500-501.

Giglioli, G. 1948. An investigation of the house-frequenting habits of mosquitoes of the British Guiana coastland in relation to the use of DDT. Am J. Trop. Med. 28: 43-70.

Gillies, M.T. 1956. The problems of exophily in *Anopheles gambiae*. Bull. Wld. Hlth. Organ. 15:437-449.

Gillies, M.T. 1970. Assessment of control, some problems in the measurement of anopheline populations. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Am. 7(1):156-167.

Gillies, M.T. 1988. Anopheline mosquitoes: vector behaviour and bionomics. In: *Malaria, Principals and Practice of Malariology.* W.H. Wernsdorfer and I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 453-485.

Hadaway, A.B. 1950. Observations on mosquito behaviour in native huts. Bull. Entomol. Res. 41:63-78.

Hadaway, A.B., F. Barlow, J.E.H. Grose, C.R. Turner and L.S. Flower. 1970. Evaluation of compounds for insecticidal activity on adult mosquitoes. 1. Responses of adult mosquitoes to some new insecticides. Bull. Wld. Hlth. Organ. 42:353-368.

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. In: *Conference on Anopheline Biology and Malaria Eradication.* Misc. Pubs. Entomol. Soc. Amer. 7(1):28-41.

Harrison, G. 1978. *Mosquitoes, Malaria and Man: A History of the Hostilities Since 1880.* John Murrey, London. 314 pp.

Hartshorn, G., L. Nicolait, L. Hartshorn, G. Bevier, R. Brightman, J. Cal, A. Cawich, W. Davidson, R. DuBois, C. Dyar, J. Gibson, W. Hawley, J. Leonard, R. Nicolait, D. Weyer. H. White and C. Wright. 1984. Belize Country Environmental Profile A Field Study. R. Nicolait & Associates, Belize City, Belize. 151 pp.

Haworth, J. 1988. The global distribution of malaria and the present control effort. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer and I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 1379-1420.

Hecht, O. and J. Hernandez. 1960. Resena de las investigaciones sobre la irritacion de Anofelinos por el contacto con superficies cubiertas con DDT. [Review of investigations on the irritation of anophelines by contact with DDT-treated surfaces.] Bol. Com. Nac. Errad. Paludismo. 4:93-106.

Heinemann, S.J. and J.N. Belkin. 1977. Collection records of the project "Mosquitoes of Middle America". 8. Central America: Belize (BH), Guatemala (GUA), El Salvador (SAL), Honduras (HON), Nicaragua (NI, NIC). Mosq. Syst. 9:403-454.

Horsfall, W. 1955. *Mosquitoes: their Bionomics and Relation to Disease.* Ronald, New York. 723 pp.

Hudson, J.E. 1984. *Anopheles darlingi* Root (Diptera: Culicidae) in the Suriname rain forest. Bull. Entomol. Res. 74:129-142.

Karabatsos, N. (Ed.) 1985. International Catalogue of Arbovirusues (and certain other viruses of vertebrates). 3rd edition. American Society of Triopical Medicine and Hygiene, San Antonio, TX. 1147 pp.

Kartman, L. and M.M. da Silveira. 1946. Effect of short contact with DDT residues on *Anopheles gambiae*. J. Econ. Entomol. 39:356-359.

Kennedy, J.S. 1946. The excitant and repellent effects on mosquitos of sublethal contacts with DDT. Bull. Entomol. Res. 37:593-607.

King, R.B., I.C. Baillie, T.M.B. Abell, J.R. Dunsmore, D.A. Gray, J.H. Pratt, H.R. Versey, A.C.S. Wright and S.A. Zisman. 1992. Land resource assessment of Northern Belize. Natural Resources Institute Bulletin 43. Kent, United Kingdom. 513 pp.

Klowdon, M.J. 1996. Vector Behavior. In: *The Biology of Disease Vectors*. B.J. Beaty and W.C. Marquardt (Eds.). University Press, Colorado. pp. 34-50.

Knight, K.L and A. Stone. 1977. *A Catalog of the Mosquitoes of the World*. Thomas Say Foundation. Volume 6. 611 pp.

Komp, W.H.W. 1940. The occurrence of *Anopheles darlingi* Root in British Honduras and Guatemala. Science. 91:522-523.

Komp, W.H.W. 1942. The anopheline mosquitoes of the Caribbean region. U.S. Public Health Service, Nat. Inst. Hlth. Bull. No.179. 195 pp.

Krogstad, D.J. 1996. Malaria as a reemerging disease. Epidemiol. Rev. 18:77-89.

Kuhlow, F. 1962. Field experiments on the behaviour of malaria vectors in an unsprayed hut and in a hut sprayed with DDT in northern Nigeria. Bull. Wld. Hlth Organ. 26:93-102.

Kumm, H.W. and L.M. Ram. 1941. Observations on the *Anopheles* of British Honduras. Am. J. Trop. Med. 21:559-566.

Kumm, H.W. and H. Zuniga. 1944. Seasonal variations in the numbers of *Anopheles albimanus* and *A. pseudopunctipennis* caught in stable traps in Central America. Am. J. Hyg. 39:8-15.

Lockwood, J.A., T.C. Sparks and R.N. Story. 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30:41-51.

Loyola, E.G., M.H. Rodriquez, L. Gonzalez, J.L. Arredondo, D.N. Bown and M.A. Vaca. 1990. Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of *Anopheles pseudopunctipennis* in Mexico. J. Am. Mosq. Control Assoc. 6:635-640.

Loyola, E.G., M.A. Vaca, D.N. Bown, E. Perez and M.H. Rodriguez. 1991a. Comparative use of bendiocarb and DDT to control *Anopheles pseudopunctipennis* in a malarious area of Mexico. Med. Veter. Entomol. 5:233-242.

Loyola, E.G., J.I. Arredondo-Jimenez, M.H. Rodriguez, D.N. Bown and M. A. Vaca Marin. 1991b. *Anopheles vestitipennis*, the probable vector of *Plasmodium vivax* in the Lacandon forest of Chiapas, Mexico. Trans. Roy. Soc. Trop. Med. Hyg. 85:171-174.

Loyola, E.G., L. Gonzalez-Ceron, M.H. Rodriquez, J.I. Arredondo-Jimenez, S. Bennett and D.N. Bown. 1993. *Anopheles albimanus* (Diptera: Culicidae) host selection patterns in three ecological areas of the coastal plains of Chiapas, southern Mexico. J. Med. Entomol. 30:518-523.

Macdonald, G. 1957. *The Epidemiology and Control of Malaria*. Oxford Univ. Press.

Manguin, S., D.R. Roberts, R.A. Andre, E. Rejmankova and S. Hakre. 1996. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. J. Med. Entomol. 33:205-211.

Marquetti, M.C., A. Navarro and J.A. Bisset. 1990. Estudio de la actividad hematofagica de *Anopheles vestitipennis* Dyar & Knab, 1906 (Diptera: Culicidae). [Hematophagous activity of *Anopheles vestitipennis* Dyar & Knab, 1906 (Diptera: Culicidae)]. Rev. Cub. Med. Trop. 42:101-108.

Mattingly, P.F. 1962. Mosquito behaviour in relation to disease eradication programmes. Annu. Rev. Entomol. 7:419-436.

Mekuria, Y., M.A. Tidwell, D.C. Williams and J.D. Mandeville. 1990. Bionomic studies of the *Anopheles* of Dajabon, Dominican Republic. J. Am. Mosq. Control Assoc. 6:651-657.

Mekuria, Y., R. Granados, M.A. Tidwell, D.C. Williams, R.A. Wirtz and D.R. Roberts. 1991. Malaria transmission potential by *Anopheles* mosquitoes of Dajabon, Dominican Republic. J. Am. Mosq. Control Assoc. 7:456-461.

Metcalf, R.L., A.D. Hess, C.E. Smith, G.M. Jeffrey and G.L. Ludwig. 1945. Observations on the use of DDT for the control of *Anopheles quadrimaculatus*. U.S. Publ. Hlth. Rep. 60:753-774.

Molineaux, L., G.R. Shidrawi, J.L. Clarke, J.R. Boulzaguet and T.S. Ashkar. 1979. Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition. Bull. Wld, Hlth. Organ. 57:265-274.

Mpofu, S.M., P. Taylor and J. Govere. 1988. An evaluation of the residual lifespan of DDT in malaria control. J. Am. Mosq. Control Assoc. 4:529-535.

Muirhead-Thomson, R.C. 1947. The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. Bull. Entomol. Res. 38:449-464.

Muirhead-Thomson, R.C. 1949. DDT and "Gammexane" as residual insecticides against *Anopheles gambiae* in African houses. Nature 163:109-110.

Muirhead-Thomson, R.C. 1950. DDT and Gammexane as residual insecticides against *Anopheles gambiae* in African houses. Trans. Roy. Soc. Trop. Med. Hyg. 43:401-412.

Muirhead-Thomson, R.C. 1951. *Mosquito Behaviour in Relation to Malaria Transmission and Control in the Tropics.* Edward Arnold, London. 219 pp.

Muirhead-Thomson, R.C. and E.C. Mercier. 1952. Factors in malaria transmission by *Anopheles albimanus* in Jamaica. Part I. Ann. Trop. Med. Parasitol. 46:103-116.

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. Wld. Hlth. Organ. 22:721-734.

Muirhead-Thomson, R.C. 1968. *Ecology of Insect Vector Populations.* Academic Press, London. 174 pp.

Muirhead-Thomson, R.C., 1991. *Trap Responses of Flying Insects.* Acedemic Press, London. 299 pp.

Navarro O., A., M. Del Carmen Marquetti, J.A. Bisset Lazcano and F.A. Garcia. 1986. Estudio estacional de sitios de reposo de anophelinos (Diptera: Culicidae). [Studies of the resting places of anophelines (Diptera: Culicidae).] Informe preliminar. Rev. Cub. Med. Trop. 38:21-26.

Nutsathapana, S., P. Sawasdiwongphorn, U. Chitprarop and J.R. Cullen. 1986. The behaviour of *Anopheles minimus* Theobald (Diptera: Culicidae) subjected to differing levels of DDT selection pressure in northern Thailand. Bull. Entomol. Res. 76:303-312.

Olliaro, P., J. Cattani and D. Wirth. 1996. Malaria, the submerged disease. J. Am. Med. Assoc. 275(3):230-233.

Padilla, N., P. Molina, J. Juarez, D. Bown and C. Cordon-Rosales. 1992. Potential malaria vectors in northern Guatemala. J. Am. Mosq. Control Assoc. 8:307-308.

[PAHO] Pan American Health Organization 1994. Prohibition of DDT. Med. Veter. Entomol. 8:164

[PAHO] Pan American Health Organization. 1996. Biology and ecology of *Anopheles albimanus* Weidmann in Central America. Tech. Rep. Ser. No. 43. Washington, D.C. 44 pp.

Pant, C.P. 1988. Malaria vector control: imagociding. In: *Malaria, Principals and Practice of Malariology*. W.H. Wernsdorfer and I. McGregor, (Eds.). Churchill Livingstone, New York. pp. 1173-1212.

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

Rachou, R.G., G. Lyons, M. Moura-Lima and J.A. Kerr. 1965. Synoptic epidemiological studies of malaria in El Salvador. Am. J. Trop. Med. Hyg. 14:1-62.

Rachou, R.G. and M. Moura-Lima. 1966. Preliminary note on the epidemiological studies made in El Salvador to determine the causes of the failure of residual spraying to interrupt the transmission of malaria. Rev. Brasil. Malariol. Doencas Trop. 18(3/4):763-779.

Rachou, R.G., L.A. Schinazi and M.M. Lima. 1973. An extensive epidemiological study of the causes for the failure of residual DDT-spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages on the coastal plain of El Salvador, Central America. Rev. Bras. Malariol. Doencas Trop. 25:3-293.

Read, R.G., A.J. Adames and P. Galindo. 1978. A model of microenvironment and man biting tropical insects. Environ. Entomol. 7:547-552.

Read, R.G. and A.J. Adames. 1980. Atmospheric stimulation of man-biting activity in tropical insects. Environ. Entomol. 9:677-680.

Reid, J.A. and R.H. Wharton. 1956. Trials of residual insecticides in window-trap huts against Malayan mosquitos. Bull. Entomol. Res. 47:433-468.

Rejmankova, E., D.R. Roberts, R.E. Harbach, J. Pecor, E.L. Peyton, S. Manguin, R. Krieg, J. Polanco and L. Legters. 1993. Environmental and regional determinants of *Anopheles* (Diptera: Culicidae) larval distribution in Belize, Central America. Environ. Entomol. 22:978-992.

Rejmankova, E., D.R. Roberts, A. Pawley, S. Manguin and J. Polanco. 1995. Predictions of adult *Anopheles albimanus* densities in villages based on distances to remotely sensed larval habitats. Am. J. Trop. Med. Hyg. 53:482-488.

Rejmankova, E., D.R. Roberts, S. Manguin, K.O. Pope, J. Komarek and R.A. Post. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. Environ Entomol. 25:1058-1067.

Rejmankova, E., K.O. Pope, D.R. Roberts, M.G. Lege, R. Andre, J. Greico and Y. Alonzo. 1998. Characterization and detection of *Anopheles vestitipennis* and *Anopheles punctimacula* (Diptera: Culicidae) larval habitats in Belize with field survey and SPOT satellite imagery. J. Vect. Ecol. 23:74-88.

Ribbands, C.R. 1946. The use of residual films of DDT and Gammexane in malaria control. Bull. Entomol. Res. 37:567-592.

Richards, F.O., Jr., R.Z. Flores, J.D. Sexton, R.F. Beach, D.L. Mount, C. Cordon Rosales, M. Gatica and R.E. Klein. 1994. Effects of permethrin-impregnated bed nets on malaria vectors in northern Guatemala. Bull. Pan Am. Hlth. Organ. 28:112-121.

Roberts, D.R., W.D. Alecrim, A.M. Tavares and K.M. McNeill. 1984. Influence of physiological condition on the behavioral response of *Anopheles darlingi* to DDT. Mosq. News 44:357-362.

Roberts, D.R., W.D. Alecrim, A.M. Tavares and M.G. Radke. 1987. The house frequenting, host-seeking and resting behavior of *Anopheles darlingi* in southeastern Amazonas, Brazil. J. Am. Mosq. Control Assoc. 3:433-441.

Roberts, D.R. and W.D. Alecrim. 1991. Behavioral response of *Anopheles darlingi* to DDT-sprayed house walls in Amazonia. Bull. Pan Am. Hlth. Organ. 25:210-217.

Roberts, D.R. 1993. Insecticide repellancy in malaria vector control: a position paper. VBC Report no. 81131. USAID, Arlington, VA. 72 pp.

Roberts, D.R., O. Chan, J. Pecor, E Rejmankova, S. Manguin, J. Polanco and L.J. Legters. 1993. Preliminary observations on the changing roles of malaria vectors in southern Belize. J. Am. Mosq. Control Assoc. 9:456-459.

Roberts, D.R. and R.G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. Am. J. Trop. Med. Hyg. 50(Suppl):21-34.

Roberts, D.R. (ed.) 1997. Remote sensing for research and control of malaria in Belize. Progress report 1996. (unpub. doc.) 413 pp.

Roberts, D.R., L.L. Laughlin, P. Hsheih and L.J. Letgers. 1997a. DDT: global strategies, and a malaria control crisis in South America. Emerg. Inf. Dis. 3:295-302.

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

Roberts, D.R., W.D. Alecrim, R.G. Andre and P. Hsheih. A stochastic model of vector behavior: effects of DDT repellency, irritancy and toxicity in malaria control. Emerg. Inf. Dis. (unpub. doc.).

Rodriguez, M.H., D.N. Bown, J.I. Arredondo-Jimenez, C. Villarreal, E.G. Loyola, and C.E. Frederickson. 1992. Gonotrophic cycle and survivorship of *Anopheles albimanus* (Diptera:Culicidae) in southern Mexico. J. Med. Entomol. 29:395-399.

Rodriguez, A.D., M.H. Rodriguez, R.A. Meza, J.E. Hernandez, E. Rejmankova, H.M. Savage, K.O. Pope, D.R. Roberts and L. Legters. 1993. Dynamics of population densities and vegetation associations of *Anopheles albimanus* larvae in a coastal area of southern Chiapas, Mexico. J. Am. Mosq. Control Assoc. 9:46-58.

Rozeboom, L.E. 1941. Distribution and ecology of the *Anopheles* mosquitoes of the Caribbean region. In: *A symposium on human malaria with special reference to North America and the Caribbean region*. F.R. Moulton, (Ed.). Am. Assoc. Adv. Sci., Pub. No. 15. Washington, D.C. pp. 98-107.

Rozendaal, J.A., J.P.M. van Hoof, J. Voorham and B.F.J. Oostburg. 1989. Behavioral responses of *Anopheles darlingi* in Suriname to DDT residues on house walls. J. Am. Mosq. Control Assoc. 5:339-350.

Rozendaal, J.A. 1990. Epidemiology and control of malaria in Suriname, with special reference to *Anopheles darlingi*. Leiden. ICG Printing, Dordrecht. 171 pp.

Russell, P.F., L.S. West, R.D. Manwell and G. Macdonald. 1963. *Practical Malariology*. 2ed. Oxford University, London. 750 pp.

Service, M.W. 1989. The importance of ecological studies on malaria vectors. Bull. Soc. Vect. Ecol. 14:26-38.

Service, M.W. 1992. Vector Control. Where are we now? Bull. Soc. Vect. Ecol. 17:94-108.

Service, M.W. 1993a. The role of ecology and ecological modeling in vector control. Bull. Soc. Vect. Ecol. 18:85-98.

Service, M.W. 1993b. *Mosquito Ecology, Field Samplig Methods*. 2ed. Elsevier, London. 988 pp.

Shalaby, A.M. 1966. Observations on some responses of *Anopheles culicifacies* to DDT in experimental huts in Gujarat State, India. Ann. Entomol. Soc. Am. 59:938-944.

Sharp, B.L., D. Le Sueur and P. Bekker. 1990. Effect of DDT on survival and blood feeding success of *Anopheles arabiensis* in northern Kwazulu, Republic of South Africa. J. Am. Mosq. Control Assoc. 6:197-202.

Simmons, J.S., G.R. Callender, D.P. Curry, S.C. Schwartz and R. Randall. 1939. *Malaria in Panama.* Am. J. Hyg. Monogr. Ser. No. 13. 326 pp.

Simmons, J.S. 1941. The transmission of malaria by the *Anopheles* mosquitoes of North America. In: *A symposium on human malaria with special reference to North America and the Caribbean region.* F.R. Moulton, (Ed.). Am. Assoc. Adv. Sci. Pub. No. 15. Washington, D.C. pp. 113-130.

Singh, P.P., A.S. Udeaan and S. Battu. 1992. DDT and HCH residues in indoor air arising from their use in malaria control programmes. Sci. Total Environ. 116:83-92.

Slooff, R. 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *Anopheles punctulatus* group. Leyden: A.W. Sythoff. 144 pp.

Smith, A. 1963. Deterrent effect of insecticides on malaria vectors. Nature. 200:861 862.

Smith, A. 1964. A review of the origin and development of experimental hut techniques used in the study of insecticides in East Africa. E. Afr. Med. J. 41:361-374.

Smith, A. 1965. A verandah-type hut for studying the house-frequenting habits of mosquitos and for assessing insecticides. I. A description of the verandah-trap and of studies on the egress of *Anopheles gambiae* Giles and *Mansonia uniformis* (Theo.) from an untreated hut. Bull. Entomol. Res. 56:161-167.

Smith, A. and D.J. Webley. 1969. A verandah-type hut for studying the house frequenting habits of mosquitoes and for assessing insecticides. III. The effect of DDT on behaviour and mortality. Bull. Entomol. Res. 59:33-46.

Smits, A., P. Roelants, W. Van Bortel and M. Coosemans. 1996. Enzyme polymorphisms in the *Anopheles gambiae* (Diptera: Culicidae) complex related to feeding and resting behavior in the Imbo Valley, Burundi. J. Med. Entomol. 33:545-553.

Smyth. T., Jr. and C.C. Roys. 1955. Chemoreception in insects and the action of DDT. Biol. Bull. 108:66-76.

Soerono, M., G. Davidson and D.A. Muir. 1965. The development and trend of insecticide-resistance in *Anopheles aconitus* Dönitz and *Anopheles sundaicus* Rodenwaldt. Bull. Wld. Hlth. Organ. 32:161-168.

Suwonkerd, W. B. Amg-Ung, K. Rimwangtrakul, S. Wongkattiyakul, B. Kattiyamongkool, U. Chitraprop and M. Takagi. 1990. A field study on the response of *Anopheles dirus* to DDT and fenitrothion sprayed to huts in Petchabun Province, Thailand. Trop. Med. (Nagasaki). 32:1-5.

Symes, C.B. and A.B. Hadaway. 1947. Initial experiments in the use of DDT against mosquitoes in British Guiana. Bull. Entomol. Res. 37:399-430.

Tarzwell, C.M. and F.W. Fisk. 1947. Observations on the nighttime resting and biting habits of anopheline mosquitoes in DDT-treated and untreated buildings. U.S. Publ. Hlth. Rep. 62:84-94.

Taylor, B. 1975. Changes in the feeding behaviour of a malaria vector, *Anopheles farauti* Lav., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. Trans. Roy. Entomol. Soc. Lond. 127:277-292.

Taylor, P., M.J. Crees and K. Hargreaves. 1981. Duration of *Anopheles arabiensis* control in experimental huts sprayed with DDT and Decamethrin. Trans. Zimbabwe Scient. Ass. 61:1-13.

Thevasagayam, E.S., C.C. Knoon and Y. Siong. 1979. Studies on the biology of *Anopheles campestris* Reid (Diptera, Culicidae) and its response to residual spraying with DDT, carried out in experimental huts in Penang, Malaysia. Med. J. Malay. 34:117-130.

Thiel, P.H. van. 1951. The repellent effect of insecticides, especially of contact insecticides. Doc. Neerl. Indones. Morb. Trop. 3:117-125.

Thiel, P.H. van and D. Metselaar. 1955. A pilot project of residual spraying as a mean of controlling malaria transmitted by anophelines of the punctulatus group in Netherlands New Guinea. Doc. Med. Geog. Trop. 7:164-181.

Trigg, P.I. and A.V. Kondrachine. 1998. Commentary: Malaria control in the 1990s. Bull. Wld. Hlth. Organ. 76:11-16.

Trapido, H. 1952. Modified response of *Anopheles albimanus* to DDT residual housing spraying in Panama. Am. J. Trop. Med. Hyg. 1:853-861.

Vargas, L. and A. Martinez-Palacios. 1956. Anofelinos mexicanos. Taxonomia y distribucion [Mexican anophelines. Taxonomy and distribution]. Mex., D.F., ecr. Salubr. Assist. CNEP/SSA, 181 pp.

Wharton, R.H. 1951. The behaviour and mortality of *Anopheles maculatus* and *Culex fatigans* in experimental huts treated with DDT and BHC. Bull. Entomol. Res. 42:1-20.

White, G.B. 1984. *Anopheles gabaldoni* as a putative malaria vector in La Mosquitia, Honduras. In: Mosquito studies at the London School of Hygiene and Tropical Medicine, Progress Report No. 45.

[WHO] World Health Organization. 1975a. Ecology and control of vectors in public health. Wld. Hlth. Organ. Tech. Rep. Ser. No. 561. 35 pp.

[WHO] World Health Organization. 1975b. Manual on practical entomology in malaria. Part II. Methods and techniques. WHO Offset Pub. No, 13. Geneva. 191 pp.

[WHO] World Health Organization. 1981a. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organphosphate and carbamate insecticides. Diagnostic Test. WHO/VBC/81.806. Geneva. 6 pp.

[WHO] World Health Organization. 1981b. Instructions for the bio-assay of insecticidal deposits on wall surfaces. WHO/VBC/81.5. Geneva. 3 pp.

[WHO] World Health Organization. 1986. Resistance of vectors and reservoirs of disease to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 737. 87 pp.

[WHO] World Health Organization. 1992. Vector resistance to pesticides. Wld. Hlth. Organ. Tech. Rep. Ser. No. 818. 62 pp.

[WHO] World Health Organization. 1994. Use of DDT in vector control. Med. Veter. Entomol. 8:113.

[WHO] World Health Organization. 1995. Vector control for malaria and other mosquito-borne diseases. Wld. Hlth. Organ. Tech. Rep. Ser. No. 857. 91 pp.

[WHO] World Health Organization. 1996. Operational manual on the application of insecticides for control of the mosquito vectors of malaria and other diseases. WHO/CTD/VBC/96.1000. 198 pp.

[WHO] World Health Organization. 1997. World malaria situation in 1994. Parts I, II, and III Wkly Epidemiol. Rec. 72:269-276, 277-284, 285-292.

Wilkerson, P.R. 1951. Distribution and fate of *Anopheles gambiae* and *A. funestus* in two different types of huts treated with DDT and BHC in Uganda. Bull. Entomol. Res. 42:45-54.

Wilkerson, R.C. 1990. Rediscription of *Anopheles punctimacula* and *An. malefactor* (Diptera: Culicidae). J. Med. Entomol. 27:225-247.

Wilkerson, R.C., D. Strickman and T.R. Litwak. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. J. Am. Mosq. Control Assoc. 6:7-34.

Wilson, H.G., D.E. Weidhaas and G.C. LaBrecque. 1973. Toxicity of insecticide residues to *Anopheles quadrimaculatus* and effects on resting behavior. Mosq. News 33:539-542.

Wirtz, R.A., T.R. Burkot and P.M. Graves. 1987. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera; Culicidae) from Papua New Guinea. J. Med. Entomol. 24:433-437.

Wolda, H. and P. Galindo. 1981. Population fluctuations of mosquitoes in the non-seasonal tropics. Ecol. Entomol. 6:99-106.

Worth, C.B. 1953. Construction and use of a simplified window trap for insects. Mosq. News 13:204-206.

Zetek, J. 1915. Behavior of *Anopheles albimanus* Wiede. and *tarsimaculata* Goeldi. Ann. Entomol. Soc. Am. 8:221-271.

Zimmerman, R.H. 1992. Ecology of malaria vectors in the Americas and future direction. Mem. Inst. Oswaldo Cruz. 87 (suppl. III):371-383.

Table 1. Monthly climate data collected daily from 10 September 1995 to 20 May 1996 near study site in Caledonia, Corozal District, northern Belize, C.A.

Month	Mean Temperature (SD)◆ Max.	Min.	Range	Rainfall✦ mm (in)	Raindays (%/mo.)	Degree Wetness▼
Sep. 1995	84(2.7)	77(1.4)	86-75	167.2 (6.7)	17 (81.0)*	135
Oct.	86(4.3)	75(2.4)	93-70	285.2 (11.4)	24 (77.4)	221
Nov.	86(2.5)	72(4.1)	90-62	105.6 (4.2)	14 (46.6)	49
Dec.	83(2.9)	69(2.6)	88-64	153.8 (6.2)	11 (35.5)	55
Jan. 1996	81(5.4)	64(5.2)	90-49	124.2 (5.0)	10 (32.2)	40
Feb.	85(4.8)	64(4.4)	92-55	27.4 (1.1)	6 (20.7)	6
Mar.	86(7.1)	66(7.2)	94-51	35.0 (1.4)	5 (16.1)	6
Apr.	92(2.2)	73(2.8)	95-68	63.9 (2.6)	9 (30.0)	19
May	92(1.2)	74(1.5)	94-72	82.1 (3.3)	9 (45.0)*	37

◆ Temperature in Fahrenheit (Standard Deviation)
✦ Rainfall measured every 24 hr.
* Period of observation: 10-30 Sept.; 1-20 May.
▼ $\frac{\text{\# raindays per month x total rainfall}}{\text{\# days in month}}$

Table 2. Adult mosquitoes* encountered during evening collections from human bait or exit traps in Caledonia Village, Corozal District, Belize, Central America, (18°13'78"N, 88°28'33"W) from August 1995 to May 1996.

Aedeomyia
Ad. squamipennis† (Lynch Arribalzaga) 1878

Aedes
Ae. (Stegomyia) aegypti (Linnaeus) 1762
Ae. (Ochlerotatus) angustivittatus (Wiedemann) 1828
Ae. (O.) fulvus Dyar & Knab 1907
Ae. (O.) scapularis (Rondani) 1848
Ae. (O.) serratus (Theobald) 1901
Ae. (O.) taeniorhynchus (Wiedemann) 1821

Anopheles
An. (Nyssorhynchus) albimanus Wiedemann 1820
An. (Anopheles) crucians Wiedemann 1828
An. (Anop.) gabaldoni Vargas 1941
An. (Anop.) punctimacula Dyar & Knab 1906
An. (Anop.) vestitipennis Dyar & Knab 1906

Coquillettidia
Cq. (Rhynchotaenia) nigricans Coquillett 1904

Culex
Cx. (Culex) coronator Dyar & Knab 1906
Cx. (Melanoconion) erraticus (Dyar & Knab) 1906
Cx. (Cx.) nigripalpus Theobald 1901
Cx. (Mel.) pilosus (Dyar & Knab) 1906
Cx. (Cx.) quinquefasciatus Say 1823
Cx. (Mel.) taeniopus (=opisthopus) Komp 1926
Cx. (Mel.) spp. (undetermined)

Limatus
Li. durhamii Theobald 1901

Mansonia
Ma. (Mansonia) dyari Belkin, Heinemann & Page 1970
Ma. (Ma.) titillans (Walker) 1848

Psorophora
Ps. (Janthinsoma) albipes (Theobald) 1907
Ps. (Psorophora) ciliata (Fabricius) 1794
Ps. (Grabhamia) confinnis group (Lynch Arribalzaga) 1891
Ps. (Jan.) ferox (Von Humboldt) 1819

Uranotaenia
Ur. (Uranotaenia) lowii Theobald 1901
Ur. (Ur.) socialis† Theobald 1901

* based on Knight & Stone (1977); † captured in experimental hut exit traps only.

Table 3. Comparison of Hut-1 and Hut-2 pre-DDT treatment human-landing catches under matched and unmatched hut conditions for indoor access by mosquitoes in Caledonia, Belize. Each trial represents 1 collection night during December 1995 to January 1996.

	Conditions Hut-1/ Hut-2						Ratio Hut-1/Hut-2 (sample size)		
Trial	**W2**	**W4**	**door**	**eave**	**walls**	***An. albimanus***	***An. vestitipennis***	***Ma. dyari***	**Sum**
1	1/1	0/0	0/0	1/1	0/1	0.5 (6)	0.47 (128)	0.34 (131)	265
2	0/1	0/0	0/0	1/1	0/1	0.0 (7)	0.26 (157)	0.29 (107)	271
3	0/0	0/0	0/0	0/1	0/1	0 (1)	0.17 (14)	0.07 (29)	44
4	0/0	1/1	0/0	0/1	0/1	0.52 (46)	1.47 (464)	0.85 (313)	823
5	0/0	0/0	0/0	0/1	0/1	0.17 (7)	0.04 (111)	0.04 (166)	284
6	0/0	0/0	0/0	1/1	0/1	0.0 (6)	0.125 (9)	0.28 (59)	74
7	1/1	0/0	0/0	1/1	0/1	0.4 (7)	0.43 (149)	0.34 (163)	319
						Sum 80	1,032	968	2,080
8	0/0	0/0	1/1	1/1	1/1	0.29 (9)	1.29 (94)	1.44 (100)	203
9	0/0	0/0	0/0	1/1	1/1	0.29 (9)	0.52 (32)	0.75 (21)	62
10	1/1	0/0	0/0	1/1	0/0	1.57 (36)	2.67 (121)	1.93 (290)	447
11	1/1	0/0	0/0	1/1	0/0	0.5 (12)	0.87 (56)	0.52 (108)	176
12	0/0	1/1	0/0	1/1	0/0	0.12 (19)	0.97 (118)	0.64 (141)	278
						Sum 85	421	660	1,166

0= closed; 1= open. Paired trials 1-7 (unmatched conditions), # 8-12 (matched conditions). Trials 1, 2, 3, 4, 6 13 hr collections, all others 6 hr.

Table 4. Proportion analysis of paired human-landing collections in pre-DDT treatment Hut-1 v. Hut-2 under variable test conditions in Caledonia, Belize. Each trial represents 1 collection night during December 1995 to January 1996 (see Table 3).

Trial 1	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.33	0.32	0.252
n	06	128	131
z-stat	-0.883	-4.073	-5.677
***p*-value**	0.405	**<0.001****	**<0.001****
Trial 2	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0	0.20	0.22
n	07	157	107
z-stat	-2.646	-7.518	-5.793
***p*-value**	**0.008****	**<0.001****	**<0.001****
Trial 3	***An. albimanus*§**	***An. vestitipennis*§**	***Ma. dyari*§**
(P) Hut 1	0	0.143	0.068
n	1	14	29
z-stat	-1	-2.672	-4.653
***p*-value**	0.317	**<0.0076****	**<0.001****
Trial 4	***An. albimanus***	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	348	0.595	0.46
n	46	464	313
z-stat	-2.062	4.093	-1.415
***p*-value**	**<0.0392***	**<0.001****	0.157
Trial 5	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.143	0.036	0.036
n	07	111	166
z-stat	-1.889	-9.777	-11.956
***p*-value**	0.058	**<0.001****	**<0.001****

(Continued)

Continued Table 4.

Trial 6	***An. albimanus*§**	***An. vestitipennis*§**	***Ma. dyari***
(P) Hut 1	0	0.11	0.22
n	6	9	59
z-stat	-2.449	-2.34	-4.301
***p*-value**	**<0.014***	**<0.019***	**<0.001****

Trial 7	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.28	0.3	0.25
n	7	149	163
z-stat	-1.164	-4.883	-6.384
***p*-value**	0.244	**<0.001****	**<0.001****

(P)= proportion; n=sample size; z stat= test statistic; *p*-value * significant, ** highly significant; §=sample too small

Table 5. Proportion analysis of paired human-landing collections in pre-DDT treatment Hut-1 v. Hut-2 under identical test conditions in Caledonia, Belize. Each trial represents 1 collection night during December 1995 to January 1996 (see Table 3).

Trial 1	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.22	0.56	0.59
n	09	94	100
z-stat	-1.68	1.163	1.8
***p*-value**	0.093	0.244	0.072
Trial 2	***An. albimanus*§**	***An. vestitipennis*§**	***Ma. dyari*§**
(P) Hut 1	0.22	0.34	0.43
n	09	32	21
z-stat	-1.68	-1.81	1.8
***p*-value**	0.093	0.070	0.521
Trial 3	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.61	0.72	0.65
n	36	121	290
z-stat	1.32	4.84	5.109
***p*-value**	0.187	**<0.001****	**<0.001****
Trial 4	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.33	0.46	0.34
n	12	56	108
z-stat	-1.178	-0.60	-3.326
***p*-value**	0.238	0.549	**<0.001****
Trial 5	***An. albimanus*§**	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.1	0.49	0.39
n	19	118	141
z-stat	-3.487	-0.217	-2.612
***p*-value**	**<0.001****	0.828	**<0.001****
Combined	***An. albimanus***	***An. vestitipennis***	***Ma. dyari***
(P) Hut 1	0.374	0.556	0.528
n	85	421	660
z-cal	-2.323	2.298	1.439
***p*-value**	**0.020***	**0.021***	0.1502

(P)= proportion in Hut-1; n=sample size; z stat= test statistic; *p*-value * significant, ** highly significant. §=sample too small.

Table 6. Bioassay with proportion mortality and knockdown (KD) after 1-hr contact on walls treated with DDT and untreated (control) experimental huts at 1, 6 and 12 weeks post-spray in Caledonia, Corozal District, Belize.

Post-spray		Sprayed Hut			Control Hut		
Weeks	**N**	**# Dead**	**P**	**KD**	**N**	**# Dead**	**P**
Week 1							
An. albimanus	52	52	1.00	1.00	42	2	0.047
An. vestitipennis	30	30	1.00	1.00	35	0	0.000
An. crucians	18	18	1.00	1.00	23	1	0.043
Sum	100	100	1.00	1.00	100	3	0.030
Week 6							
An. albimanus	41	41	1.00	1.00	32	3	0.093
An. vestitipennis	27	27	1.00	1.00	24	1	0.042
An. crucians	19	19	1.00	1.00	7	0	0.000
Sum	75	75	1.00	1.00	75	4	0.053
Week 12							
An. albimanus	73	69	0.945	0.849	65	1	0.015
An. vestitipennis	19	17	0.895	0.789	17	0	0.000
An. crucians	8	8	1.00	1.00	15	0	0.000
Sum	100	94	0.94	0.85	97	1	0.010

All *An. gabaldoni* (11) and *An. punctimacula* (7) found susceptible over the 12-wk period. N= sample size; # Dead after 24-hr post-contact; P= proportion mortality; KD= knockdown after 1-hr contact (treated hut only).

Table 7. Post-spray proportion analysis comparing 5 all night human-landing collections (HLC)§ of *Anopheles vestitipennis* in Hut-1 (DDT-treated) v. Hut-2 (control) by collection hour in Caledonia, Belize during April-May 1996.

Time	Hut-1			Hut-2			Sum n	z-stat	*p*-value
	[n	HLC	P	n	HLC	P]			
1	0	0	0	0	0	0	0	ND	ND
2	05	0.1	0.357	09	1.8	0.643	14	-0.070	0.285
3	12	2.4	0.500	12	2.4	0.500	24	0	1.0
4	11	2.2	0.333	22	4.4	0.667	33	-1.919	0.055
5	14	2.8	0.500	14	2.8	0.500	28	0	1.0
6	12	2.4	0.364	21	4.2	0.636	33	-1.563	0.118
7	08	1.6	0.296	19	3.8	0.704	27	-2.120	**0.034***
8	06	1.2	0.200	24	4.8	0.800	30	-3.286	**0.001****
9	03	0.6	0.130	20	4.0	0.870	23	-3.549	**0.001****
10	01	0.2	0.053	18	3.6	0.947	19	-3.897	**0.001****
11	0	0	0.000	16	3.2	1.000	16	-4.000	**0.001****
12	02	0.4	0.200	08	1.6	0.800	10	-1.897	0.058
Sum	74	14.8	0.289	183	36.6	0.711	257	-7.507	**<0.001****

§ mean 5 (12-hr) collections, Time 1=1800-1845 to 12=0500-0545. P= proportion; n= sample size; z-stat= test statistic; *p*-value * significant, ** highly significant. ND= no data.

Table 8. Post-spray proportion analysis comparing 5 all night human-landing collections (HLC)§ of *Mansonia dyari* in Hut-1 (DDT-treated) v. Hut-2 (control) by collection hour in Caledonia, Belize during April-May 1996.

Time	Hut-1			Hut-2			Sum n	z-stat	*p*-value
	n	HLC	P	n	HLC	P			
1	26	5.2	0.388	41	8.2	0.612	67	1.834	0.067
2	104	20.8	0.406	152	30.4	0.594	256	-3.008	**0.002****
3	108	21.6	0.420	149	29.8	0.580	257	-2.565	**0.010***
4	92	18.4	0.472	103	20.6	0.528	195	-0.782	0.434
5	42	8.4	0.382	68	13.6	0.618	110	-2.475	**0.013***
6	30	0.6	0.517	28	5.6	0.483	58	0.259	0.796
7	16	3.2	0.457	19	3.8	0.543	35	-0.509	0.611
8	10	2.0	0.357	18	3.6	0.643	28	-1.513	0.130
9	08	1.6	0.286	20	4.0	0.714	28	-2.265	**0.024***
10	11	2.2	0.524	10	2.0	0.476	21	0.220	0.826
11	02	0.4	0.125	14	2.8	0.875	16	-3.000	**0.003****
12	11	2.2	0.367	19	3.8	0.633	30	-1.457	0.145
Sum	460	92	0.418	641	128.2	0.582	1101	-5.532	**<0.001****

§ mean 5 (12-hr) collections, Time 1=1800-1845 to 12=0500-0545. P= proportion; n= sample size; z-stat= test statistic; *p*-value * significant, ** highly significant. ND= no data.

Table 9. Hourly proportion decline and cumulative rate of decline mean human-landing collections between DDT treated and control huts averaged over 5 all night collections for *Anopheles vestitipennis* in April-May 1996 in Caledonia, Belize.

Hour	Treated Hut-1 (*Tn*)	Control Hut-2 (*Cn*)	A	B	C	D
1	0.001	0.001	0	0	0	0
2	1.0	1.8	0.555	0.4444	0.0771	0.0711
3	2.4	2.4	1.0	0	0	0.0711
4	2.2	4.4	0.5	0.5	0.0801	0.1512
5	2.8	2.8	1.0	0	0	0.1512
6	2.4	4.2	0.5714	0.4286	0.0686	0.2198
7	1.6	3.8	0.4210	0.5790	0.0927	0.3125
8	1.2	4.8	0.25	0.75	0.1201	0.4326
9	0.6	4.0	0.15	0.85	0.1361	0.5687
10	0.2	3.6	0.0555	0.9444	0.1512	0.7199
11	0	3.2	0	1.0	0.1600	0.8799
12	0.4	1.6	0.25	0.75	0.1201	1.0
	14.801	**36.601**	**4.7536**	**6.2464**		

A= Treated/Control (*Tn* / *Cn*) (post-spray ratio); **B**= 1- post-spray ratio; **C**= 1- post-spray ratio / sum B (proportion decline by hour); **D**= Cumulative rate of decline.

Table 10. Hourly proportion decline and cumulative rate of decline mean humna-landing collections between DDT treated and control huts during 5 all night collections of *Mansonia dyari* in April-May 1996 in Caledonia, Belize.

Hour	Treated Hut-1 (*Tn*)	Control Hut-2 (*Cn*)	A	B	C	D
1	5.2	8.2	0.6341	0.3658	0.0974	0.0974
2	20.8	30.4	0.6842	0.3158	0.0840	0.1814
3	21.6	29.8	0.7248	0.2752	0.0734	0.2548
4	18.4	20.6	0.8932	0.1068	0. 0284	0.2832
5	8.4	13.6	0.6176	0.3823	0.1018	0.3851
6	6	5.6	1.0714	- 0.0714	- 0.0190	0.3661
7	3.2	3.8	0.8421	0.1579	0.0420	0.4081
8	2	3.6	0.5555	0.4444	0.1183	0.5264
9	1.6	4	0.4	0.6	0.1598	0.6862
10	2.2	2	1.1	-0.1	-0.0266	0.6596
11	0.4	2.8	0.1428	0.8571	0.2282	0.8878
12	2.2	3.8	0.5789	0.4210	0.1122	1.0
	92	**128.2**	**8.2449**	**3.7550**		

A= Treated/Control (*Tn* / *Cn*) (post-spray ratio); **B**= 1- post-spray ratio; **C**= 1- post-spray ratio / sum B (proportion decline by hour); **D**= Cumulative rate of decline.

Table 11. Proportion analysis of paired mark-release-recapture data from Caledonia, Belize* of proportion escape by 2-hr intervals in DDT-treated hut v. control hut using blood-fed (BF) and non-blood fed (UF) *Anopheles albimanus*. Escape defined as mosquitoes either in window exit boxes or number missing from indoor observations at each 2-hr interval, minus previous 2-hr observations.

Hour	Control BF v. UF		Treated BF v. UF		Blood Fed Control v. Treated		Unfed Control v. Treated	
	z-stat	*p*	z-stat	*p*	z-stat	*p*	z-stat	*p*
2000	0.952	0.341	-3.613	**0.0003****	0.882	0.378	-4.01	**<0.001****
2200	0.787	0.431	-1.130	0.258	-2.145	**0.032***	-4.44	**<0.001****
2400	-0.272	0.786	-2.623	**0.001****	-1.235	0.217	-3.95	**<0.001****
0200	-2.122	**0.034***	-9.401	**<0.001****	-0.457	0.648	-8.45	**<0.001****
0400	-3.131	**0.002****	-9.939	**<0.001****	0.428	0.669	-6.96	**<0.001****
0600	-6.010	**<0.001****	-1.869	0.062	-1.496	**<0.001****	-3.294	**0.001****
0800	-6.252	**<0.001****	0.0	1.0	-6.991	**<0.001****	-2.946	**0.003****
1000	-6.291	**<0.001****	0.0	1.0	-6.596	**<0.001****	-2.40	**0.016***

* Trials during April-May 1996. z-stat= test statistic; *p*-value <0.05 = * significant; *p*<0.001= ** highly significant.
BF= 09 trials UF=12 trials. Sample size: BF sprayed= 220; UF sprayed=285; BF control= 219; UF control= 291.

Table 12. Proportion analysis of paired mark-release-recapture data from Caledonia, Belize* of proportion escape by 2-hr intervals in DDT-treated hut v. control hut using blood-fed (BF) and non-blood fed (UF) *Anopheles vestitipennis.* Escape defined as mosquitoes either in window exit boxes or number missing from indoor observations at each 2-hr interval, minus previous 2-hr observations.

Hour	Control BF v. UF		Treated BF v. UF		Blood Fed Control v. Treated		Unfed Control v. Treated	
	z-stat	p	z-stat	p	z-stat	p	z-stat	p
2000	3.254	0.0012**	-3.616	0.0003**	0.661	0.509	-6.42	<0.001**
2200	5.686	<0.001**	-3.038	0.0024**	0.644	0.520	-8.374	<0.001**
2400	7.921	<0.001**	-2.178	0.0294*	2.867	0.004**	-7.692	<0.001**
0200	8.166	<0.001**	-4.863	<0.001**	0.191	0.848	-13.50	<0.001**
0400	8.188	<0.001**	-1.730	0.084	-1.273	0.203	-12.12	<0.001**
0600	-2.916	0.0036**	-1.575	0.116	-2.445	0.014*	-1.576	0.115
0800	-2.755	0.0058**	0.0	1.0	-2.221	0.026*	0.0	1.0
1000	-1.584	0.114	0.0	1.0	-1,276	0.202	0.0	1.0

* Trials during April-May 1996. z-stat= test statistic; p-value <0.05 = * significant; p<0.001= ** highly significant.
BF= 07 trials UF=10 trials. Sample size: BF sprayed= 162; UF sprayed=247; BF control= 159; UF control= 250.

Table 13. Summary of blood meal analysis by sandwich ELISA from outdoor resting collections* in Caledonia, Corozal District, Belize.

Species	N	Human	Bovine	Pig	Fowl	Canine	Unk	H/A†
An. albimanus	31	10	18	0	0	1	2	1:2.1
An. vestitipennis	38	26	12	0	0	0	0	2.2:1
An. crucians	7	2	5	0	0	0	0	1:2.5
An. punctimacula	1	1	0	0	0	0	0	—
Total	77	39	35	0	0	1	2	1.02:1

* Collections from December 1995 –January 1996
†H/A= Human to Animal blood ratio.
Unk= Unknown

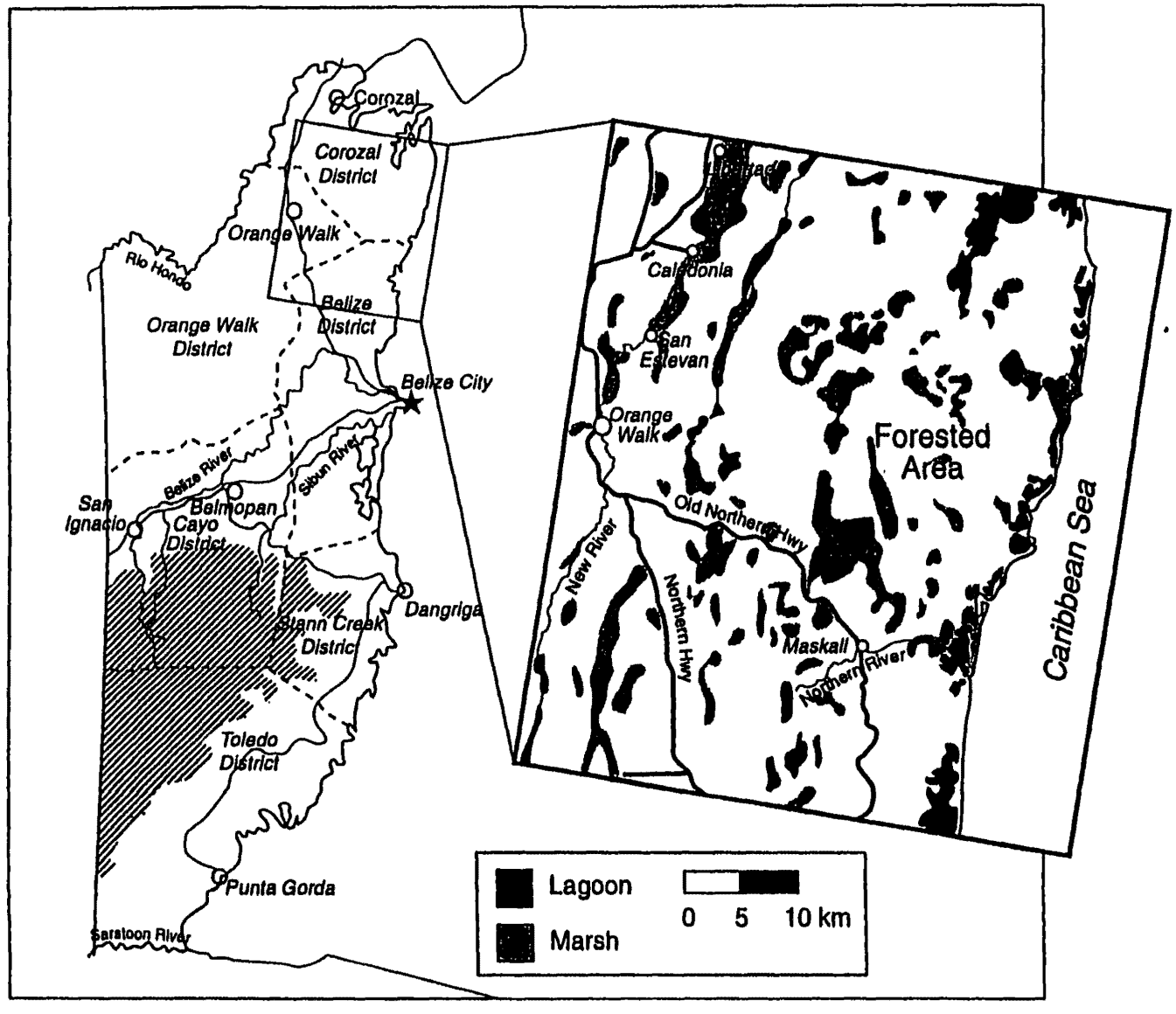


Figure 1. Map of Belize, depicting general location of study site, Caledonia Village, located in the northern district (Corozal) along the western side of the New River.

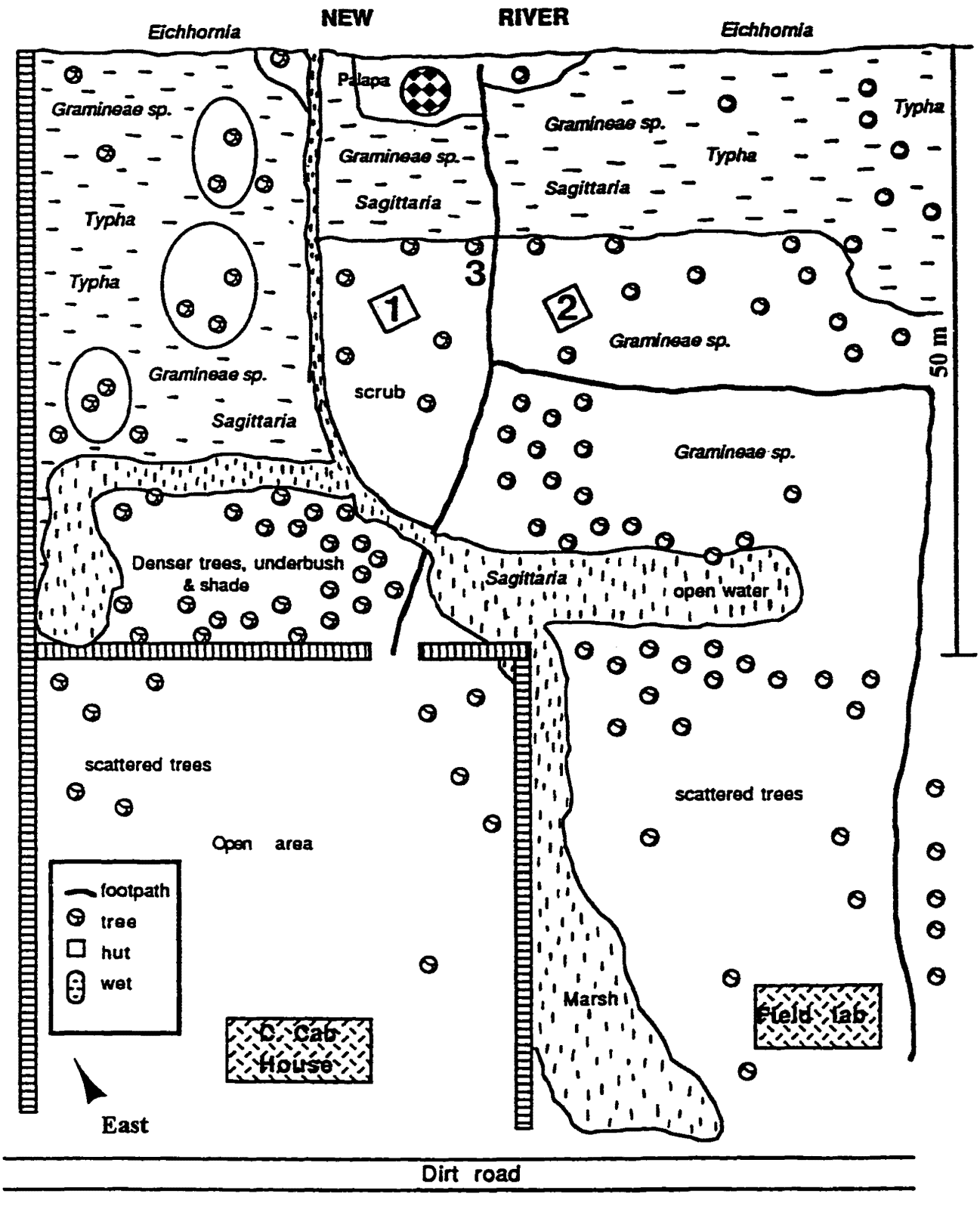


Figure 2. Immediate study site located in Caledonia Village, northern Belize, showing placement of experimental huts (1 & 2) and outdoor collection site (3) with field laboratory and surrounding environment near the New River.

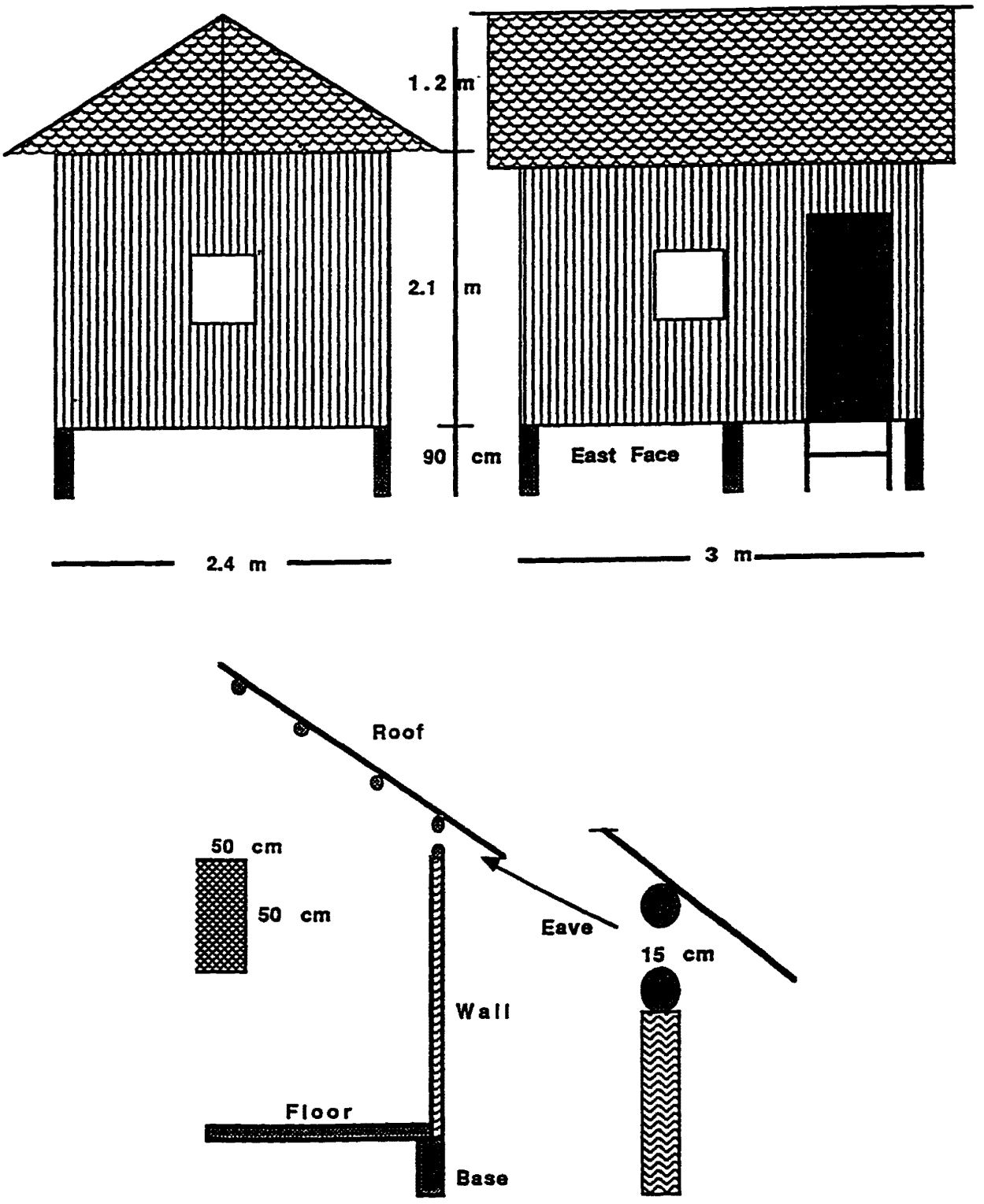


Figure 3. Representation of experimental huts: design, size dimensions and position of windows and door.

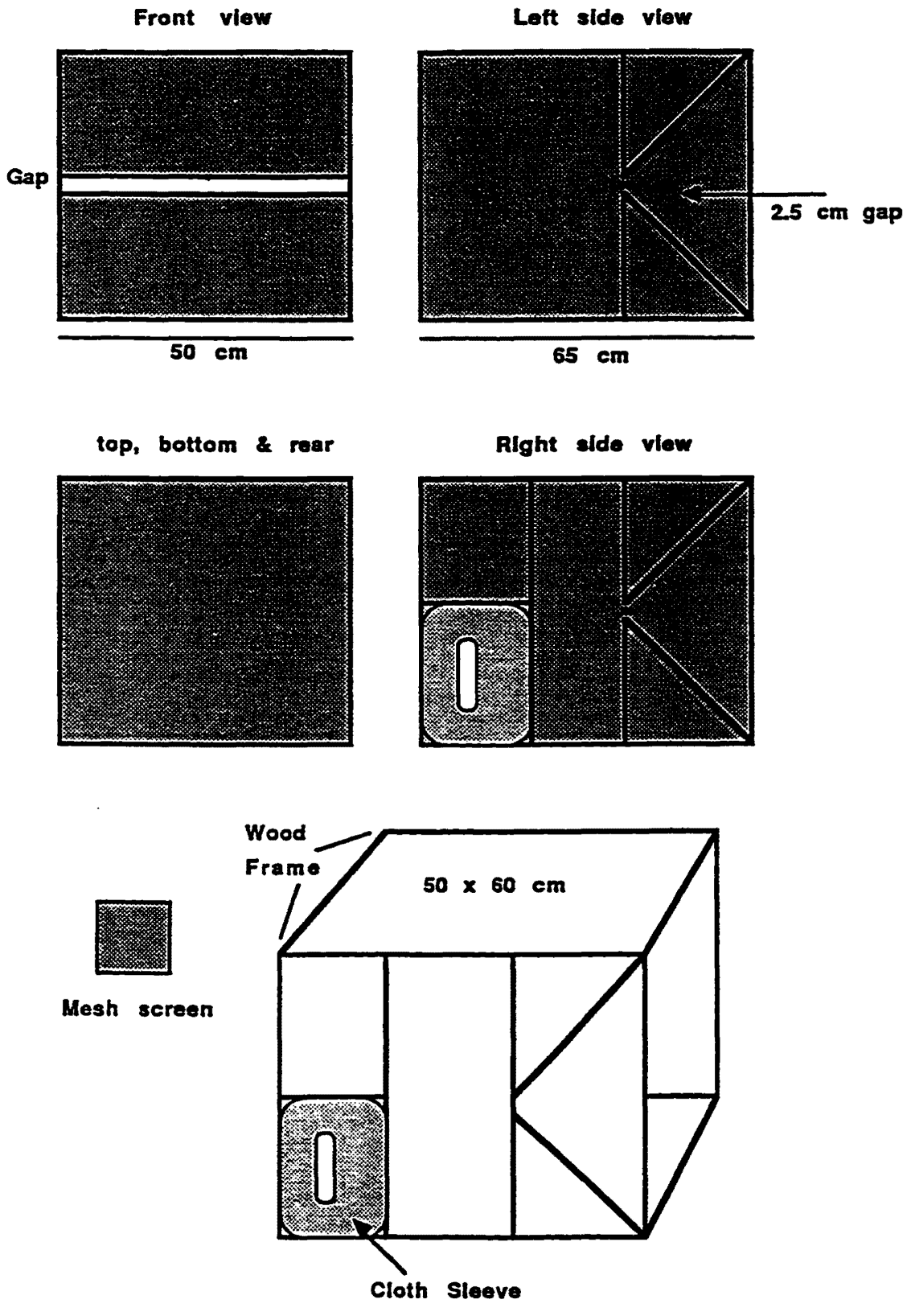


Figure 4. Representation of entry/exit interception traps: design and size dimensions.

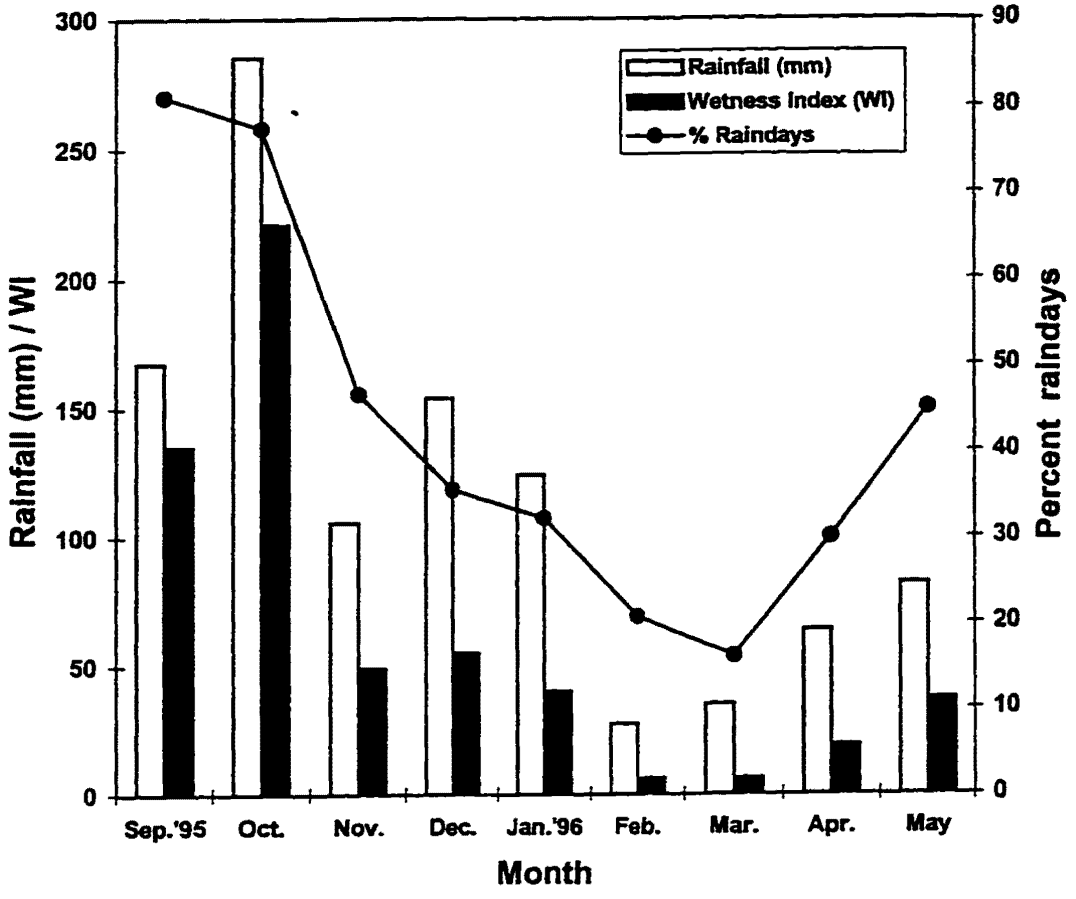


Figure 5. Monthly total rainfall, percent raindays, and wetness index from 10 September 1995 to 20 May 1996 in Caledonia Village, northern Belize.

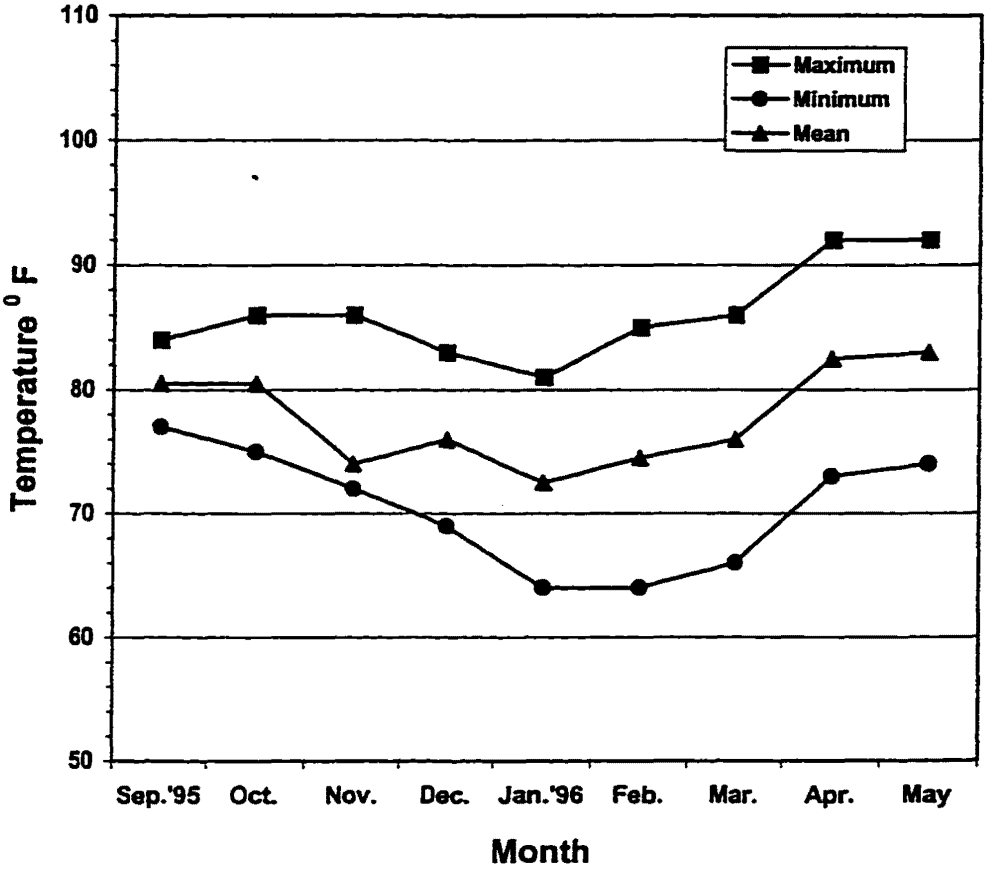
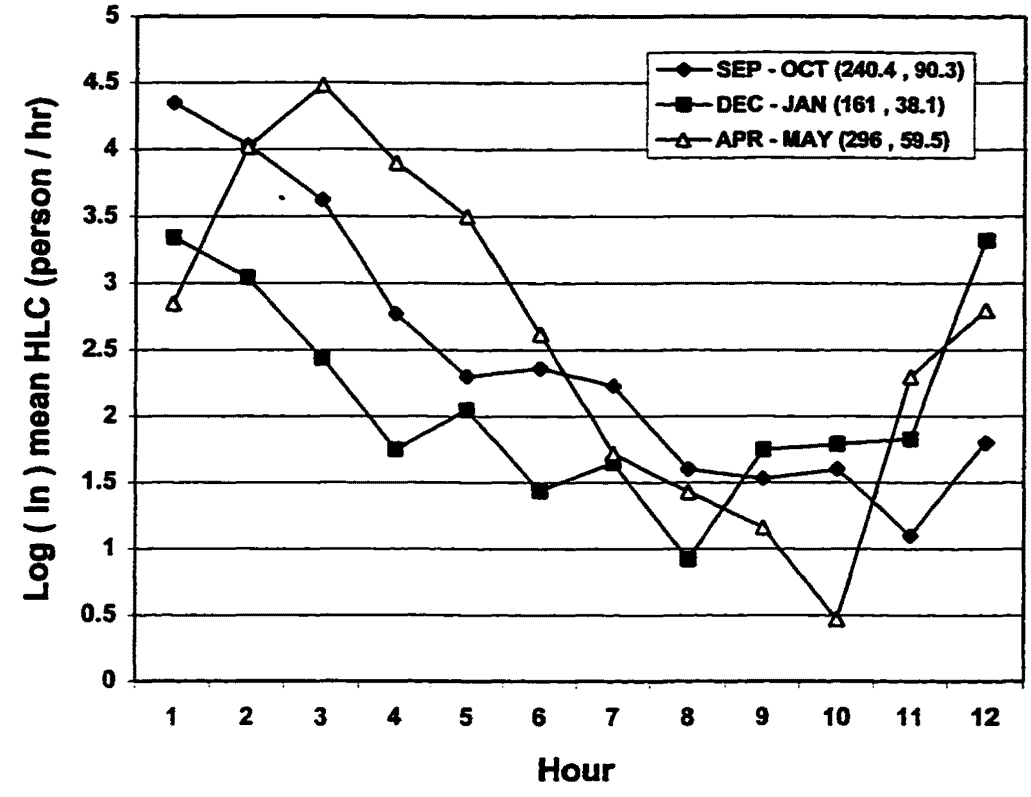


Figure 6. Monthly maximum, minimum, and mean ambient air temperatures from 10 September 1995 to 20 May 1996 in Caledonia Village, northern Belize.


SEP - OCT (240.4 , 90.3)
DEC - JAN (161 , 38.1)
APR - MAY (296 , 59.5)
Log (ln) mean HLC (person / hr)
Hour
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
1
2
3
4
5
6
7
8
9
10
11
12

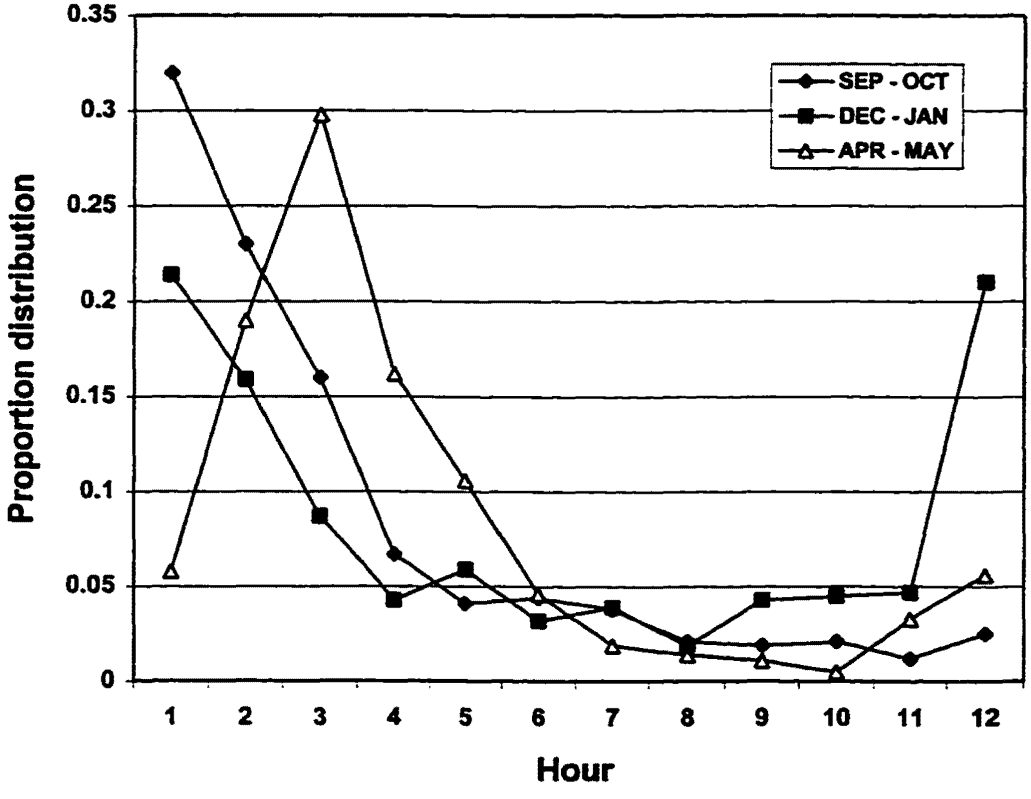
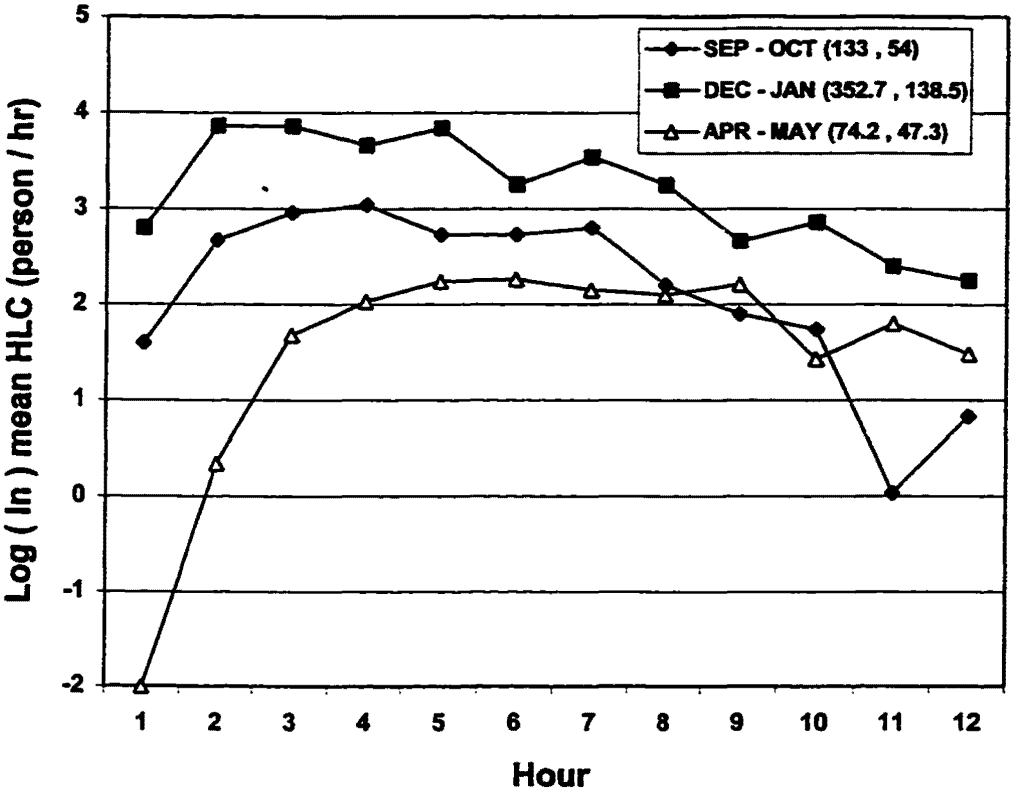

SEP - OCT
DEC - JAN
APR - MAY
Proportion distribution
Hour
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
1
2
3
4
5
6
7
8
9
10
11
12

Figure 7. (Top) Seasonal outdoor distribution of *Anopheles albimanus* in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches (HLC) per person/hour, and (Bottom) mean proportional seasonal distribution by collection hour. Sep.-Oct. and Apr.-May hourly periods: 1= 1800-1845 to 12= 0500-0545. Dec.-Jan. included 13-hr per collection, periods 1 and 2 (1730-1915 hr) represent a combined average for hr-1; hr-12= 0530-0615. Sample size (mean and SE) of HLC per person/night for Sep.-Oct. derived from 6 collection nights, Dec.-Jan.= 4 nights, and Apr.-May= 5 nights.


SEP - OCT (133 , 54)
DEC - JAN (352.7 , 138.5)
APR - MAY (74.2 , 47.3)
Log (ln) mean HLC (person / hr)
5
4
3
2
1
0
-1
-2
1
2
3
4
5
6
7
8
9
10
11
12
Hour

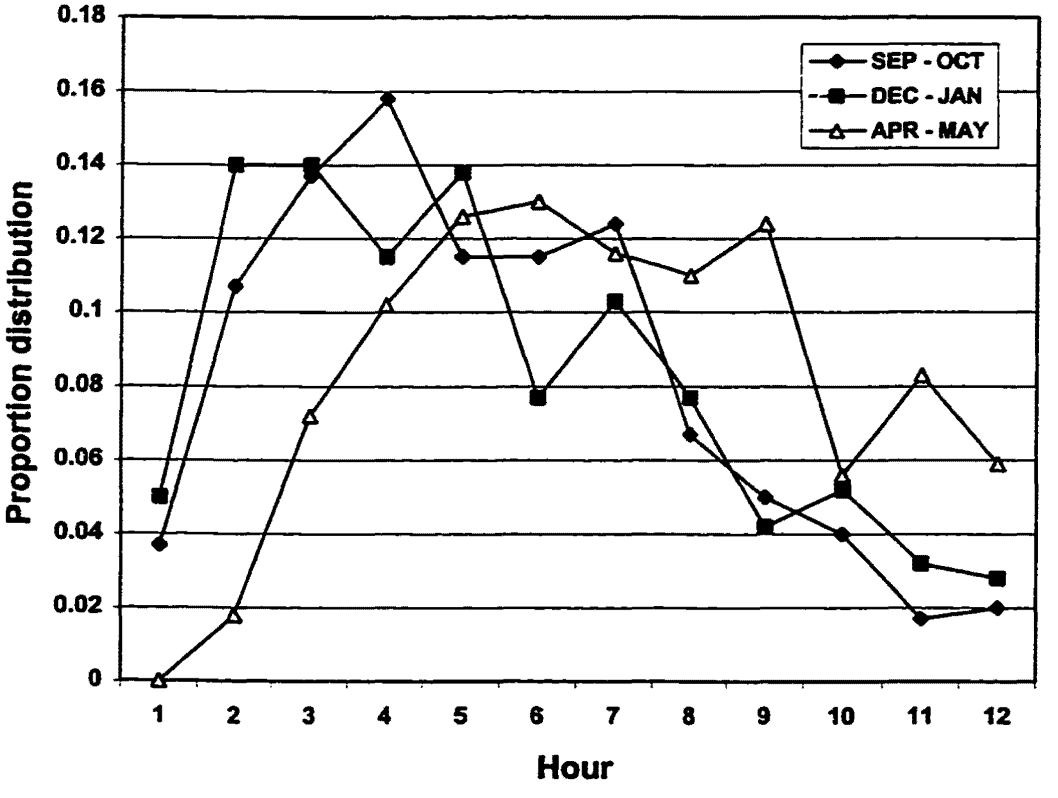
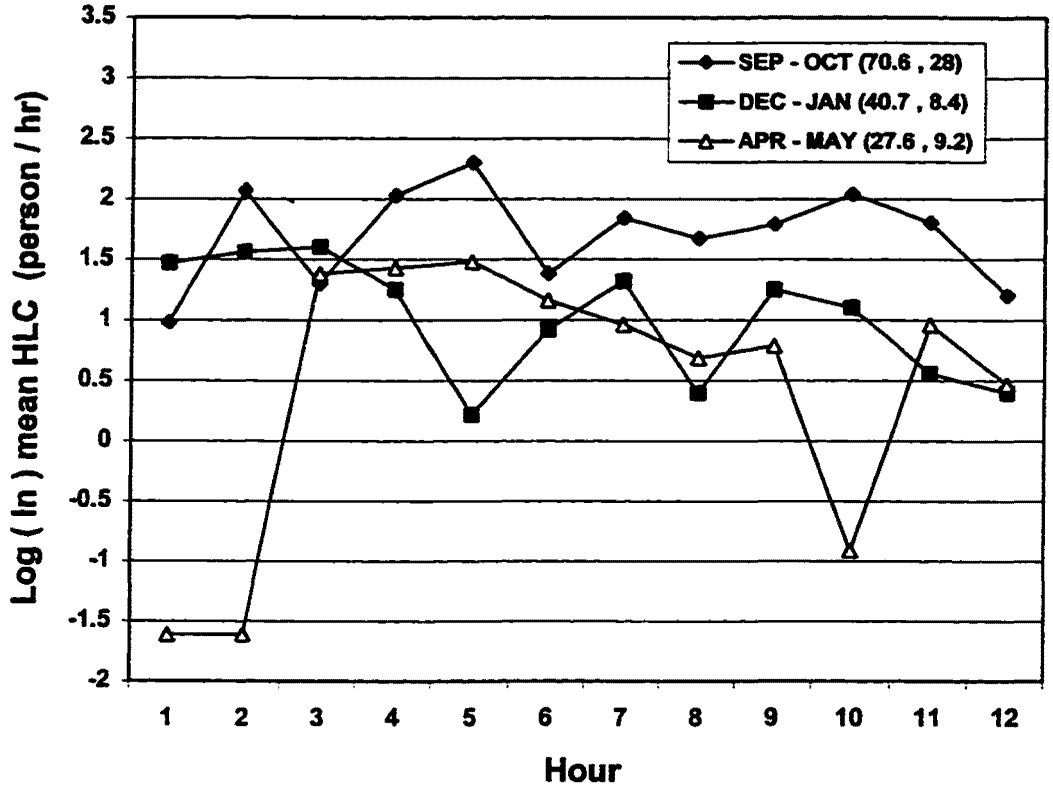

SEP - OCT
DEC - JAN
APR - MAY
Proportion distribution
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1
2
3
4
5
6
7
8
9
10
11
12
Hour

Figure 8. (Top) Seasonal outdoor distribution of *Anopheles vestitipennis* in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches (HLC) per person/hour, and (Bottom) mean proportional seasonal distribution by collection hour. Sep.-Oct. and Apr.-May hourly periods: 1= 1800-1845 to 12= 0500-0545. Dec.-Jan. included 13-hr per collection, periods 1 and 2 (1730-1915 hr) represent a combined average for hr-1; hr-12= 0530-0615. Sample size (mean and SE) of HLC per person/night for Sep.-Oct. derived from 6 collection nights, Dec.-Jan.= 4 nights, and Apr.-May= 5 nights.


SEP - OCT (70.6 , 28)
DEC - JAN (40.7 , 8.4)
APR - MAY (27.6 , 9.2)
Log (ln) mean HLC (person / hr)
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
1 2 3 4 5 6 7 8 9 10 11 12
Hour

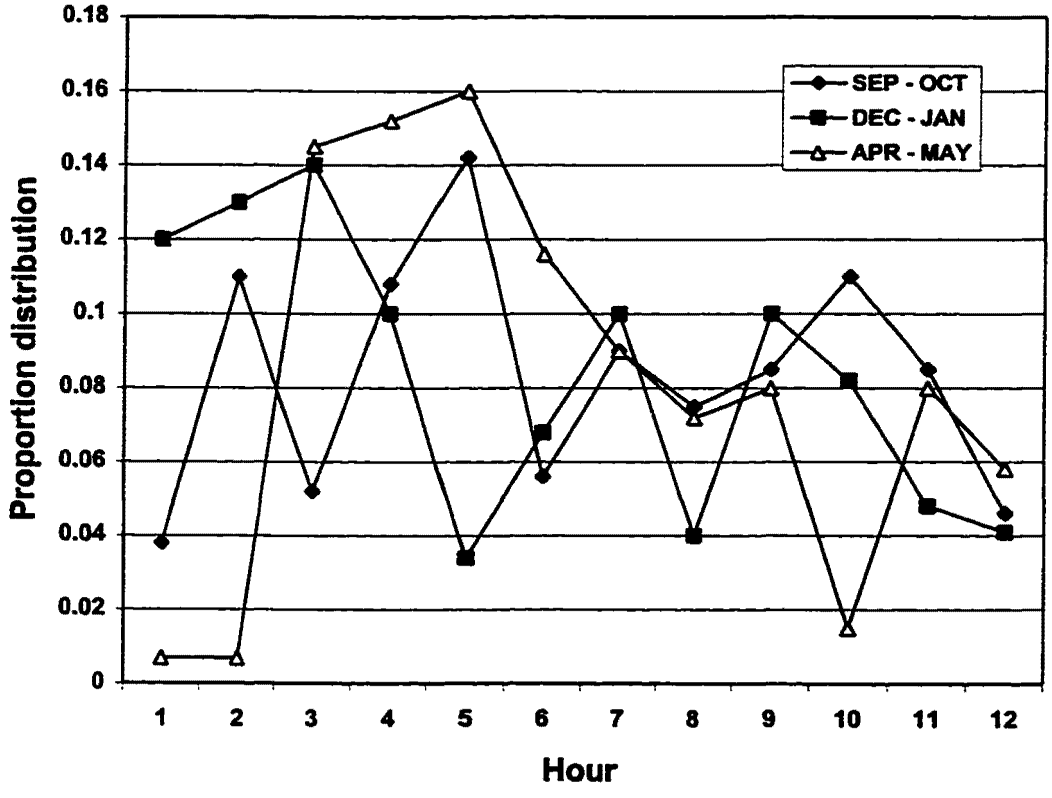
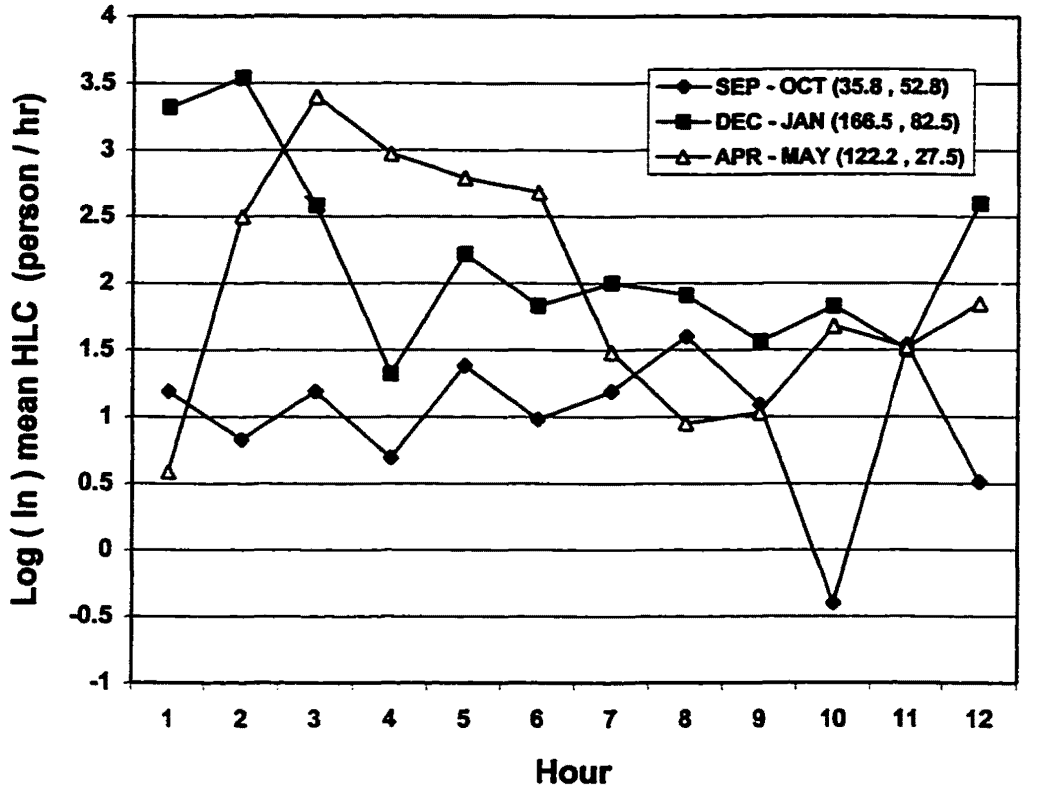

SEP - OCT
DEC - JAN
APR - MAY
Proportion distribution
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1 2 3 4 5 6 7 8 9 10 11 12
Hour

Figure 9. (Top) Seasonal outdoor distribution of *Anopheles crucians* in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches (HLC) per person/hour, and (Bottom) mean proportional seasonal distribution by collection hour. Sep.-Oct. and Apr.-May hourly periods: 1= 1800-1845 to 12= 0500-0545. Dec.-Jan. included 13-hr per collection, periods 1 and 2 (1730-1915 hr) represent a combined average for hr-1; hr-12= 0530-0615. Sample size (mean and SE) of HLC per person/night for Sep.-Oct. derived from 6 collection nights, Dec.-Jan.=4 nights, and Apr.-May=5 nights.


SEP - OCT (35.8 , 52.8)
DEC - JAN (166.5 , 82.5)
APR - MAY (122.2 , 27.5)
Log (ln) mean HLC (person / hr)
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
1 2 3 4 5 6 7 8 9 10 11 12
Hour

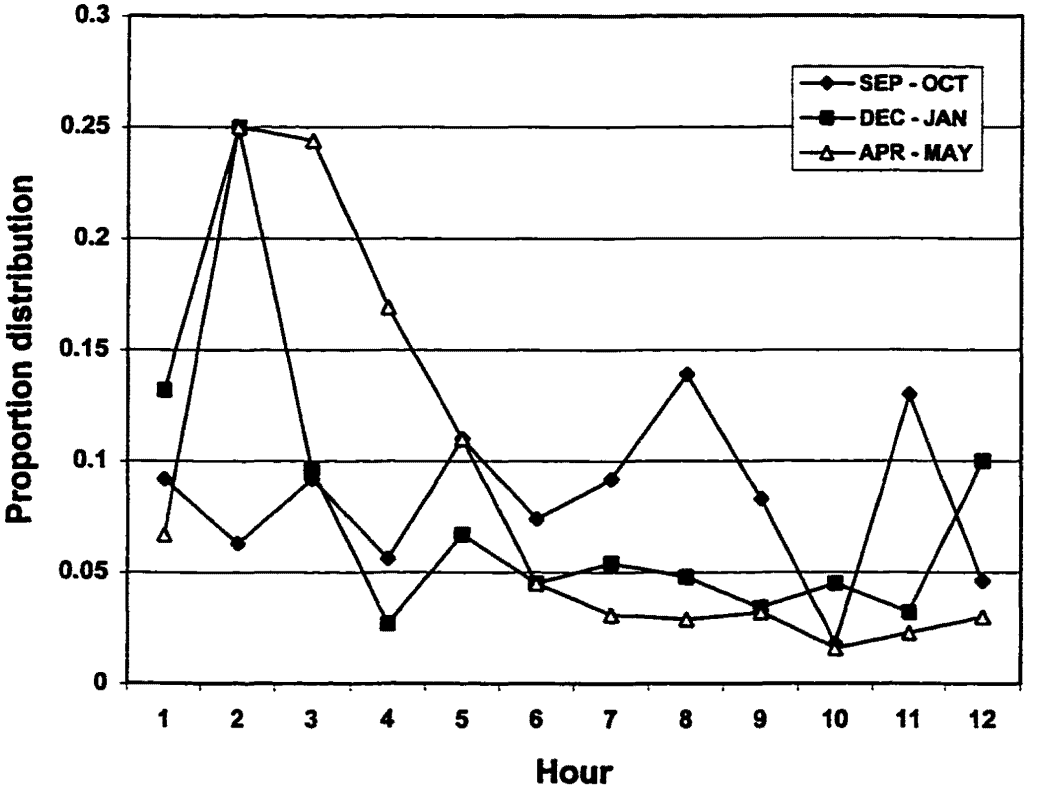

SEP - OCT
DEC - JAN
APR - MAY
Proportion distribution
0.3
0.25
0.2
0.15
0.1
0.05
0
1 2 3 4 5 6 7 8 9 10 11 12
Hour

Figure 10. (Top) Seasonal outdoor distribution of *Mansonia dyari* in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches (HLC) per person/hour, and (Bottom) mean proportional seasonal distribution by collection hour. Sep.-Oct. and Apr.-May hourly periods: 1= 1800-1845 to 12= 0500-0545. Dec.-Jan. included 13 collection periods per collection, periods 1 and 2 (1730-1915 h) represent a combined average for hour 1; hour 12= 0530-0615. Sample size (mean and SE) of HLC per person/night for Sep.-Oct. derived from 6 collection nights, Dec.-Jan.=4 nights, and Apr.-May=5 nights.

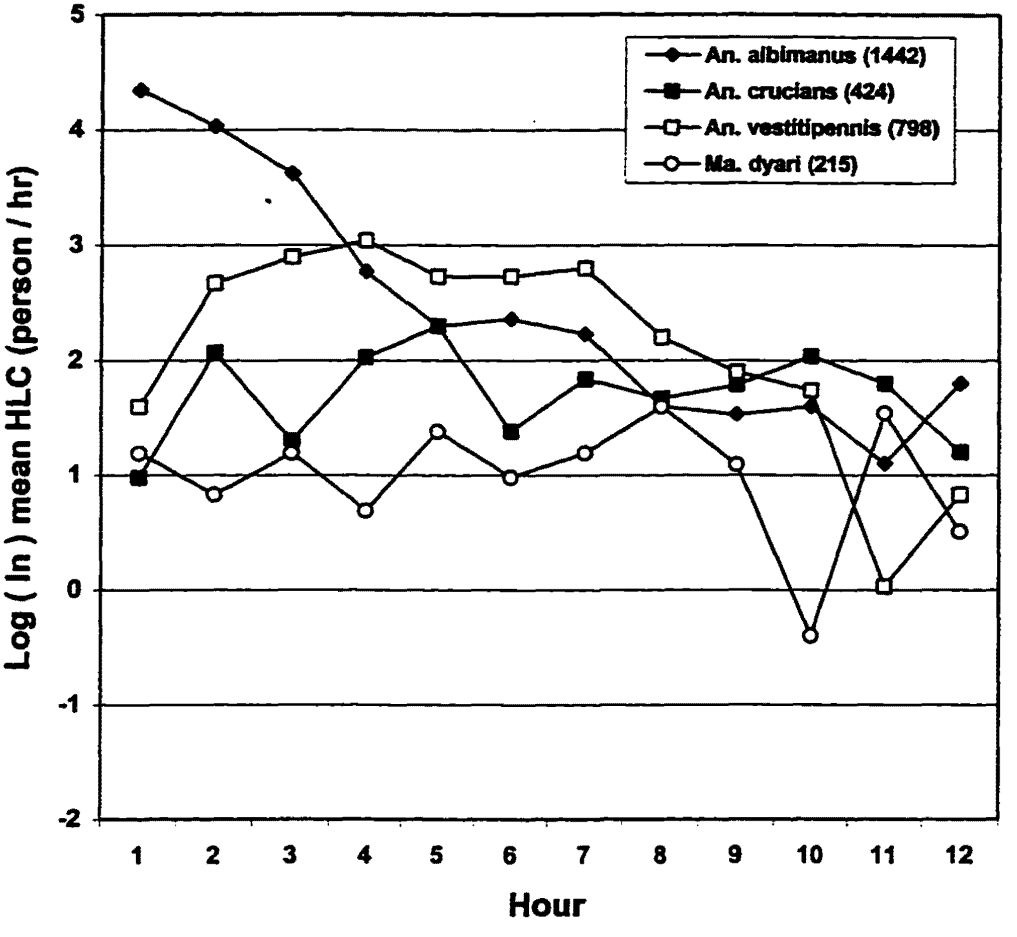


Figure 11. Outdoor distribution of 3 *Anopheles* and 1 *Mansonia* species in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches per person/hour during September - October 1995. 1= 1800-1845 to 12= 0500-0545. Sampling nights= 6, with total sample size by species in parenthesis.

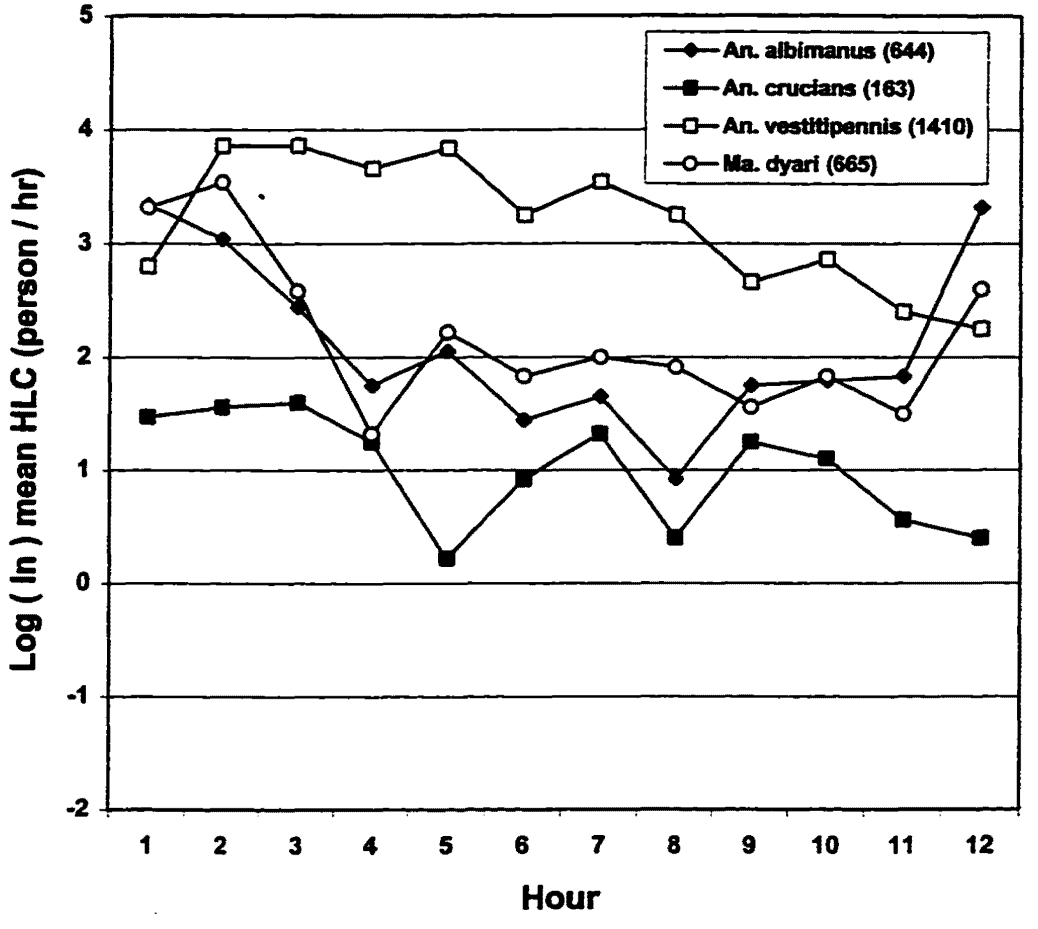


Figure 12. Outdoor distribution of 3 *Anopheles* and 1 *Mansonia* species in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches per person/hour during December 1995 - January 1996. 13 hours per collection night, periods 1 and 2 (1730-1915 h) represent combined average for hour 1, hour 12= 0530-0615. Sampling nights= 4, with total sample size by species in parenthesis.

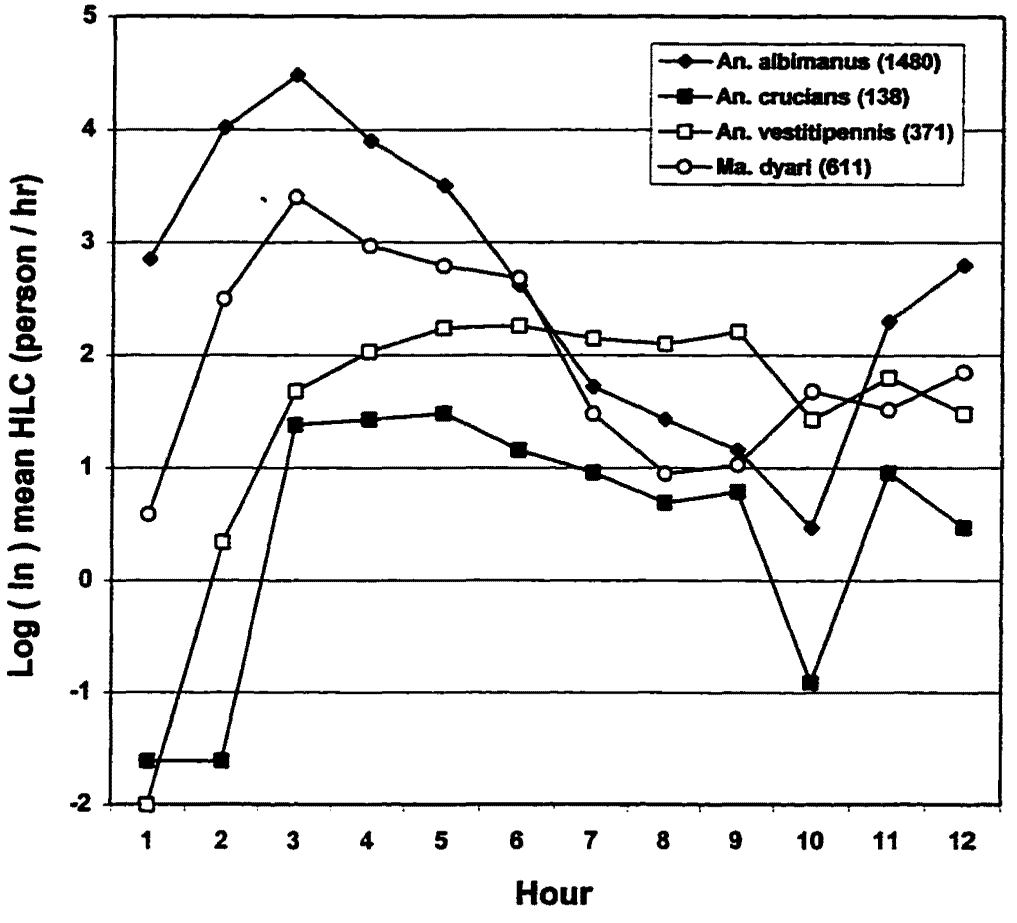
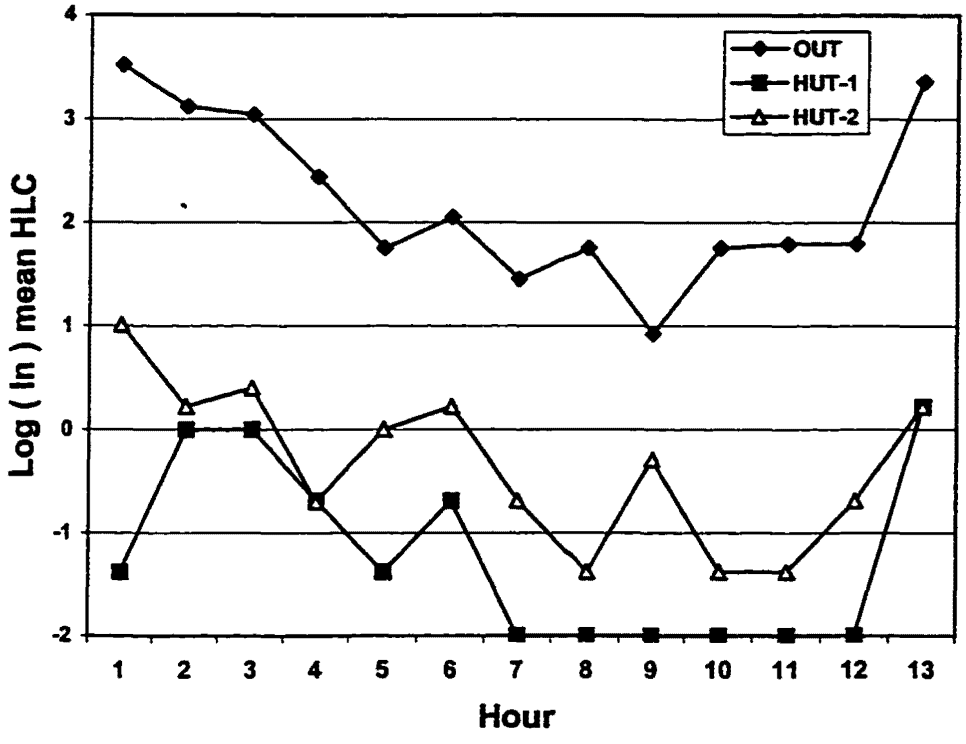


Figure 13. Outdoor distribution of 3 *Anopheles* and 1 *Mansonia* species in Caledonia, Belize expressed as natural log (ln) means of all-night hourly human-landing catches per person/hour during April - May 1996. 1= 1800-1845 to 12= 0500-0545. Sampling nights=5, with total sample size by species in parenthesis.


OUT
HUT-1
HUT-2
Log (ln) mean HLC
4
3
2
1
0
-1
-2
1
2
3
4
5
6
7
8
9
10
11
12
13
Hour

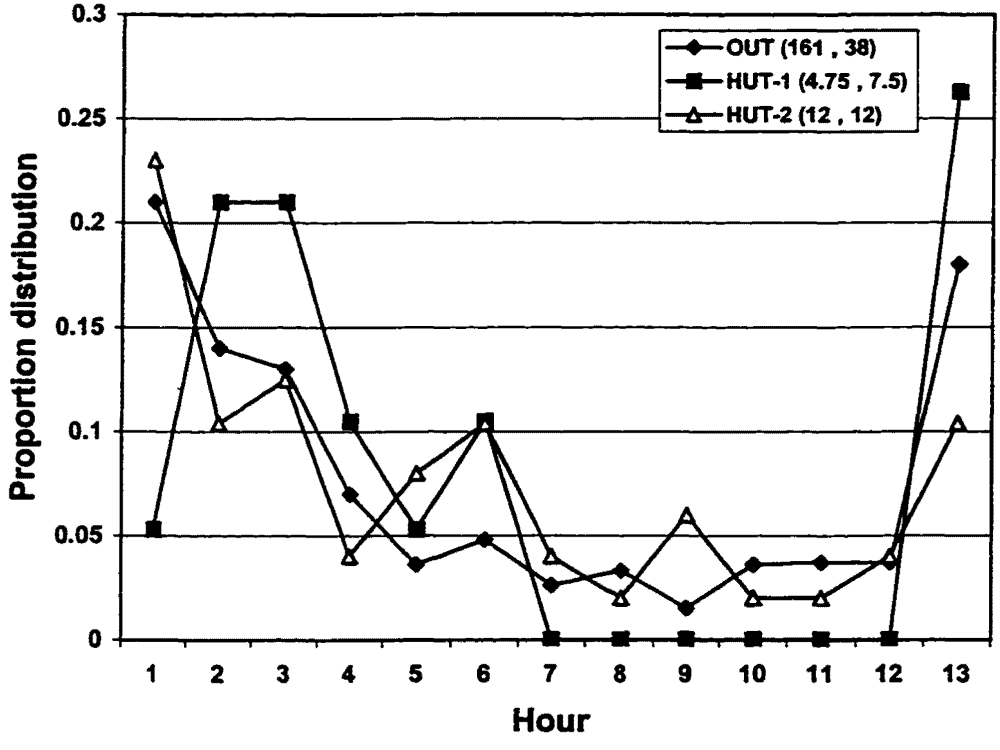
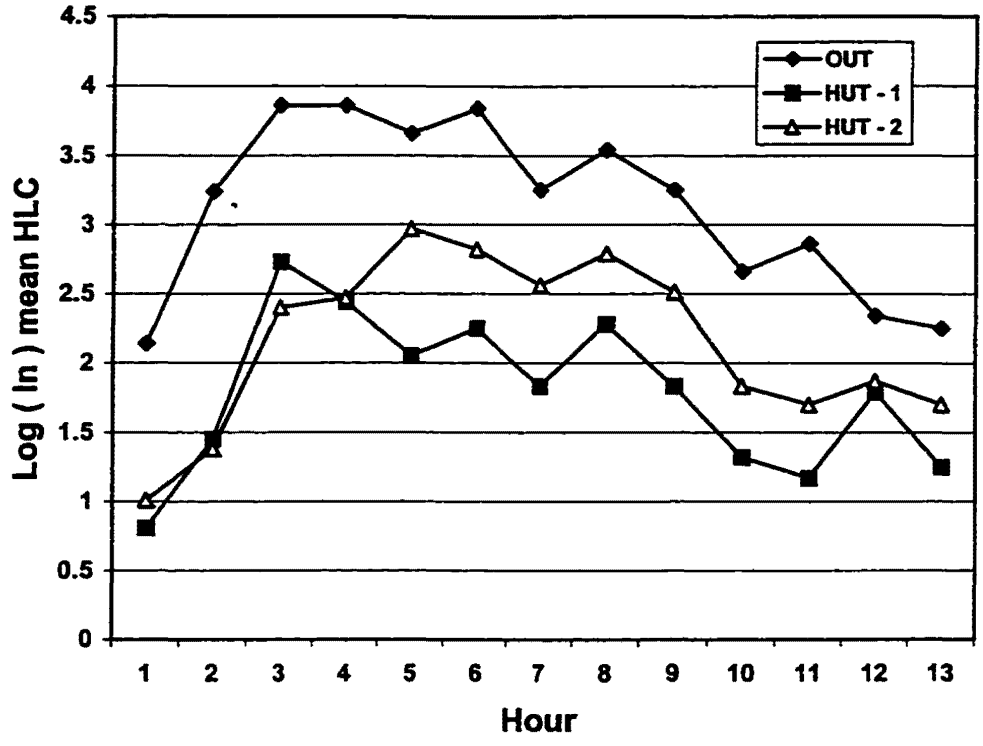

OUT (161 , 38)
HUT-1 (4.75 , 7.5)
HUT-2 (12 , 12)
Proportion distribution
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Hour

Figure 14. Pre-DDT treatment human-landing catches (HLC), outdoors (Out) and indoor Hut-1 and Hut-2, for *Anopheles albimanus* in Caledonia, Belize. Data (Top) expressed hourly as natural log (ln) mean HLC, and (Bottom) mean proportional distribution of mosquitoes per person/hr. 1= 1730-1815 to 13= 0530-0615 hr (45-min intervals). Sample size (mean, SE) for HLC by collection site during 4 (Dec.- Jan.) collection nights.


OUT
HUT - 1
HUT - 2
Log (ln) mean HLC
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Hour

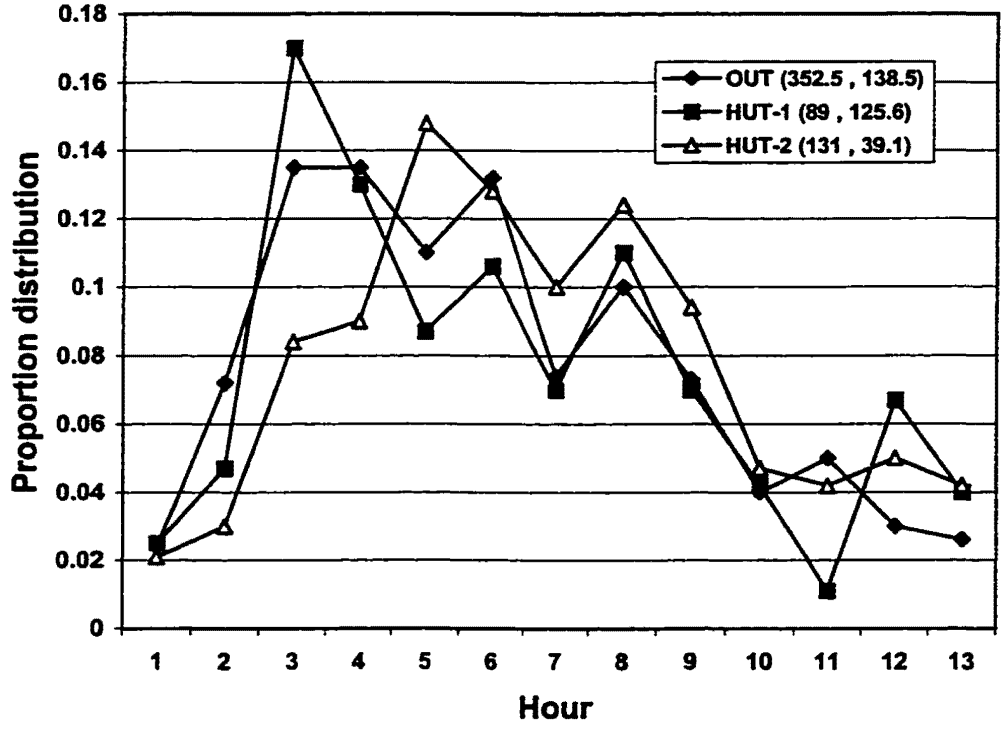
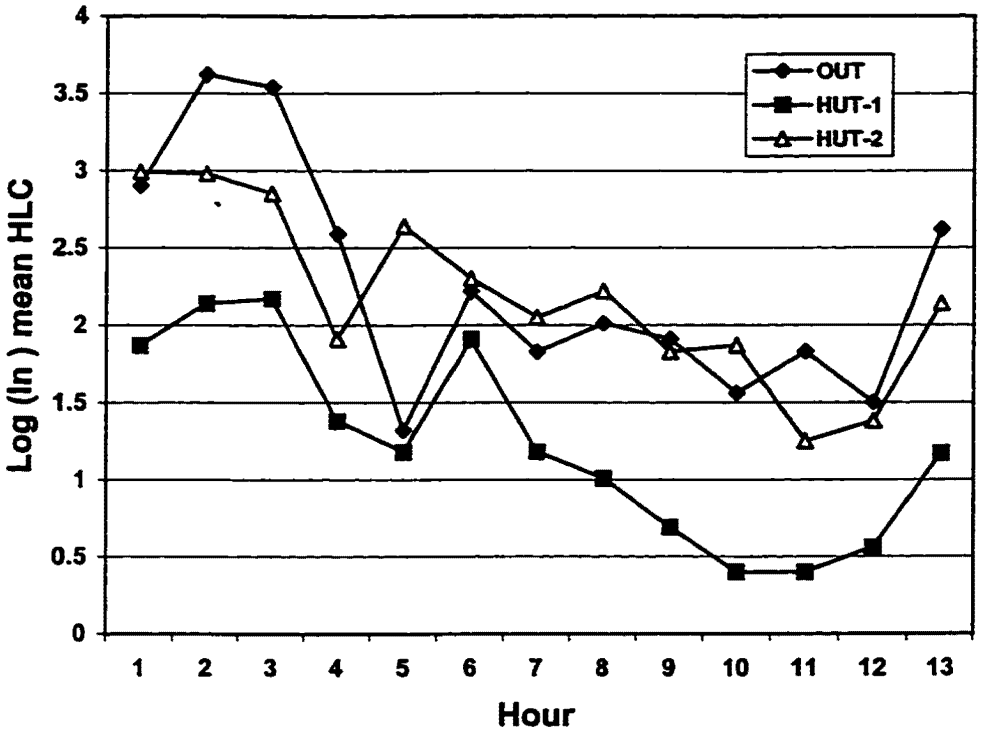

OUT (352.5 , 138.5)
HUT-1 (89 , 125.6)
HUT-2 (131 , 39.1)
Proportion distribution
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Hour

Figure 15. Pre-DDT treatment human-landing catches (HLC), outdoors (Out) and indoor Hut-1 and Hut-2, for *Anopheles vestitipennis* in Caledonia, Belize. Data (Top) expressed hourly as natural log (ln) mean HLC, and (Bottom) mean proportional distribution of mosquitoes per person/hr. 1= 1730-1815 to 13= 0530-0615 hr (45-min intervals). Sample size (mean, SE) for HLC by collection site during 4 (Dec.- Jan.) collection nights.


Log (ln) mean HLC
Hour
OUT
HUT-1
HUT-2
0
0.5
1
1.5
2
2.5
3
3.5
4
1
2
3
4
5
6
7
8
9
10
11
12
13

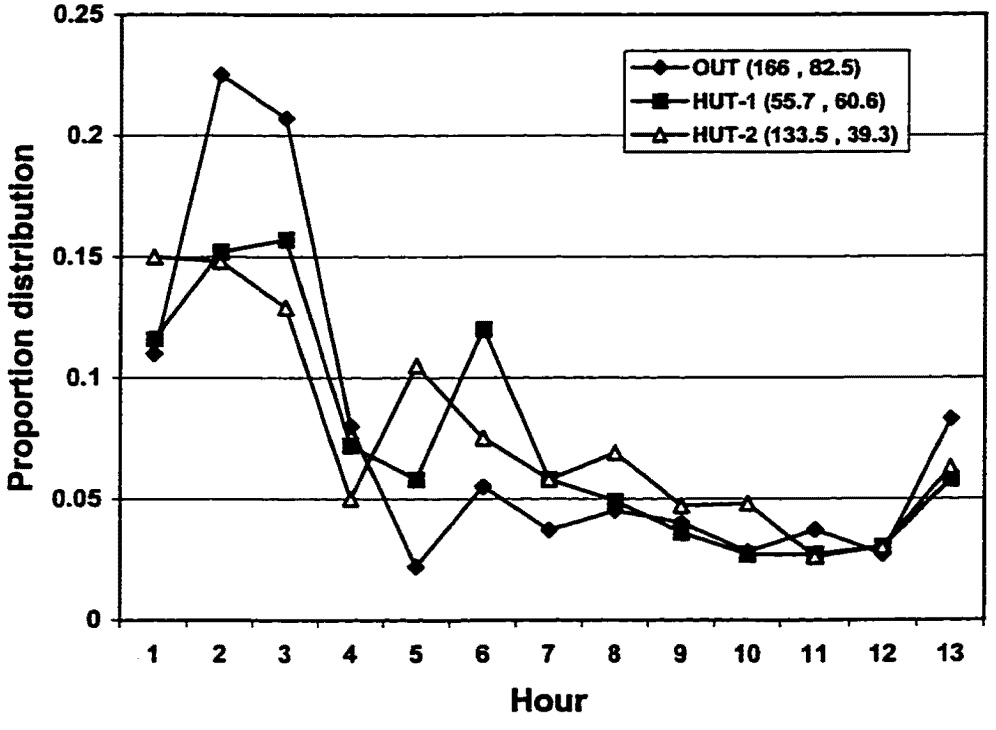
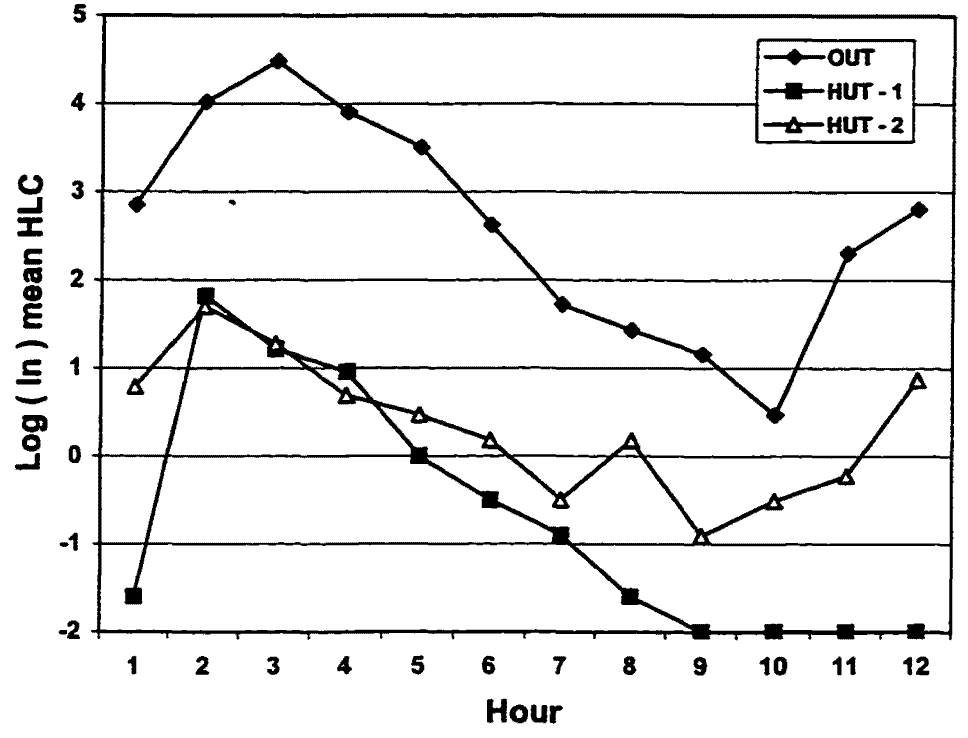

Proportion distribution
Hour
OUT (166 , 82.5)
HUT-1 (55.7 , 60.6)
HUT-2 (133.5 , 39.3)
0
0.05
0.1
0.15
0.2
0.25
1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 16. Pre-DDT treatment human-landing catches (HLC), outdoors (Out) and indoor Hut-1 and Hut-2, for *Mansonia dyari* in Caledonia, Belize. Data (Top) expressed hourly as natural log (ln) mean HLC, and (Bottom) mean proportional distribution of mosquitoes per person/hr. 1= 1730-1815 to 13= 0530-0615 hr (45-min intervals). Sample size (mean, SE) for HLC by collection site during 4 (Dec.- Jan.) collection nights.


OUT
HUT - 1
HUT - 2
Log (ln) mean HLC
5
4
3
2
1
0
-1
-2
1
2
3
4
5
6
7
8
9
10
11
12
Hour

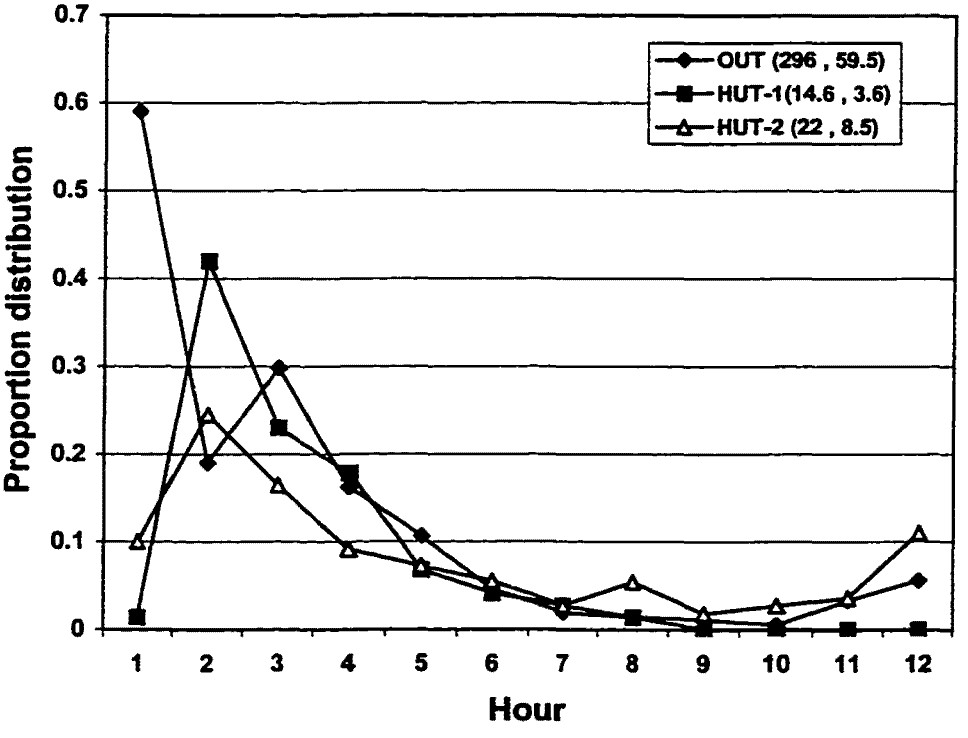
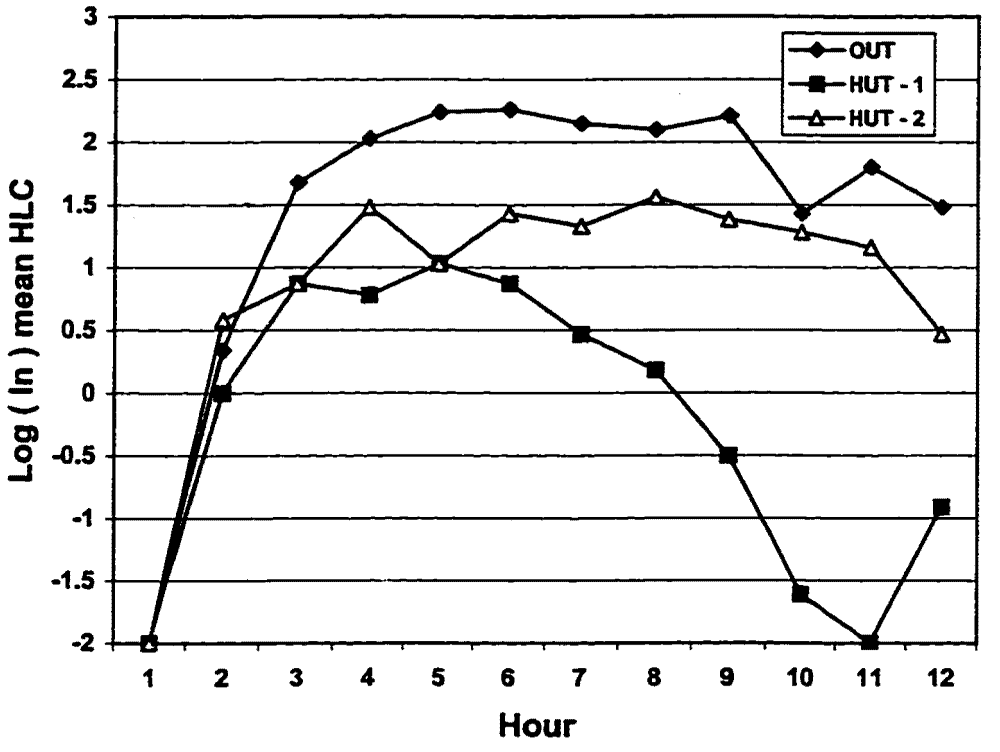

OUT (296 , 59.5)
HUT-1(14.6 , 3.6)
HUT-2 (22 , 8.5)
Proportion distribution
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
12
Hour

Figure 17. Post-DDT treatment human-landing catches (HLC), outdoors (Out) and indoor Hut-1 (DDT) and Hut-2 (control) for *Anopheles albimanus* during Apr.-May 1996 in Caledonia, Belize. Data (Top) expressed hourly as natural log (ln) mean HLC, and (Bottom) mean proportional distribution of mosquitoes per person/hr. 1= 1800-1845 to 12= 0500-0545 hr (45-min intervals). Sample size during 5 collection nights: Out (1480), Hut-1 (73), Hut-2 (110), with (mean, SE) by collection site.


OUT
HUT - 1
HUT - 2
Log (ln) mean HLC
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
1
2
3
4
5
6
7
8
9
10
11
12
Hour

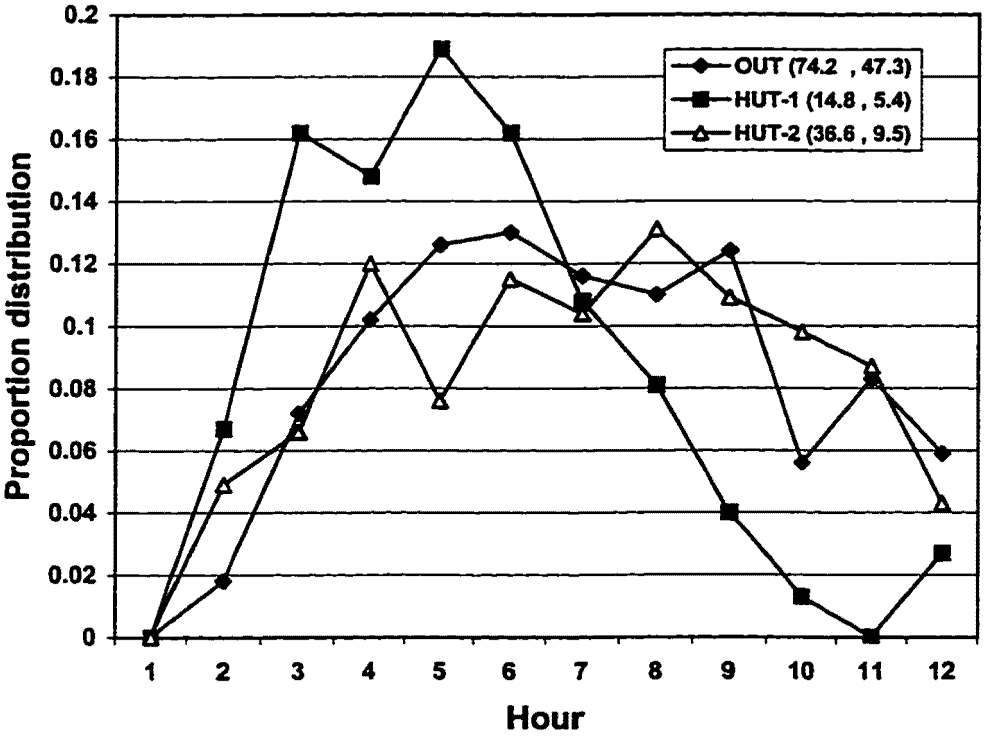
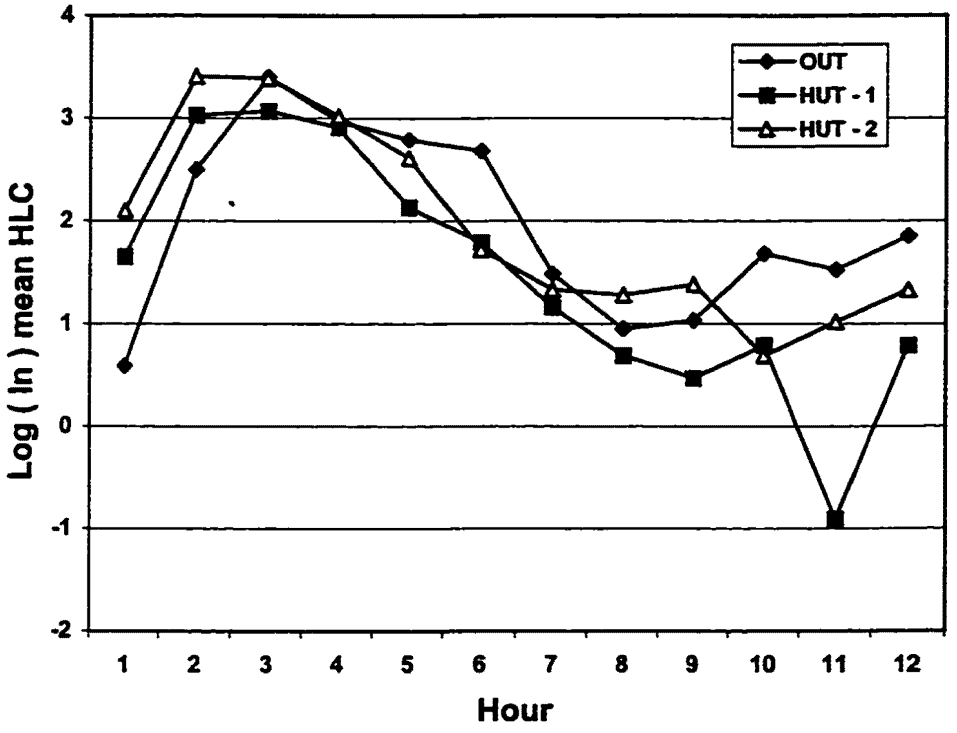

OUT (74.2 , 47.3)
HUT-1 (14.8 , 5.4)
HUT-2 (36.6 , 9.5)
Proportion distribution
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1
2
3
4
5
6
7
8
9
10
11
12
Hour

Figure 18. Post-DDT treatment human-landing catches (HLC), outdoors (Out) and indoor Hut-1 (DDT) and Hut-2 (control) for *Anopheles vestitipennis* during Apr.-May 1996 in Caledonia, Belize. Data (Top) expressed hourly as natural log (ln) mean HLC, and (Bottom) mean proportional distribution of mosquitoes per person/hr. 1= 1800-1845 to 12= 0500-0545 hr (45-min intervals). Sample size during 5 collection nights: Out (371), Hut-1 (74), Hut-2 (183), with (mean, SE) by collection site.


OUT
HUT - 1
HUT - 2
Log (ln) mean HLC
4
3
2
1
0
-1
-2
1
2
3
4
5
6
7
8
9
10
11
12
Hour

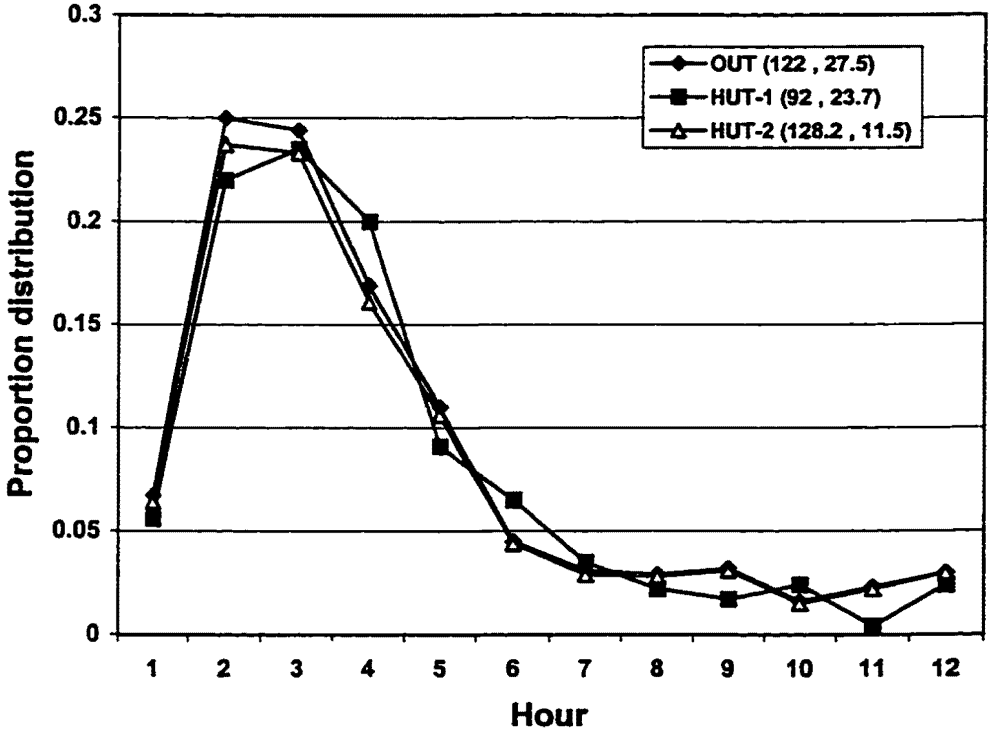
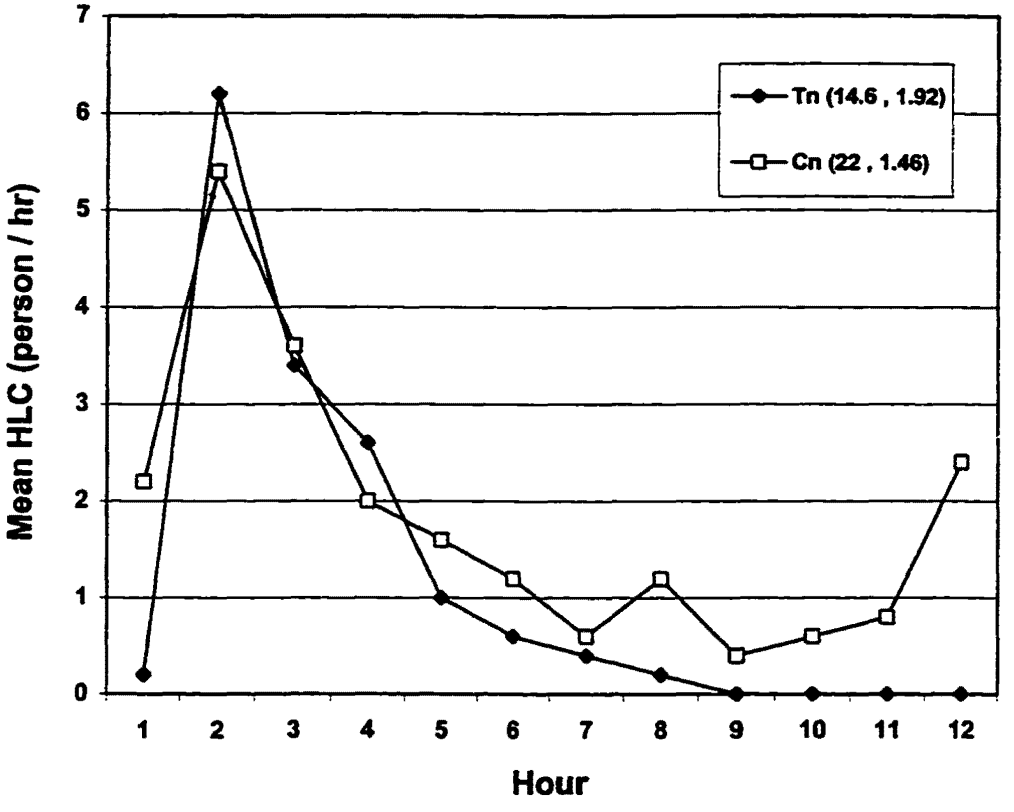

OUT (122 , 27.5)
HUT-1 (92 , 23.7)
HUT-2 (128.2 , 11.5)
Proportion distribution
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
Hour

Figure 19. Post-DDT treatment human-landing catches (HLC), outdoors (Out) and indoor Hut-1 (DDT) and Hut-2 (control) for *Mansonia dyari* during Apr.-May 1996 in Caledonia, Belize. Data (Top) expressed hourly as natural log (ln) mean HLC, and (Bottom) mean proportional distribution of mosquitoes per person/hr. 1= 1800-1845 to 12= 0500-0545 hr (45-min intervals). Sample size during 5 collection nights: Out (611), Hut-1 (460), Hut-2 (641), with (mean, SE) by collection site.


Tn (14.6 , 1.92)
Cn (22 , 1.46)
Mean HLC (person / hr)
Hour
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
11
12

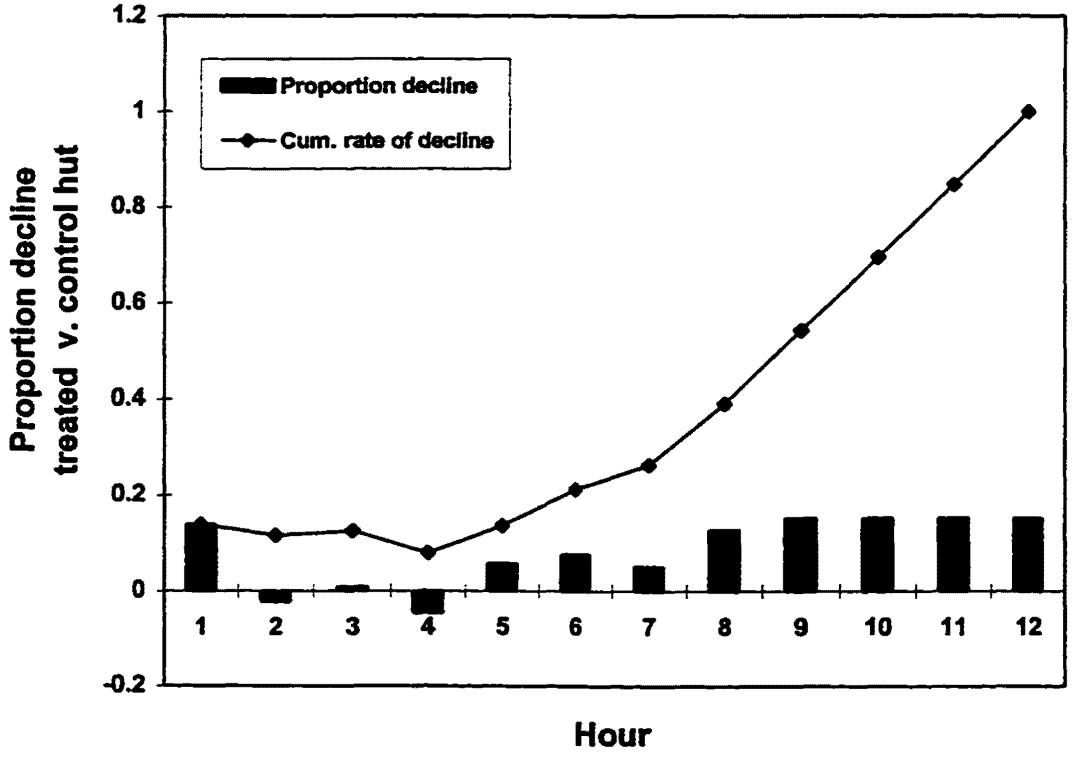
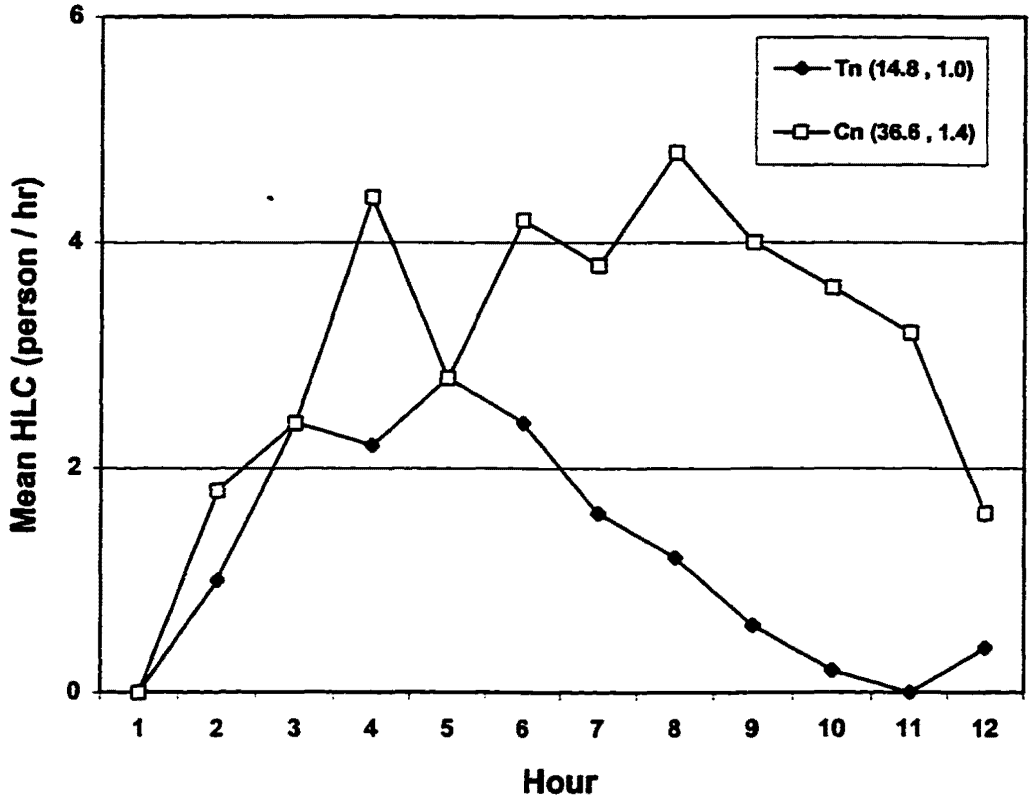

Proportion decline
Cum. rate of decline
Proportion decline treated v. control hut
Hour
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
1
2
3
4
5
6
7
8
9
10
11
12

Figure 20. (Top) Post-DDT treatment mean human-landing catches (HLC) per person/hr for *Anopheles albimanus* in treated and control huts by hr in Caledonia, Belize. Tn= treated; Cn= control; (mean, SE) per person hr. Sample size during 5 collection nights during Apr.-May: Hut-1 (73), Hut-2 (110). (Bottom) post-spray proportion decline or increase (bars) and cumulative rate of decline (line) in HLC by hr inside the treated hut compared to control. Hour 1= 1800-1845 to 12= 0500-0545 hr.


Tn (14.8 , 1.0)
Cn (36.6 , 1.4)
Mean HLC (person / hr)
0
2
4
6
1
2
3
4
5
6
7
8
9
10
11
12
Hour

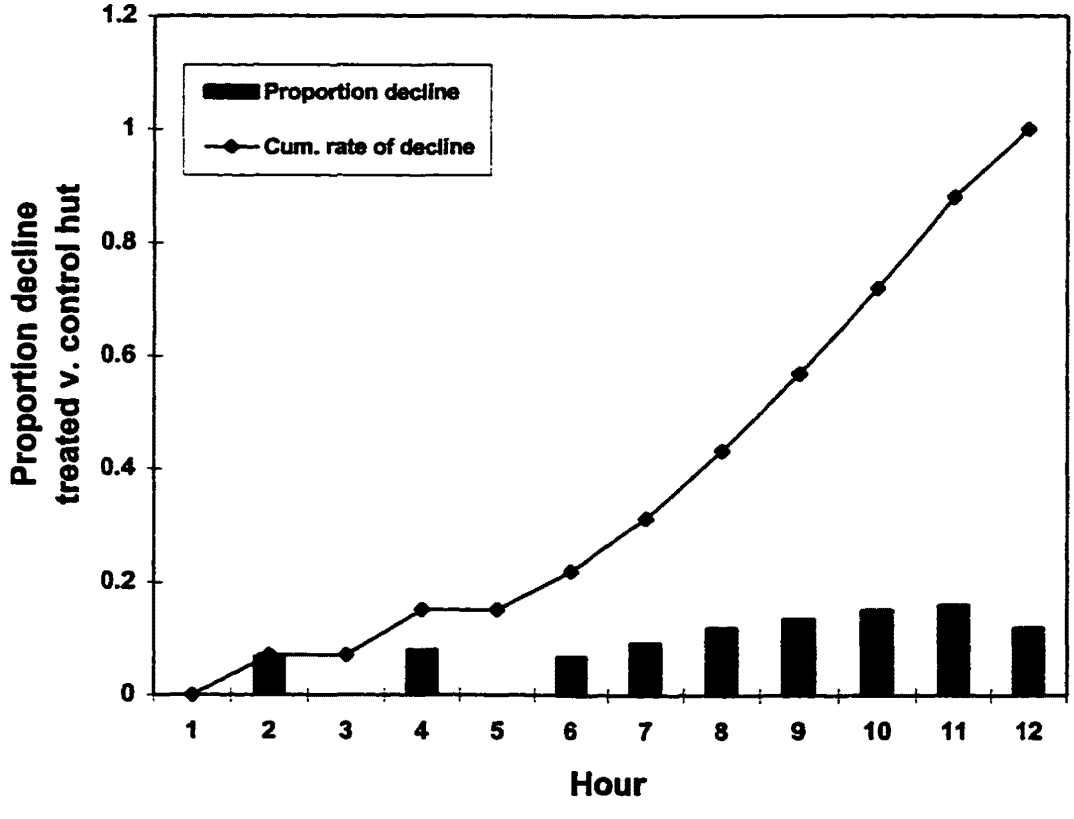

Proportion decline
Cum. rate of decline
Proportion decline treated v. control hut
0
0.2
0.4
0.6
0.8
1
1.2
1
2
3
4
5
6
7
8
9
10
11
12
Hour

Figure 21. (Top) Post-DDT treatment mean human-landing catches (HLC) per person/hr for *Anopheles vestitipennis* in treated and control huts by hr in Caledonia, Belize. Tn= treated; Cn= control; (mean, SE) per person hr. Sample size during 5 collection nights during Apr.-May: Hut-1 (74), Hut-2 (183). (Bottom) post-spray proportion decline or increase (bars) and cumulative rate of decline (line) in HLC by hr inside the treated hut compared to control. Hour 1= 1800-1845 to 12= 0500-0545 hr.

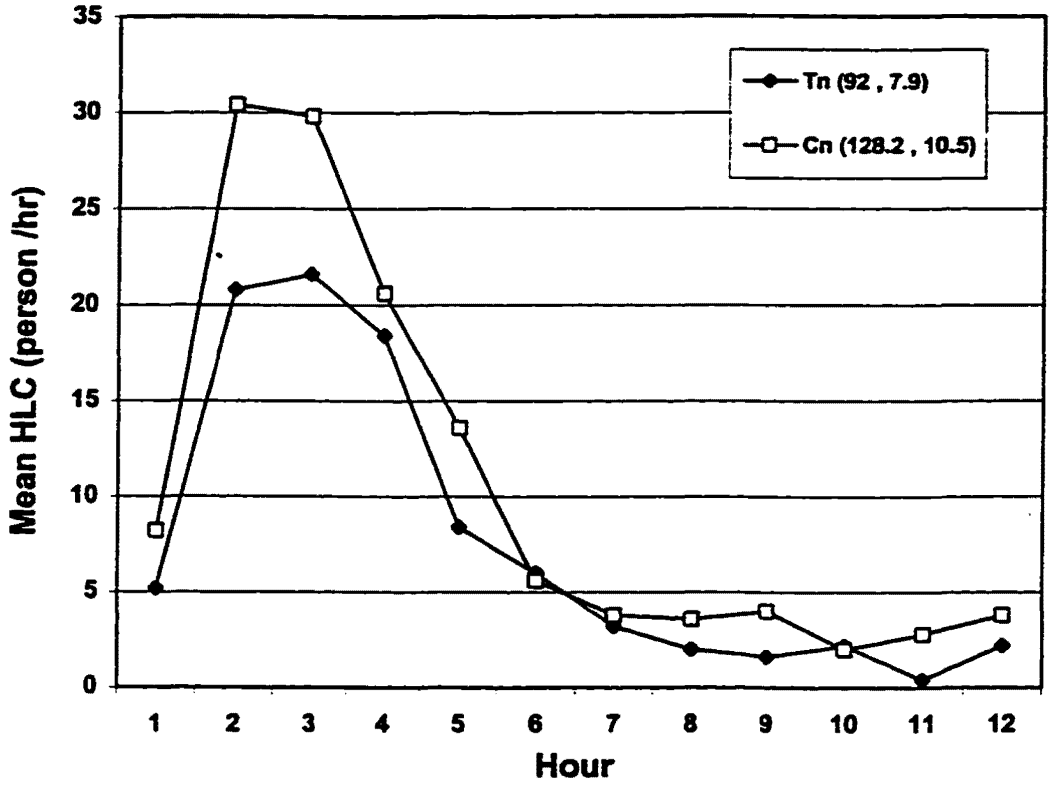


Figure 22. Post-DDT treatment mean human-landing catches (HLC) per person/hr for *Mansonia dyari* in treated and control huts by hr in Caledonia, Belize. Tn= treated; Cn= control; (mean, SE) per person hr. Sample size during 5 collection nights during Apr.-May in Caledonia: Hut-1 (460), Hut-2 (641). Hour 1= 1800-1845 to 12= 0500-0545 hr.

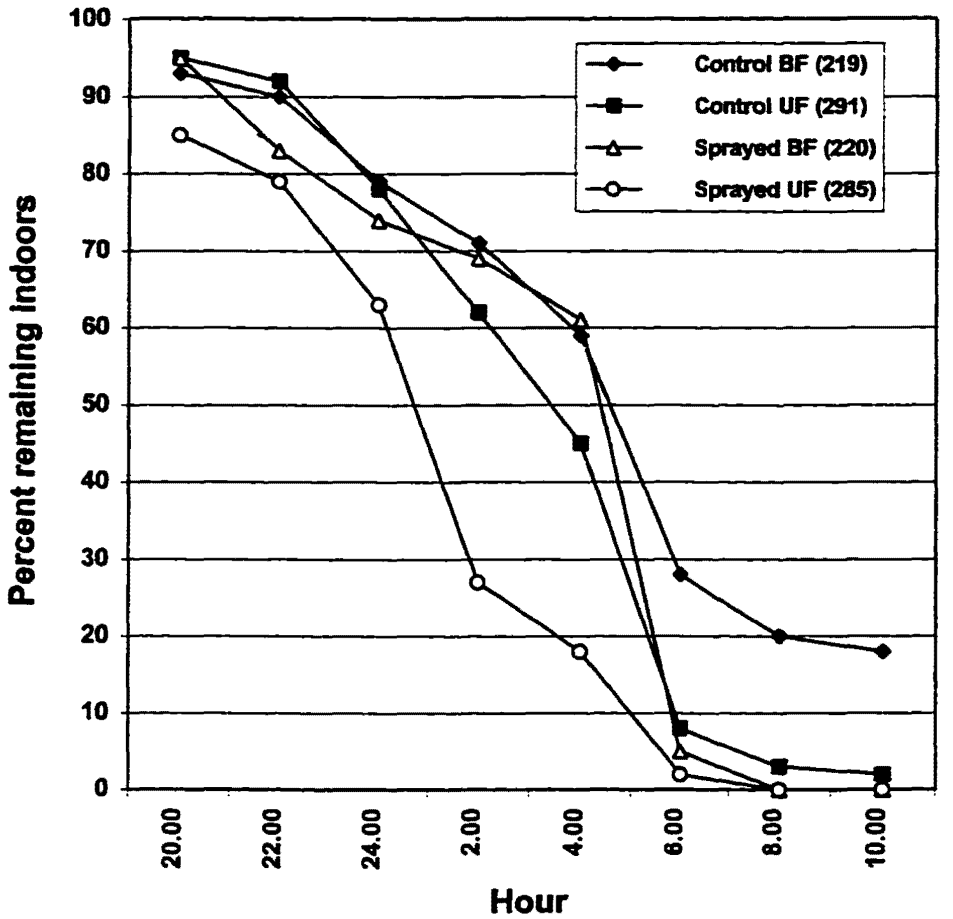


Figure 23. Mark-release study comparing effect of physiological condition and response to DDT in experimental huts 12-15 weeks post-spray during Apr.-May 1996 in Caledonia, Belize. Declining percentage of fresh blood-fed and unfed female *An. albimanus* remaining indoors during evening and morning hours, comparing paired post-spray experimental huts (DDT treated and control), using mosquitoes simultaneously released into huts equipped with window traps. Approximately 25 BF and 25 UF fluorescent powder-marked mosquitoes were monitored at 2-hr intervals from 2000 to 1000 hr in each hut. Total sample size in parenthesis over the following number of trial nights per physiological condition and hut: Treated hut BF=220, UF=285; Control hut BF=219, UF=291.

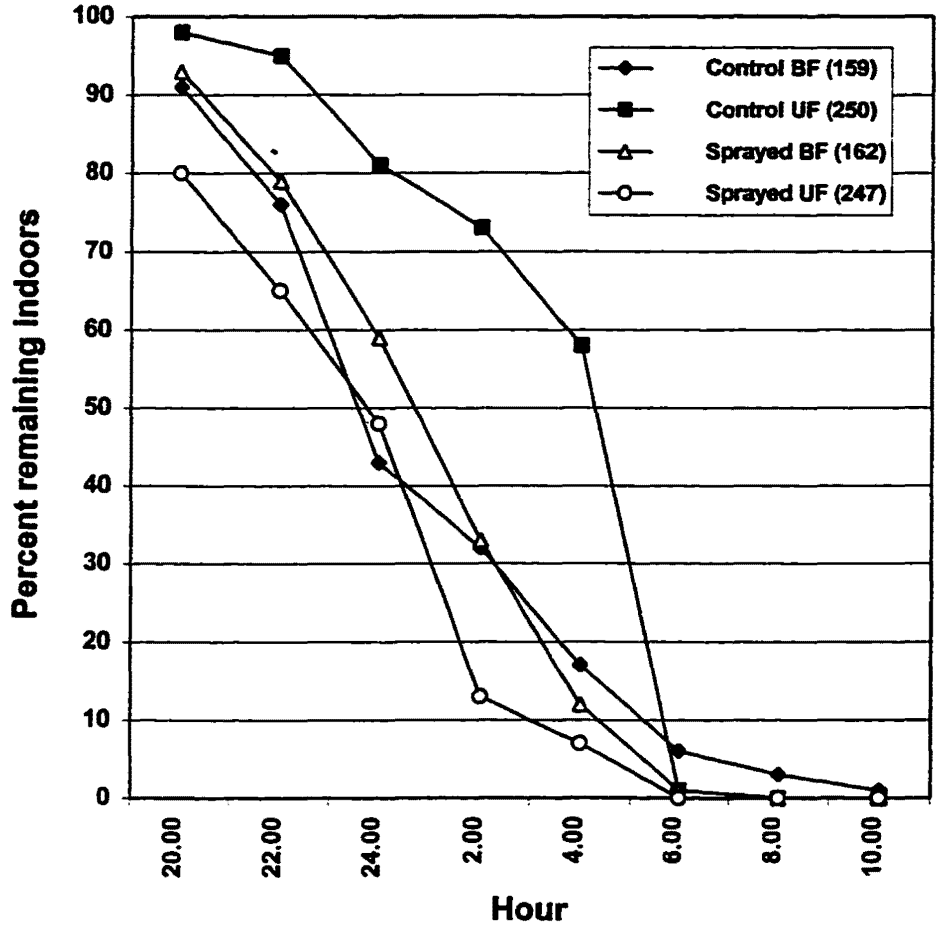


Figure 24. Mark-release study comparing effect of physiological condition and response to DDT in experimental huts 12-15 weeks post-spray during Apr.-May 1996 in Caledonia, Belize. Declining percentage of fresh blood-fed and unfed female *An. vestitipennis* remaining indoors during evening and morning hours, comparing paired post-spray experimental huts (DDT treated and control), using mosquitoes simultaneously released into huts equipped with window traps. Approximately 25 BF and 25 UF fluorescent powder-marked mosquitoes were monitored at 2-hr intervals from 2000 to 1000 hr in each hut. Total sample size in parenthesis over the following number of trial nights per physiological condition and hut: Treated hut BF=162, UF=247; Control hut BF=159, UF=250.

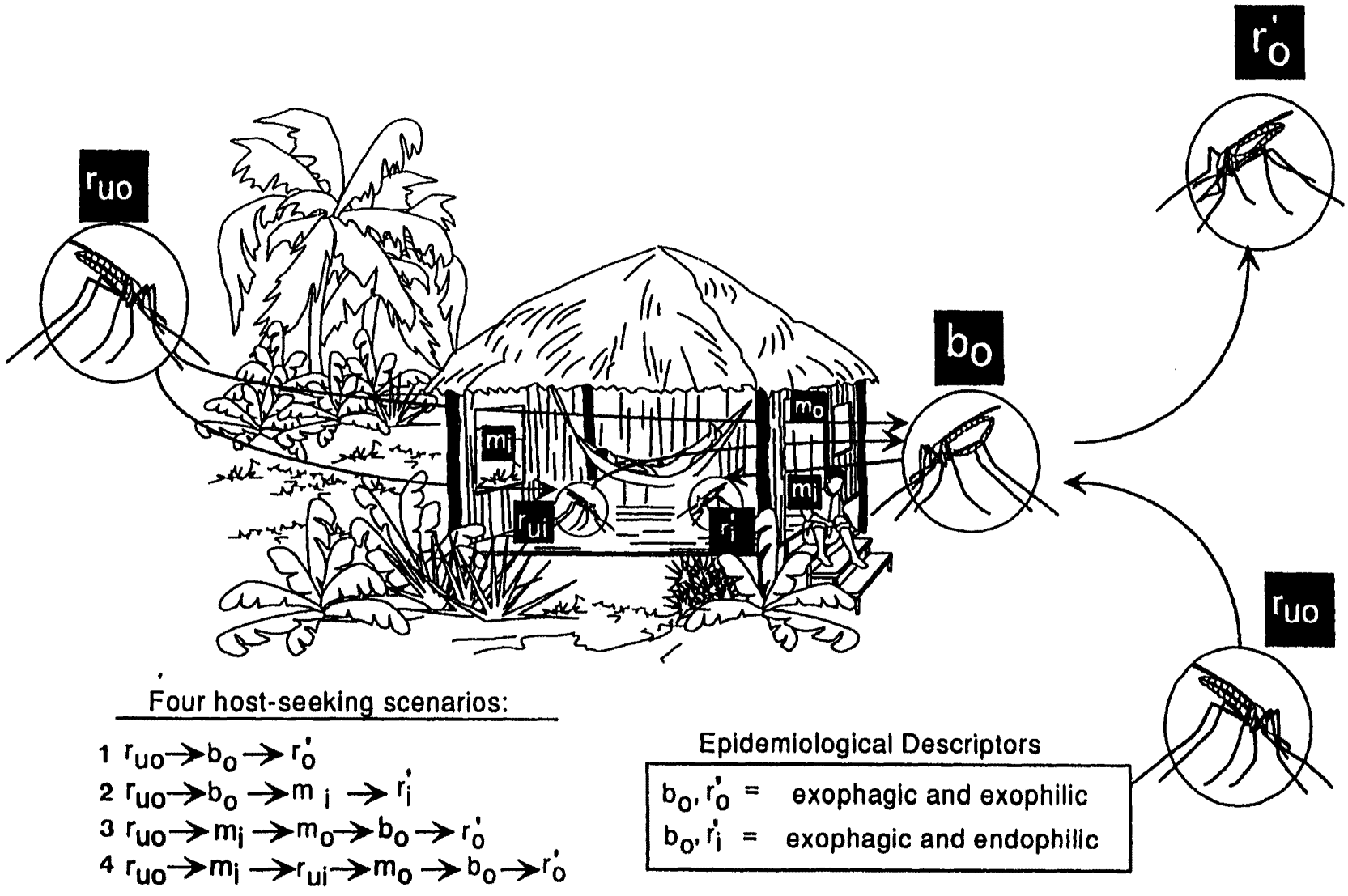
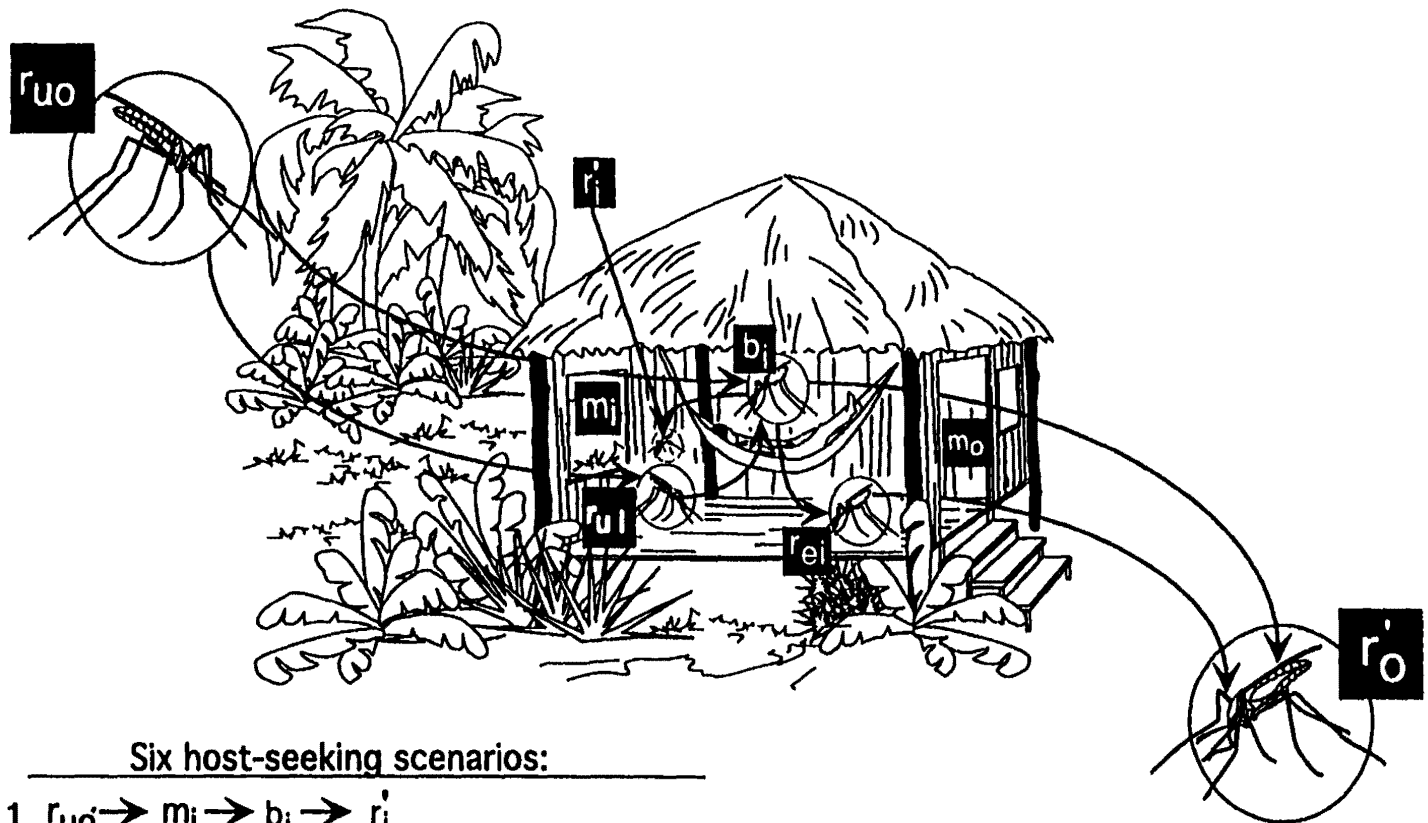


Figure 25. Patterns of outdoor host-seeking behaviors of *Anopheles* mosquitoes, depicting 4 primary scenarios of exophagic behavior, with or without entering a house. Symbols: *r*= resting; *r'*= resting during gonotrophic cycle; *u*= unengorged; *o*= outdoors; *b*= biting; *m*= movement; *i*= indoors; *e*= engorged. (after D.R. Roberts)



Six host-seeking scenarios:

1 $r_{uo} \rightarrow m_i \rightarrow b_i \rightarrow r'_i$

2 $r_{uo} \rightarrow m_i \rightarrow b_i \rightarrow m_o \rightarrow r'_o$

3 $r_{uo} \rightarrow m_i \rightarrow b_i \rightarrow r_e \rightarrow m_o \rightarrow r'_o$

4 $r_{uo} \rightarrow m_i \rightarrow r_{ui} \rightarrow b_i \rightarrow r'_i$

5 $r_{uo} \rightarrow m_i \rightarrow r_{ui} \rightarrow b_i \rightarrow m_o \rightarrow r'_o$

6 $r_{uo} \rightarrow m_i \rightarrow r_{ui} \rightarrow b_i \rightarrow r_e \rightarrow m_o \rightarrow r'_o$

Epidemiological Descriptors

b_i, r'_o	=	endophagic and exophilic
b_i, r'_i	=	endophagic and endophilic

Figure 26. Patterns of indoor host-seeking behaviors of *Anopheles* mosquitoes, depicting 6 primary scenarios of endophagic behavior, with or without entering a house. Symbols: *r*= resting; *r'*= resting during gonotrophic cycle; *u*= unengorged; *o*= outdoors; *b*= biting; *m*= movement; *i*= indoors; *e*= engorged. (after D.R. Roberts)

CHAPTER 5

Epilogue

CONCLUDING REMARKS

" The mechanism of malaria transmission is so complicated, and delicate that it has never been able to resist any long-continued sabotage. Persistence is more important than perfection and whether control is a partial failure or a partial success depends on the point of view. Above all, let us not allow ourselves to be discouraged by theorists...and fight the disease now with the weapons already proved useful, albeit imperfect, rather than fold the hands while awaiting a problematical therapia magna *of the future."* **G.W. Hackett, 1937.**

"*But it is necessary not to forget that with the means we have available today, if properly used, malaria need no longer be the scourge that has ravaged and continues to desolate large numbers of people in vast tropical areas*" **A. Gabaldon, 1978.**

"*Perhaps the world's people are not yet ready to unite in waging a global war against the* Plasmodium-*mosquito axis; but in my opinion, a day will come when the havoc inflicted on the human race by malaria will create anew the determination to eliminate this scourge from the earth.*" **M.A. Farid, 1991.**

Fieldwork, on the level undertaken in this investigation, is difficult and time consuming and it should come as no surprise that so few studies of this kind are attempted. This study represents the first construction and use of experimental huts for

the study of known and potential malaria vectors in the country of Belize. This study also marks the first attempt at longitudinal collections using systematic human-landing data of *Anopheles* and culicines from a characterized location in the country.

Although the Caledonia investigation was of relatively short duration (< 9 mo.), some important contributions to the vector literature have been made. Despite nearly 4 decades of near routine indoor DDT residual spraying in Caledonia, there was no evidence that any level of selection for physiological resistance had developed in the 3 principal anophelines tested (*An. albimanus*, *An. vestitipennis*, and *An. crucians*). This is significant, if for no other point, that the use of DDT for malaria control, remains toxicologically effective. The successful use of excito-repellency (ER) test boxes (Roberts, et al. 1997a) in the field confirms their utility and value in investigating contact irritant and noncontact repellent components of pesticides used in public health (Chareonviriyaphap, et al. 1997). I also showed that the effect of DDT and avoidance behavior on local vectors in Caledonia was substantial. Based on indoor resting observations and human-landing data, significantly fewer mosquitoes fed or remained in the DDT- treated hut throughout the evening, a clear indication of the value of excito-repellency in reducing human-vector contact. In short, DDT residuals exhibited a pronounced deterrent effect on normal indoor mosquito activities and altered sequences of host-seeking and resting behavior. The use of ER boxes and experimental huts also provided strong complimentary findings, and represents one of the few published accounts combining both experimental approaches in the same study.

Longitudinal information on vector abundance, human-landing rates and sporozoite-infected mosquitoes had not previously been available from northern Belize.

Studies in Caledonia represent a true beginning of longitudinal vector information for this lowland coastal ecosystem from which future studies can be advanced and compared. Epidemiologically, this study implicated *An. albimanus* and *An. vestitipennis* as important malaria vectors in Caledonia and most likely in similar areas in northern Belize.

The apparent absence of *An. darlingi* from HLCs and other trapping methods (e.g., CO_2 -baited traps-data not presented) attests to its relatively uncommon and focal occurrence along the northern stretch of the New River (Manguin, et al. 1996). Ecological conditions in the riverine areas between San Estevan and Libertad do not seem conducive to maintaining sufficiently large populations of this important mosquito species. Lastly, deltamethrin was clearly shown to be a very potent compound, both in toxicity and hyperirritability, at a concentration 16-fold below the current recommended operational (diagnostic) lethal dosage.

Although the information on *An. albimanus* was, for the most part, unremarkable, thus adding to an already long list of citations from the Americas, this investigation contributed to the relatively sparse literature on this species from Belize (Roberts, et al. 1993). However, the information gathered on the other species, in particular, *An. vestitipennis*, is original and will hopefully spur further interest in this provocative species, to include vector incrimination and it's relative contribution in the transmission of malaria wherever it is present.

Much remains to be accomplished regarding disease transmission incrimination and bionomics of vectors in Belize. Although *An. albimanus* is undoubtedly an important, if not primary, vector in the country, its reputed vector status has yet to be

solidly confirmed by either natural field-isolated sporozoite salivary gland positive captures or by experimental infection. If time and assistance had permitted, studies on adult survivorship, gonotrophic cycle, dispersal, and larval habitat characterization, would have added greatly to these infrequently studied species, *An. vestitipennis*, *An. gabaldoni*, and *An. punctimacula*.

Malaria control by anti-mosquito measures has 4 basic objectives: exclusion, interception, reduction, and if possible, elimination of the vector (Gabaldon, 1949). The consequence of true excito-repellency functions as both exclusion and interception from human contact. However, no claim is made that this effect is absolute, and some transmission would still be expected to occur, albeit at much lower levels. The ultimate goal of insecticides is to reduce malaria transmission and disease incidence in the community, and not necessarily a reduction in larval or adult vector densities (Trapido, 1946; Gabaldon, 1953). Some of the prominent early workers in the control of malaria voiced concern on the presumed reduced efficacy of insecticides, like DDT, that exhibited profound excito-repellency in the vector populations (Muirhead-Thomson, 1947; Bertram, 1950; de Zulueta, 1962). It was widely perceived that avoidance of sprayed structures and other behavioral responses would possibly counter any hope of providing beneficial effects from indoor spraying of insecticides (Hadaway, 1950). This concern was based on the assumption that control was a singular product of toxic actions of insecticides. It is interesting to note, that with time and experience, both attitudes changed and many of the predicted control shortfalls proved wrong (Muirhead-Thomson, 1960; Parajuli, et al. 1981) (see addendum). Slowly, there has been a greater appreciation

of the relative importance and the effects of chemicals on mosquito behavior in reducing transmission (Roberts & Andre, 1994).

There has been much renewed discussion, added to recent adverse publicity, concerning public health and environmental risks because of past and continued use of DDT. However, the fact that DDT might carry with it certain hazards should not be allowed to obscure its immense and proven advantages in vector-borne disease control. During the last half century, DDT has conferred incalculable benefits to millions of people by reducing or eliminating their burden of disease, improving their well-being and opening up vast areas to economic development (Brown, 1976; WHO, 1995). Vector control for decades has provided the backbone to malaria control efforts worldwide. These benefits should be carefully and objectively weighted against the alleged health risks directly attributed to DDT and other insecticides. Only if it is clearly shown that the disadvantages outweigh the advantages should DDT, or chemical control in general, be modified or discarded.

In apparent contradiction to the alarming increase in malaria worldwide, a new "Global Malaria Control Strategy" was initiated in 1993 by the World Health Assembly, that effectively de-emphasized vector control (not necessarily excluding it) and emphasized early diagnosis and prompt treatment (Trigg & Kondrachine, 1998). This policy shift, from an organized vector control campaign with dedicated trained personnel, to one incorporated loosely within the local primary health care system has been implemented in a number of countries with varying levels of success. Politics and subjectivity continue to overpower the debate and conspire to reduce effective malaria control practices, while immediate concerns of quality of life and human health are at

stake (Farid, 1991). Clearly, acceptable risks, including the prudent and safe use of insecticides must remain available, or there can be no progress in combating disease.

With regards to DDT and the controversy surrounding its continued use in public health, the strategy remains simple. Until something better and more sustainable becomes available to supercede vector control activities, the use of DDT and other chemicals should be considered whenever appropriate for the control of larval and adult vectors. Use of DDT for strict public health purposes, specifically indoor residual spraying of structures, presents lower health risks than the overall impact of malaria infection on community health and human welfare. Conclusive evidence for long-term detrimental effects of DDT on human health and significant environmental contamination from use in public health, is presently unfounded or tenuous, at best. (Brown, 1972; Davidson, 1989). Over the past several decades, the consequences of reducing or eliminating indoor residual insecticide spraying programs, primarily DDT, appears likely to have contributed to the dramatic increases in malaria rates (Roberts, et al. 1997b). The recently proposed strategy of placing significant limitations or a complete ban on insecticide use by various international health bodies would ultimately exacerbate the already grave health problems in the majority of developing countries experiencing endemic malaria. Without acceptable, realistic, and functional alternatives in place, vector control should remain a viable option.

This preliminary body of work forms a small, but hopefully important, piece to the complex mosaic of malaria epidemiology and control. It has helped to substantiate and confirm the profound behavioral responses of mosquitoes to insecticides, evidence of which was described over 50 years ago (Kennedy, 1946). Further studies are clearly

needed to help clear the air of subjectivity and conjecture on the attributes of DDT. But until the complex interplay between vector and insecticide is thoroughly defined and accepted by species and specific locality, it will remain difficult to quantify all the possible epidemiological effects of this interaction. It is sincerely hoped that this dissertation has contributed meaningful information to the voluminous vector/malaria literature, and will spark further interest encouraging others to pursue much needed field work in the area of vector behavior and ecology in the continuing pursuit to control the deadly scourge, malaria.

REFERENCES CITED

Bertram, D. M. 1950. A critical evaluation of DDT and 'Gammexane' in malaria control in upper Assam over 5 years, with particular reference to their effect upon *Anopheles minimus*. Ann. Trop. Med. Parasitol. 44:242-254.

Brown, A.W.A. 1972. The ecological implications of insecticidal usage in malaria programs. Am. J. Trop. Med. Hyg. 21:829-834.

Brown, A.W.A. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13:1-25.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.H. Harlan, S. Manguin and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus* (Diptera: Culicidae), a malaria vector in the Americas. J. Am. Mosq. Control Assoc. 13:171-183.

Davidson, G. 1989. Insecticide usage: An anti-alarmist view of its advantages and disadvantages. Trop. Dis. Bull. 6:R1-6.

De Zulueta, J. 1962. The irritability of mosquitos to DDT and its importance in malaria eradication. Riv. Malariol. 41:169-178.

Farid, M.A. 1991. Views and reflections on anti-malaria programmes in the world. Kaohsiung J. Med. Sci. 7:243-255.

Gabaldon, A. 1949. The nation-wide campaign against malaria in Venezuela. Trans. Roy. Soc. Trop. Med. Hyg. 43:113-160.

Gabaldon, A. 1953. Possible effects of residual insecticides on the interruption of malaria transmission. Riv. Malariol. 32:155-171.

Gabaldon, A. 1978. What can and cannot be achieved with conventional anti-malaria measures. Am. J. Trop. Med. Hyg. 27:653-658.

Hackett, G.W. 1937. Malaria in Europe. London: Oxford Univ. Press.

Hadaway, A.B. 1950. Observations on mosquito behaviour in native huts. Bull. Entomol. Res. 41:63-78.

Kennedy, J.S. 1946. The excitant and repellent effects on mosquitos of sublethal contacts with DDT. Bull. Entomol. Res. 37:593-607.

Manguin, S., D.R. Roberts, R.A. Andre, E. Rejmankova and S. Hakre. 1996. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. J. Med. Entomol. 33:205-211.

Muirhead-Thomson, R.C. 1947. The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. Bull. Entomol. Res. 38:449-464.

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. Wld. Hlth. Organ. 22:721-734.

Parajuli, M.B., S.L. Shrestha, R.G. Vaidya and G.B. White. 1981. Nation-wide disappearance of *Anopheles minimus* Theobald, 1901, previously the principal malaria vector in Nepal. Trans. Roy. Soc. Trop. Med. Hyg. 75:603.

Roberts, D.R., O. Chan, J. Pecor, E Rejmankova, S. Manguin, J. Polanco and L.J. Legters. 1993. Preliminary observations on the changing roles of malaria vectors in southern Belize. J. Am. Mosq. Control Assoc. 9:456-459.

Roberts, D.R. and R.G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. J. Am. Trop. Med. Hyg. 50(Suppl.):21-34.

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

Roberts, D.R., L.L. Laughlin, P. Hsheih and L.J. Letgers. 1997b. DDT: global strategies, and a malaria control crisis in South America. Emerg. Inf. Dis. 3:295-302.

Trapido, H. 1946. The residual spraying of dwellings with DDT in the control of malaria transmission in Panama, with special reference to *Anopheles albimanus*. Am. J. Trop. Med. 26:383-415.

Trigg, P.I. and A.V. Kondrachine. 1998. Commentary: Malaria control in the 1990s. Bull. Wld. Hlth. Organ. 76:11-16.

[WHO] World Health Organization. 1995. Vector control for malaria and other mosquito-borne diseases. Wld. Hlth. Organ. Tech. Rep. Ser. No. 857. 91 pp.

Addendum

"The irritant effect of this particular form of DDT treatment [5% DDT + kerosene] evidently prevents full use being made of the known lethal properties of the insecticide. Until this obstacle has been overcome, the striking reduction in the number of mosquitoes resting in houses treated with DDT in kerosene cannot be accepted as evidence of effective control." **R.C. Muirhead-Thomson, 1947.**

"It is now recognized that although some mosquitoes appear to escape the effects of residual spraying by the fact that they are irritated without lethal contact, nevertheless their subsequent behaviour may be such as to reduce their biting activity and drive them out of human habitations. If this regular contact with man is sufficiently interrupted this way, then there appears a distinct possibility that transmission of malaria will also be interrupted." **R.C. Muirhead-Thomson, 1960.**

"The results of an experiment on the excitatory effects of the insecticides are in accordance with the findings of Muirhead-Thomson (1949). It appears that, for the control of malaria transmitted by *An. minimus*, DDT can be no more recommended than it can for the control of malaria transmitted by *An. gambiae*." **D.M. Bertram, 1950.**

"As a result of DDT house-spraying, *A. minimus* disappeared from most parts of Nepal during the 1960s." **M.B. Parajuli, et al., 1981.**

APPENDIX I

Key to the female *Anopheles* (Diptera: Culicidae) of Caledonia Village, northern Belize, Central America

Key to the Female *Anopheles** (Diptera: Culicidae) of Caledonia Village, Northern Belize, Central America

1. Hind leg with few or no pale spots 2

   Hind leg with many large pale spots 4

2. Hind legs all dark, wings with dark patches, costa all dark with pale scales at fringe tip of wing ..... ***An. crucians***

   Tip of hind leg (tarsomeres 3-5) all or mostly white, wing with pale scale patches on costa 3

3. Hind leg, tarsomere 5 with a small dark band..... ***An. albimanus***

   Hind leg, tarsomere 5 all white..... ***An. darlingi***†

4. Scutum mostly dark, without distinct spots..... 5

   Scutum pale with 3 large dark spots..... ***An. punctimacula***

5. Abdomen usually without scale tufts on side, with few dark and pale scales on venter..... ***An. vestitipennis***

   Abdomen with scale tufts on side, with many dark and pale scales on venter..... ***An. gabaldoni***

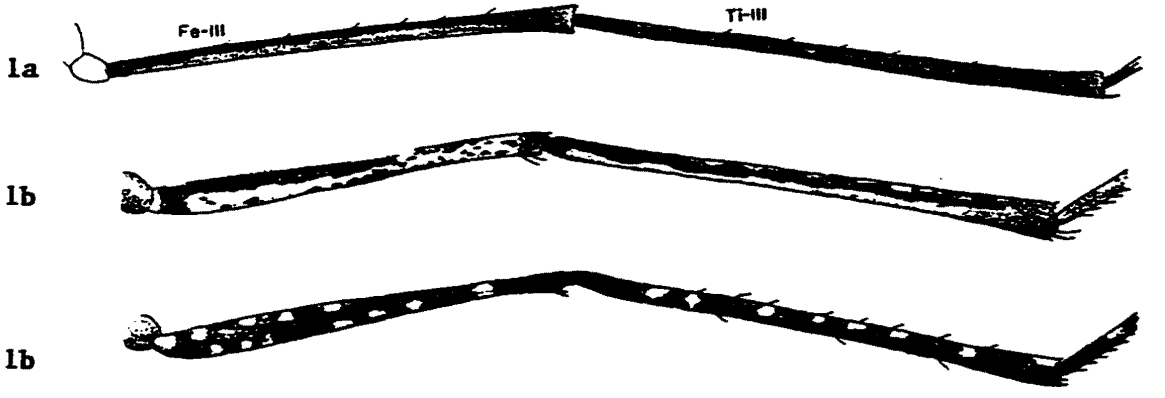
---

* based on Wilkerson, Strickman & Litwak (1990). J. Am. Mosq. Control Assoc. 6:7-34.
† not detected in Caledonia but found along New River.

KEY TO THE FEMALE *ANOPHELES* OF CALEDONIA VILLAGE, NORTHERN BELIZE, CENTRAL AMERICA

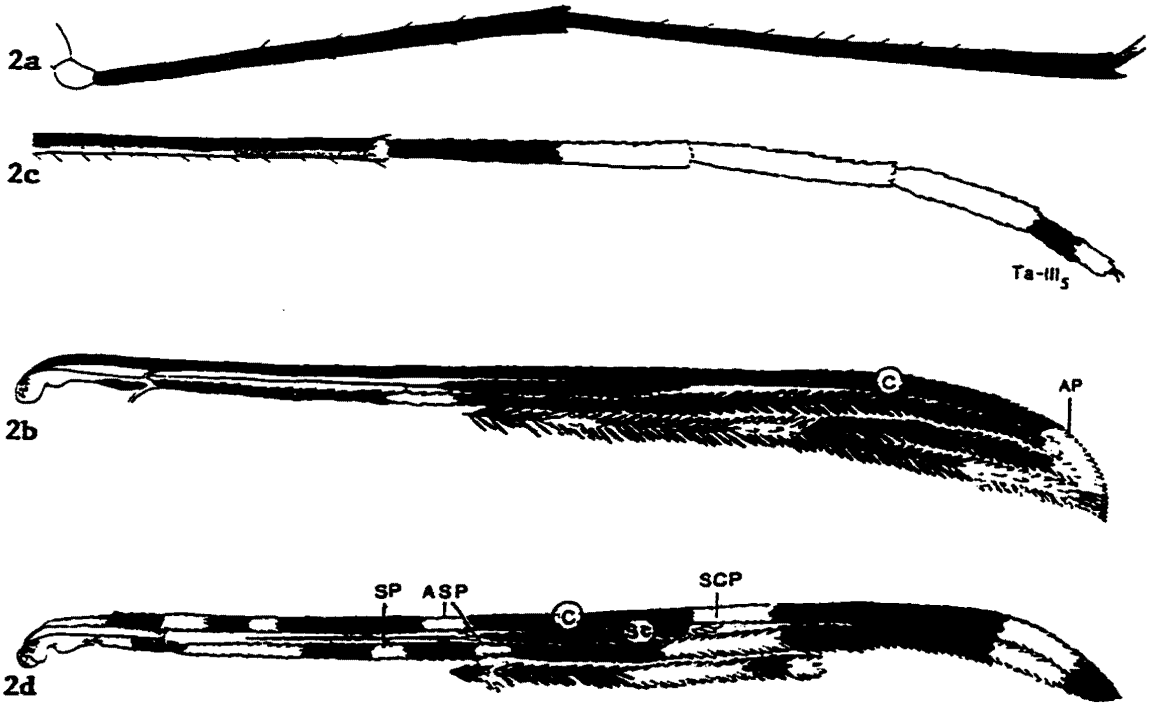
1. Hind leg with few or no pale spots (1a).....2

   Hind leg with many large pale spots (1b).....4



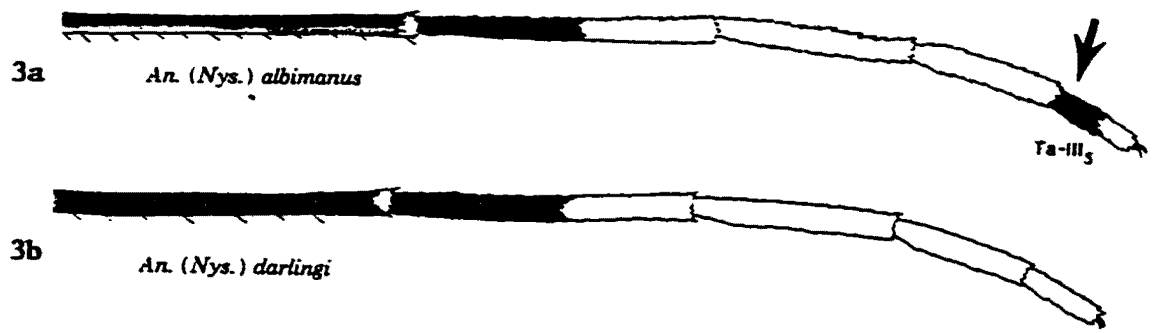
2. Hind legs all dark (2a), wings with dark patches, costa all dark with pale scales at tip of wing (2b).....*An. crucians*

   Tip of hind leg all or mostly white (2c), wing with pale patches on costa (2d).....3



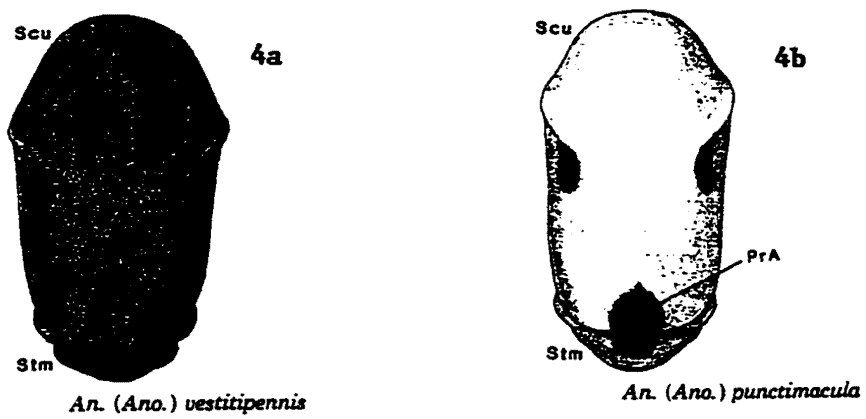
3. Tip of hind leg with a small dark band (3a).....*An. albimanus*

Tip of hind leg all white (3b).....*An. darlingi*



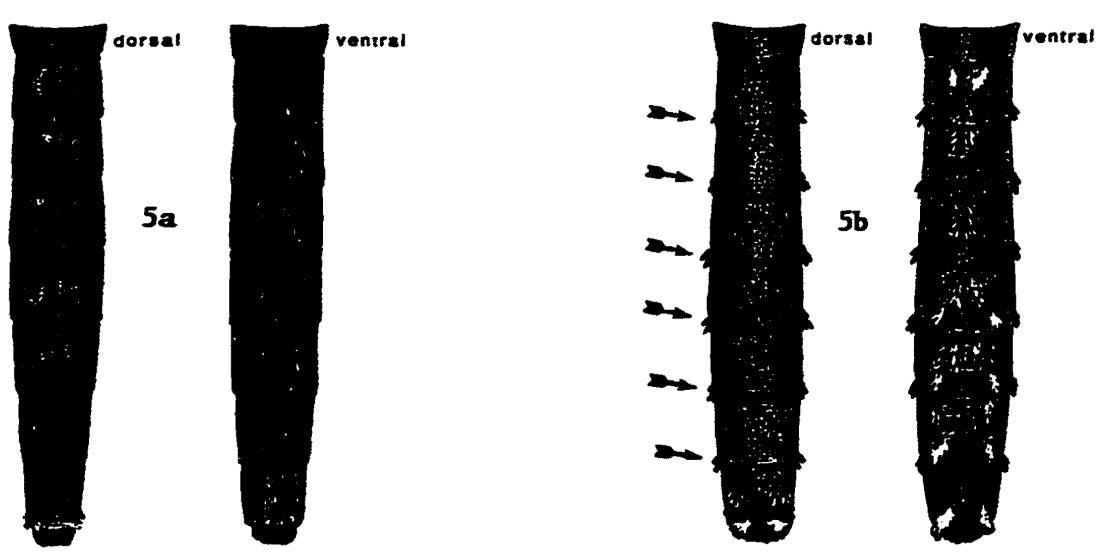
4. Scutum mostly dark, without distinct spots (4a).....5

Scutum with 3 large dark spots (4b).....*An. punctimacula*



5. Abdomen <u>without</u> scale tufts on side and few dark and pale scales below (5a).....*An. vestitipennis*

Abdomen <u>with</u> scale tufts on side and many dark and pale scales below (5b).....*An. gabaldoni*



APPENDIX II

Key to the fourth stage larvae *Anopheles* (Diptera: Culicidae) of Caledonia Village, northern Belize, Central America

Key to the Fourth Stage Larvae *Anopheles** (Diptera: Culicidae) of Caledonia Village, Northern Belize, Central America

1. Posterolateral spiracular lobe with seta 13 extremely long.....***An. darlingi***†

   Seta 13 small to medium.....2

2. Seta 3-C with 4 or more branches.....3

   Seta 3-C single .....***An. albimanus***

3. Seta 3-C with 20 or more branches; seta 0-IV, V large, with 4 or more branches, equal in size to 2-IV, V.....***An. crucians***

   Seta 3-C with 15 or less branches, seta 0-IV, V minute, much smaller than 2-IV, V, simple.....4

4. Setae 9,10,12-P unbranched, seta 3-C subequal to 2-C.....***An. punctimacula***

   Setae 9,10,12-P branched; seta 3-C distinctly shorter than 2-C.....5

5. Large and small spines of pecten plate in regular alternating order; seta 3-C with 2 main branches, each subdivided apically.....***An. gabaldoni***

   Large and small spines on pectin plate alternating irregularly; seat 3-C with 4 or more long subdivided branches.....***An..vestitipennis***

---

* based on Clark-Gil and Darsie (1983). Mosq. Syst. 15:151-284.

† not detected in Caledonia but found along the New River.

APPENDIX III

Human use information and agreement: Consent form for the voluntary participation in a scientific investigation of malaria mosquito vectors in Caledonia, Belize, Central America

HUMAN USE INFORMATION AND AGREEMENT

Consent Form for voluntary participation in a scientific investigation of malaria mosquito vectors in Belize, Central America

Study Title: 'Observations on the bionomics and response of *Anopheles* mosquitoes to DDT in Belize, Central America'

Background/Purpose: Malaria is a serious disease problem in Belize. Because Anopheles mosquitoes transmit malaria, the best and most efficient way to control this disease is to control the mosquito. Effective control using insecticides or other means requires detailed knowledge of the biology and behavior of these mosquitoes. *Anopheles albimanus* is the most important malaria vector in Central America. Despite it's importance, we have little information on this species in Belize, its contribution to the transmission of malaria or on which to base effective control measures. The purpose of this longitudinal investigation is to assess the susceptibility and behavioral response to DDT. Other studies will be carried out at the same time to obtain valuable information on the biology and life history of this and other mosquitoes. The information obtained from this project, because of your participation, will be of great benefit to your community and the country of Belize in the control of malaria.

Participation information: You have been asked to voluntarily participate in a scientific research study to be performed in Belize, with the understanding of the following points:

1) Your participation is voluntary. **2)** You will be compensated at an agreed upon daily rate for each collection period. **3)** You may withdraw from participation in this study or any part of the study at any time. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. **4)** The details of this study will be explained to you. You will be free to ask questions at any time before or during the study that will allow you to clearly understand aspects of the investigation. **5)** There will be no cost to you for your participation.

Duration of participation: The study will last for a period of up to one year, with varying intervals of activity and tasks depending on the study requirements. Your participation can continue until you withdraw, are removed earlier by a physician because of health reasons or for inability to perform the required tasks.

Procedures: Before agreement to participate, you will be interviewed and informed of the full scope of activity requirements and risks involved. If acceptable by both parties, you will be given the opportunity to be trained and begin the study.

Activities: You will be asked to collect mosquitoes from your exposed lower legs using a mouth aspirator and flashlight. Collections will be performed both outside and inside of local houses or specially constructed experimental huts during evening hours. Maximum periods per collection will be 6 hours (18.00 - 24.00 or 24.00 - 06.00 hours). Collections will involve no more than 3 night collection periods per week. You will be provided with all necessary supplies (flashlights, batteries, cups, aspirators, etc.).

Risks, Hazards and Discomforts: You will be asked to collect mosquitoes landing on your exposed skin. During the collection period, you will not be allowed to apply mosquito repellents or smoke tobacco. Depending on the number of mosquitoes landing on your legs and your skill of capture, some of these mosquitoes will have the opportunity to bite and cause local irritation. Other exposed parts of your body (i.e., face, hands, etc.) may be exposed to bites as well. Depending on the amount of malaria in the study area, you may be at an increased risk of acquiring malaria parasites from the infective bite of certain mosquitoes.

Benefits: Upon request or if you fall ill, your blood will be checked for malaria infection. If you are infected, you will be given treatment by a physician monitor until you are completely cured in accordance with the policies of the Belize Ministry of Health. Malaria treatment will be free of charge.

Confidentiality: All records related to your participation as a research subject will remain confidential. Your name will not be used in any report resulting from the study without your consent. A statement of your participation in the study will be given to you.

Circumstances under which your participation may be terminated without your consent:

1) Conditions, which might occur, that would make participation detrimental to your health.

2) You are unable to comply with the activity requirements.

Maximum number of study participants: 8

Medical care for injury or illness: You will be entitled to free medical care through a government health care facility for the treatment of clinically diagnosed malaria any injury as a direct result of your participation in this study. Points of contact are available if you have any questions concerning the medical research or your rights as a subject under this agreement. If you become ill during the study, either due to malaria or to the medications used to prevent or treat malaria, please contact one of the listed investigators.

Points of Contact:

1. Michael J. Bangs (Principal Investigator)
2. Dr. E. Vanzie, MD (Director, Vector Control Programme)

I have read/had read to me the Consent Form in a language I understand. Additionally, I have been given the opportunity to ask questions and have received satisfactory answers to all questions. The general purpose of this study, and the risks and benefits to me are fully understood. I understand that participation will entitle me to malaria treatment should the need arise and that I may withdraw from this study without prejudice at any time.

Having been fully informed and I consent to participate in this study based on the above conditions.

_____
Subject
Signature and Date

_____
Project Supervisor
Signature and Date

_____
Witness
Signature and Date

Version 1: English

APPENDIX IV

Pre-study summary collection records for site selection in Belize, Central America

Summary Collection Record: Site Selection Survey

Belize, Central America
12 June – 14 July 1995

DATE: 12 JUNE 1995
SITE: MASKALL, Belize District, near school teachers house along Northern River. 17°52.750'N, 88°18.710'W, Alt 200 ft (?), PDOP 3.4 *
NO. COLLECTORS: 2
WEATHER: Calm, partly cloudy, 3/4 moon (1806)
SUNSET: 1840
FINDINGS:

ADULTS: 90 minute collection (1830-2000); outdoors, low numbers of *An. albimanus*, no other anophelines captured.

DATE: 13 JUNE 1995
SITE: GALVEZ'S RANCH, Cayo District, near Lagoon (~120 x 30 m). Collection near house not possible because of fire and smoke.
NO. COLLECTORS: 2
WEATHER: Calm, scattered clouds, occasional slight breeze, full moon (1909)
SUNSET: 1840
FINDINGS:

LARVAE: Sibun River, shaded, floating leaves + *An. darlingi*; Sibun River exposed, shallow w/algae + *An. pseudopunctipennis*; Lagoon, shaded, steep bank, 0.5 m deep clear water + *An albimanus*; Lagoon, exposed, shallow, algae/submerged grasses + *An. albimanus*.

ADULTS: 90 min. collection (1830-2000) *An. darlingi* (38) 0.21/human/min; *An. albimanus* (23); *An. pseudopunctipennis* (1).

DATE: 14 JUNE 1995
SITE: MASKALL, Belize District, opposite side of Northern River, road to Bomba, near Nago Bank, approximately 1.5 km from Highway bridge, near house on left. 17°53.518'N, 88°17.746'W, Alt 100 ft (?), PDOP 3.0.
NO. COLLECTORS: 1
WEATHER: Calm, scattered clouds, 3/4 moon (2008), slight breeze to none.
SUNSET: 1840
FINDINGS:

ADULTS: 120 min. collection (1830-2030), primarily pestiferous *Aedes* spp. (many *Ae. taeniorhynchus*), *An. albimanus* (15), *An. crucians* (3), numerous culicoides. Terrible place.

DATE: 15 JUNE 1995
SITE: NOVELLO'S ORCHARD, Cayo District. 17°08.887'N, 88°37.689'W, Alt 200ft PDOD 4.4. Collections near house.
NO. COLLECTORS: 2
WEATHER: Calm, cloudy, no breeze, 3/4 moon (2102) (mostly hidden)
SUNSET: 1840
FINDINGS:

ADULTS: 90 min collection (1830-2000), outside. Near house: *An. darlingi* (109), *An albimanus* (3), *An pseudopunctipennis* (1). ~5 m from house: *An darlingi* (76), *An albimanus* (7), *An psuedopunctipennis* (14), *An. apicimacula* (2), *An. punctimacula* (1). A few culicoides present. Peak biting 1930-2000, *An darlingi* (93) both collection sites. Overall biting rate (185 *An. darlingi*) 1.02/human/min. Many specimens teneral.

DATE: 16 JUNE 1995
SITE: CALEDONIA, Corozal District, near community health center/church, >50 m from New River. 18°13.781'N, 88°28.327'W, Alt 200 ft (?), PDOP 2.3. General area dry, all major ground depressions in village dry.
NO. COLLECTORS: 2
WEATHER: Calm, partly cloudy, 3/4 moon (2152), generally hidden
SUNSET: 1841
FINDINGS:

ADULTS: 90 min. collection (1830-2000). Moderate densities of *Aedes* spp., *An. albimanus* only (87), 0.48/human/min. Many specimens appear aged.

DATE: 19 JUNE 1995
SITE: SANTA CRUZ, Corozal District, near New River. Area dry, major ground depressions lacking standing water. GPS not functional.
NO. COLLECTORS: 1
WEATHER: Calm, cloudy, occasional light rain, 1/2 moon (2358), mostly hidden.
SUNSET: 1841
FINDINGS:
ADULTS: 90 minute collection (1830-2000). High densities *Aedes*, low *An. albimanus* (23), 0.25/human/min, mostly an older population.

DATE: 20 JUNE 1995
SITE: CHURCHYARD (MONKEY BAY), Cayo District, Mile 30, Western Highway, outside near house located near Sibun River on right branch of road, small creek behind house in steep gully. 17°18.155'N, 88°33.601'W Alt 100ft, PDOP 0.0. Road further back flooded in areas with deep water, not safe to pass.
WEATHER: Heavy clouds, light to heavy rain, 1/2 moon (2438)
FINDINGS: Washout, collection aborted

DATE: 22/23 JUNE 1995
SITE: NOVELLO'S
WEATHER: Clear to partly cloudy on 22nd, rain on 23rd. Creek in road overflowing, not passable with vehicle both days.

DATE: 26 JUNE 1995
SITE: NOVELLO'S (as above)
NO. COLLECTORS: 4
WEATHER: Calm to slight breeze, partly cloudy initially to clear by 2000, New moon (0407). Heavy shower (1745-1800)
SUNSET: 1843
FINDINGS:

ADULTS: 90 min collection (1830-2000), all outdoors (owner not home). Collections began slow, mostly Aedes + other culicines. Due to lack of collection experience, the 2 additional collectors captures were not included in the final tabulation. Peak anopheline period 1930-2000. *An darlingi* (37) 0.2/human/min; *An punctimacula* (6); *An. gabaldoni* (5); *An. albimanus* (3); *An. apicimacula* (1); *An pseudopunctipennis* (1). Most specimens recent. Wide assortment of culicines (*Culex, Aedes, Coquillettidia*).

DATE: 27 JUNE 1995
SITE: CHURCHYARD (as above)
NO. COLLECTORS: 2
WEATHER: Calm, partly cloudy to clear, New moon (0455).
SUNSET: 1843
FINDINGS:

ADULTS: 90 min (1830-2000), family absent, only outdoor collection. Moderate numbers of *Aedes* and *Culex*, some culicoides. Very few (mostly older) anophelines. *An. darlingi* (2), *An. punctimacula* (2), *An gabaldoni* (1).

DATE: 28 JUNE 1995
SITE: MASKALL (teacher's house, as above)
NO. COLLECTORS: 1
WEATHER: Calm, light rain, cloudy, New moon (0544).
SUNSET: 1843
FINDINGS:

ADULTS: 90 min (1830-2000). Low numbers for all genera. Only *An. albimanus* captured (23).

DATE: 30 JUNE 1995
SITE: NOVELLO'S (as above)
NO. COLLECTORS: 2
WEATHER: Calm, clear to partly overcast, 1/4 moon (0724-2029), partly hidden during evening.
SUNSET: 1843
FINDINGS:

ADULTS: 150 min collection (1830-2100). (Outdoor) and **indoor** collections. Outdoor collection (~2 m distance from house), Indoor collection (kitchen with large open window, eaves and gaped walls): *An. darlingi* (47) **14**, 0.28/human/min., peak activity 2000-2030; *An. albimanus* (48) **2**, peak 1900-1930; *An. gabaldoni* (17) **1**; *An. apicimacula* (4); *An. punctimacula* (3); *An. pseudopunctipennis* (2).

DATE: 05 JULY 1995
SITE: CHAN CHIN, Corozal District, on Northern highway. 18°25.700'N, 88°26.503'W, Alt 0, PDOP 2.2. Outside near house along edge of expansive flat marsh. Recent rains, relatively small patches of water, little or no algae.
NO. COLLECTORS: 2
WEATHER: Calm, clear and balmy, 1/4 moon (1145). Just happy to be alive.
SUNSET: 1844
FINDINGS:

ADULTS: 60 min (1830-1930). *AEDES* from Hell!! RAN away at 1930. If anophelines were present we were not able to detect them among the clouds of flood-water *Aedes*. Horse in road attacks Bronco, delirious from the biting Aedes. Nearly every house had smoky fires outside to keep the Nature's surreal wrath at bay. Estimated biting rate 120/human/min. Decided this was a bad site, not to be visited again.

DATE: 06 JULY 1995
SITE: CALEDONIA, (as above)
NO. COLLECTORS: 2
WEATHER: Calm. clear to partly cloudy. 3/4 moon (1242)
SUNSET: 1844
FINDINGS:

ADULTS: 90 min (1830-2000). Outdoor. Numerous aedines captured. Only *An. albimanus* captured (142), 0.78/human/min.

DATE: 07 JULY 1995
SITE: MASKALL, teacher's house (as above)
NO. COLLECTORS: 1
WEATHER: Heavy rain.
FINDINGS: None, collection aborted.

DATE: 10 JULY 1995
SITE: HERSHEY'S, Cayo District, edge of elevated step between mature cocoa groves and citrus trees on lower flood plain, separated to the south from Sibun River by large stand of bamboo, ~1/2 km distance. Road not passable to river due to flooding. 17°08.810'N, 88°38.070'W, Alt 100 ft, PDOP 3.1. No human habitation at site.
NO. COLLECTORS: 2
WEATHER: Calm, partly cloudy to clear, light overcast with occasional sprinkles. Full moon (1649), mostly exposed.
SUNSET: 1843
FINDINGS:

ADULTS: 90 min (1830-2000). Seven anopheline species captured. *An. darlingi* (14), 0.07/human/min; *An. albimanus* (4); *An. psuedopunctipennis* (13); *An. punctimacula* (22); *An. gabaldoni* (5); *An. vestitipennis* (2); *An. apicimacula* (1).

DATE: 11 JULY 1995
SITE: HERSHEY'S, Cayo District. Attempted to reach St. Thomas, road creek not passable. Attempted to collected at previous nights site.
FINDINGS: None, collection aborted due to intermittent moderate to heavy rain.

DATE: 12 JULY 1995
SITE: MASKALL, Belize District, near Teacher's house (as above), Northern River very high from previous 4 days rain, clear movement.
NO. COLLECTORS: 1
WEATHER: Calm, clear to partly cloudy. Full moon (1847)
SUNSET: 1843
FINDINGS:

ADULTS: 90 agonizing min. (1830-2000). AEDES with pure hate!!! Increase in *An albimanus* from previous collections (53), 0.58/human/min; *An. punctimacula* (6). No *An. darlingi* seen.

DATE: 13 JULY 1995
SITE: BDF AIRPORT CAMP, Ladyville, Belize District. BDF married officers quarters. 17°32.801'N, 88°18.270'W PDOP 2.8
NO. COLLECTORS: 1
WEATHER: Calm, scattered clouds, Full moon (1940).
SUNSET: 1843
FINDINGS:

ADULTS: Collection had to be stopped due to thermal fogging operation with malathion. *Aedes* spp. and *An albimanus* (6) captured.

DATE: 14 JULY 1995
SITE: CALEDONIA, collection near Community Health Clinic (as above)
NO. COLLECTORS: 2
WEATHER: Calm, scattered clouds. Full moon rising 2028 hrs.
SUNSET: 1843
FINDINGS:

ADULTS: 90 min collection (1830-2000), numbers for all genera much lower than previous collections on 16 June and 6 July. *An. albimanus* (45), 0.25/human/min; *An. crucians* (4); *An. vestitipennis* (1). Variety of *Culex, Aedes* and 1 *Coquillettidia.*

* *GPS (Trimble Navigation, Sunnyvale, CA, USA)*

APPENDIX V

Ancillary observations on some mosquito species attracted to humans in Caledonia, Belize, Central America

Observations on mosquito morphology:

Limited observations were made on the morphological variation seen in several of the species studied in Caledonia during collections from September 1995 to May 1996. It was noted that during the transition period from high to lower rainfall, there was a general decrease in adult body size in anophelines (e.g., *An. albimanus* and *An. vestitipennis*), and some *Aedes* species (*Ae. taeniorhynchus*). On rare occasion, aberrant dark bands occurred on hindtarsomere 4 of *An. albimanus* adult females captured in Caledonia, previously described for various members of the subgenus *Nyssorhynchus* (Faran, 1980; Harbach, et al. 1993).

Anopheles vestitipennis provided the most interesting morphological variations. A dramatic difference in body (cuticle) color was noted between dark brown 'black' and lighter toned 'brown' variants. Both occurred sympatrically, with the brown variant always less common that the black variety. This species has been reported as variable in the fourth instar stages, showing high phenotypic variability from the same locality in southern Mexico (Bonilla et al. 1996), while variations in adult body color have been mentioned over its distribution (Komp, 1942). The degrees of pale markings on legs were strikingly varied between individuals and even between legs on the same individual viewed dorsal and ventral. Darker legs appeared more common during the drier/cooler period (Dec.-Jan.). Body size variation also appeared to influence speckling on the legs- small specimens having very few, mostly indistinct spots. Additionally, some *An. vestitipennis* lacked the presubcostal pale spots (PRSCP) in the subcostal area as described by Wilkerson and Peyton (1990). It is speculated that certain environmental

determinants, including particular larval habitats, may influence body color and other polymorphic variations. Studies to determine if there is any genetical or epidemiological significance would be interesting.

On occasion, some confusion was found separating *An. vestitipennis* and *An. gabaldoni* from the Caledonia collections based on published taxonomic keys (Wilkerson et al., 1990). Both species are in the subgenus *Anopheles* (Arribalzagia series) and presumably closely related (Harbach, 1994). Between these 2 species, abdominal scale-tufts on segments 2-8 are presumably restricted to *An. gabaldoni*. A few specimens (in very good condition) possessed obvious posterolateral scale-tufts on abdominal terga (5-8 only); when other diagnostic criteria suggested these specimens were *An. vestitipennis* (as recorded in this study). It was also noted, the length and number of pale markings viewed on hind tarsi 1 were smaller and ranged from 3-6 on *An. vestitipennis* compared to > 6-9 on *An. gabaldoni*. Overall, *An. gabaldoni* had much more speckling on the legs than *An. vestitipennis* on specimens captured in Caledonia or seen elsewhere in northern Belize (Andre, et al., unpub. obs.)

Parasitic and phoretic acarines:

Water mites comprise one of the dominant forms of fauna in all fresh water ecosystems. Relative proportions of parasitized mosquitoes with particular mite species or morphs may provide valuable information on seasonal variations in mosquito larval habitat selection or other important measures in larval habitat conditions and locations (limnological ecology, proximity to breeding sites, dispersal, etc.). Larval water mites (Acariformes: Hydrachnellae) Kantz were predominately seen on anophelines and rare occasions *Culex* (*Melanoconian*) species and *Ma. titillans*. Only once was *Ma. dyari*

seen with an attached mite (1 on thorax). Four types of mites were identified. The vast majority were *Arrenurus* spp. (Arrenuridae), commonly attached to dorsal and ventral aspects of the abdomen and pleural areas of the metathorax, and occasionally coxa. Unfortunately, species identification of *Arrenurus* is possible only in the adult stage. These small, rounded mites presented in various colors (predominately carotenoid pigments), most commonly red and green (also yellow, gray and brown). *Anopheles albimanus* was the most commonly parasitized mosquito and had mites of various colors. *Anopheles vestitipennis* and *Anopheles punctimacula* had mites almost exclusively dark red in color. *An. crucians* and *Cx. pilosus* had bright, ruby red mites. Numbers of mites per mosquito were generally below 10; however, on occasion some specimens were heavily parasitized (> 25). Two specimens of *Ma. titillans* had a single red, long-legged mite *Callidosoma* sp. (Erythraeidae) attached to the hind tarsi. One *An. crucians* had a *Microtromdidium*? sp. attached to the mid-pleural (mesepimeron) region of the thorax . The oddest find was a small phoretic (non-aquatic) deutonymph (Mesostigmata: Uropodidae) attached to the thorax of one *An. vestitipennis*.

Attached mites suggested parasitized mosquitoes were 'young' teneral (recently emerged) adults, further indicating increased output from breeding sites. Mites were seen parasitizing adult mosquitoes during every collection period; however, a greater proportion of infested mosquitoes were seen in the December-January period (a period of cooler temperatures and reduced rainfall). Interestingly, mites were more common (proportion-wise) on *An. albimanus* during Sep.-Oct. and *An. vestitipennis* during Dec.-Jan., when each species was the dominant collected anopheline, respectively.

REFERENCES CITED

Bonilla, A.O., J.I. Arredondo-Jimenez and M.H. Rodriguez. 1996. Classic morphology of fourth instar larvae, pupae and adults of *Anopheles vestitipennis*. VI symposium. Mosquito Vector Control and Biology in Latin America. 62nd Ann. Mtg. Am. Mosq. Control Assoc. Norfolk, VA. (Abstract).

Faran, M. E. 1980. Mosquito studies (Diptera: Culicidae) 34. A revision of the Albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. 15:1-215.

Harbach, R.E., D.R. Roberts and S. Manguin. 1993. Variation in the hindtarsal markings of *Anopheles darlingi* (Diptera: Culicidae) in Belize. Mosq. Syst. 25:192-197.

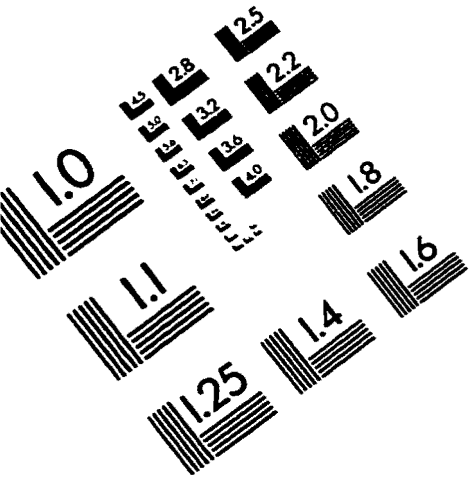
Harbach, R.E. 1994. Review of the internal classification of the genus *Anopheles* (Diptera: Culicidae): the foundation for comparative systematics and phylogenetic research. Bull. Entomol. Res. 84:331-342.

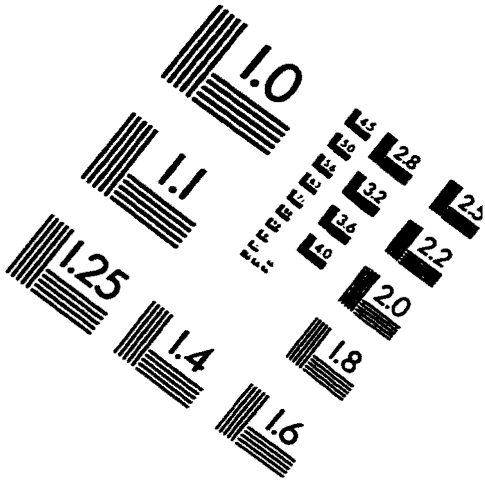
Komp, W.H.W. 1942. The anopheline mosquitoes of the Caribbean region. U.S. Publ. Hlth. Ser., Nat. Inst. Hlth. Bull. No.179. 195 pp.

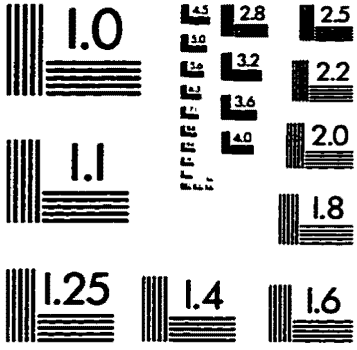
Wilkerson, R.C., D. Strickman and T.R. Litwak. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. J. Am. Mosq. Control Assoc. 6:7-34.

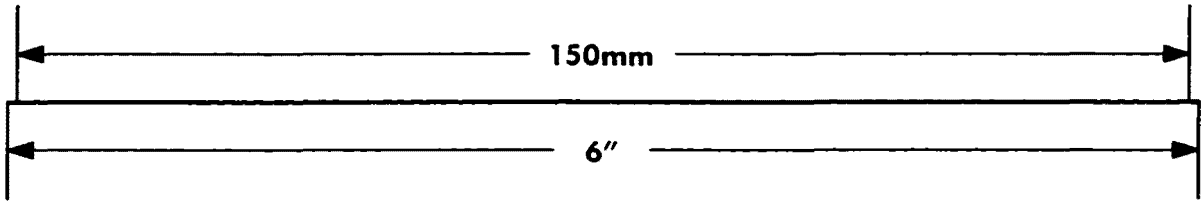
Wilkerson, R.C. and E.L. Peyton. 1990. Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). J. Med. Entomol. 27:207-224.

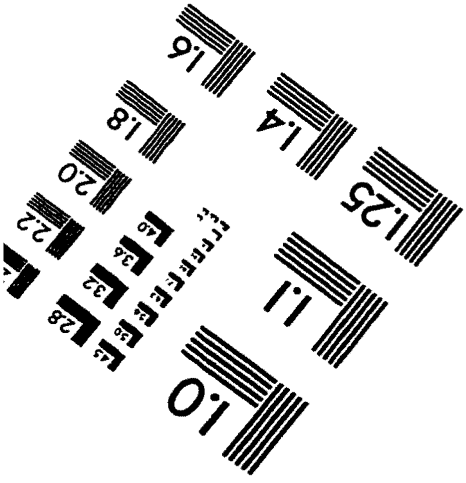
IMAGE EVALUATION
TEST TARGET (QA-3)

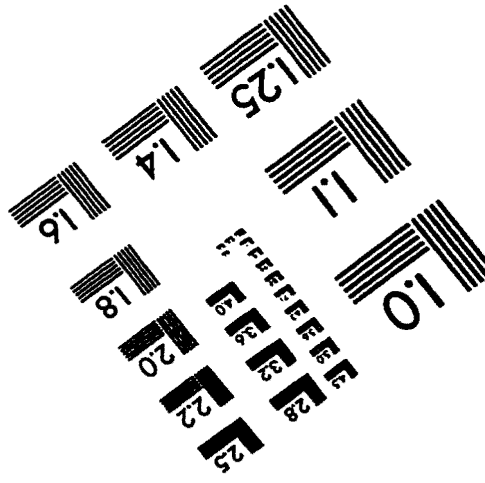












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989