

Evaluation of a lethal ovitrap for control of *Aedes aegypti* (L.)  
(Diptera: Culicidae), the vector of dengue in Costa Rica

by

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A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE

Major: Entomology

Major Professor: Wayne A. Rowley

Iowa State University

Ames, Iowa

2001

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This is to certify that the Master's thesis of  
Jennifer Lee Remmers  
has met the thesis requirements of Iowa State University

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## DEDICATION

This thesis is dedicated to Dr. Wayne A. Rowley. I developed my fascination and love for mosquitoes because of him. For six years, Dr. Rowley has been like a father to me, a mentor who has taught me more than anyone else. It was Dr. Rowley who taught me to write, research, and think about things in a critical way. He has given me many fantastic opportunities. I worked with *Aedes aegypti* mosquitoes and got to know their life cycle better than I know my own. His support and encouragement pushed me to become who I am today, both as a scientist and as a person. I thank you, Dr. Rowley, with all of my heart.

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## CHAPTER 1. GENERAL INTRODUCTION

### Thesis Organization

This thesis is composed of four chapters. The first explains the organization of this thesis and gives a general summary of the research conducted. Chapter 2 is a review of literature pertaining to the history of dengue, the dengue virus, *Aedes aegypti* mosquitoes, and the control of dengue and of *Ae. aegypti*. Chapter 3 is a manuscript that will be submitted for publication. Within this manuscript is an introduction, a description of the materials and methods used, the results found, and a discussion of these results. Chapter 4 contains general conclusions from the research and recommendations for future research. References cited are listed at the end of each of these chapters.

### General Overview

In the Americas, dengue and dengue hemorrhagic fever are of serious health concern. Worldwide, 2.5 billion people live in areas at risk for dengue. Currently, there is no vaccine and no treatment for the diseases caused by the dengue virus (World Health Organization 1998).

*Aedes aegypti* is the primary vector of the dengue virus in Central America. This mosquito co-habits with humans, often breeding in man-made containers such as tires, vases, and cisterns. Currently, control of these mosquitoes relies mainly upon the reduction of breeding sites. However, in many dengue-endemic areas, surveillance practices and control tactics are inadequate. Thus, other means of control are necessary.

In the laboratory, lethal ovitraps have been effective in killing *Ae. aegypti* adults and larvae (Zeichner and Perich 1999). These ovitraps contain a strip of paper treated with deltamethrin. They are then filled with 10% hay infusion water as an attractant (Reiter *et al.* 1991).

One objective of this study was to determine if these ovitraps could be effective in controlling *Ae. aegypti* populations in homes in Costa Rica. A further objective of this study was to determine if there was a difference in oviposition activity of the mosquitoes in indoor traps and in outdoor traps.

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## CHAPTER 2. LITERATURE REVIEW

### History of Dengue

The origin of dengue is uncertain. Some believe that it may have evolved from a forest cycle involving lower primates and canopy-dwelling mosquitoes of the Malay Peninsula. Most believe that it originated in Asia due to the presence of all four viral serotypes in this area (Smith 1956, Rudnick and Lim 1986, Halstead 1992). Only dengue-2 (DEN-2) can be found in Africa (Rudnick and Lim 1986, Cornet 1993). It is generally agreed that the disease spread as a result of commerce, as port cities were the first to be affected (Gubler 1997).

The first probable case of dengue fever was reported in the Chinese *Encyclopedia of Disease Symptoms and Remedies* which was published during the Chin dynasty of 265-420 AD (Gubler 1997). This book was edited in both 610 AD and 992 AD, dating the disease description to a minimum of 1000 years ago. This description called the disease “water poison” and indicated that it was thought to be connected to flying insects closely related to water. Though it cannot be determined if the described disease was actually dengue fever, the book’s description of the symptoms matches current descriptions of dengue and dengue hemorrhagic fever (Gubler 1997). Similar reports of probable dengue outbreaks also came from the French West Indies in 1635 and from Panama in 1699 (Howe 1977, McSherry 1982).

The first definite outbreak of dengue occurred in Philadelphia in 1780 (Carey 1971, Rosen 1977). Dr. Benjamin Rush later described this outbreak in 1789. He wrote,



“In some cases, the discharge of a few spoonful of blood from the nose accompanied a solution of the fever on the third or fourth day; while in others, a profuse hemorrhage from the nose, mouth, and bowels, on the tenth and eleventh days, preceded a fatal issue of the disease” (Rosen 1977).

More outbreaks followed in the United States, Caribbean, and South American port cities during the nineteenth century and the first thirty years of the twentieth century (Hirsch 1883, Halstead 1992). It was during this time that the disease was given the name “dengue”. On June 12, 1801, the Queen of Spain, Maria Luisa, wrote, “I was sick with a disease called ‘dengue’ and since yesterday, had bleeding” (Gubler 1997). It is possible that this name originated from Swahili. In 1823 and 1870 there were epidemics of what was possibly dengue on the east African coast and in Zanzibar. There, it was called “Ki-Dinga pepo” which meant “cramp-like pains, produced through the agency of an evil spirit” (Christie 1872, Christie 1881, Gubler 1997). This then evolved to “Dinga” or “Denga” which was later called “Dandy Fever” or “The Dandy” during an epidemic in St. Thomas in 1827. In 1828 it was referred to as “Dunga” which probably eventually evolved to Dengue (Munoz 1828, Gubler 1997). Other past names for the disease include the French’s “minauderie”, the Spanish “colorado”, the English and American “break-bone” and “broken wing”, the French “giraffe” and “bouquet”, and the Brazilian “polka fever” (Hirsch 1883).

It wasn’t until 1903 that Graham documented that the disease was transmitted by mosquitoes. In 1906, Bancroft demonstrated that *Aedes aegypti* that fed on patients in the acute phase of the disease could, after a ten day incubation period, transmit the disease to

a healthy individual. Subsequent studies in the Phillipines, Indonesia, and the Pacific showed that other efficient vectors included *Ae. albopictus* and *Ae. polynesiensis* (Gubler 1988).

During World War II, outbreaks of the disease were common, probably due to the movement of soldiers. It spread from Southeast Asia to Japan and the Pacific Islands (Halstead 1992). Scientific interest in this disease grew during this time and in 1944 the etiology of the disease was discovered and the virus was isolated from soldiers in Hawaii, Calcutta, India, and New Guinea (Gubler 1988).

In 1947, the Pan American Health Organization launched a yellow fever prevention effort that involved the eradication of *Ae. aegypti* in the United States and in Central and South America. This subsequently reduced the number of cases of dengue in these areas. However, this effort dissolved as many countries stopped their eradication efforts in the early 1960's. The *Ae. aegypti* populations as well as dengue were soon flourishing once again (World Health Organization 1994). Now most of these countries are endemic for the disease.

### **Dengue Fever**

Dengue and dengue hemorrhagic fever (DEN and DHF) are diseases caused by RNA viruses in the family Flaviviridae (Waterman and Gubler 1989). There are four distinct viruses that are designated DEN-1, DEN-2, DEN-3, and DEN-4. Each of these serotype viruses causes a strong homologous immunity but only causes very short-term cross-immunity to the other three viruses (Hay *et al.* 2000).

Dengue fever is a febrile, flu-like illness. Symptoms often include a rash, sudden onset of a high fever, severe muscle and joint pain, headache, and pain behind the eyes (World Health Organization 1998). Dengue has an incubation period of 5 to 7 days and the symptoms last approximately one week (McBride and Bielefeldt-Ohmann 2000).

The dengue virus is transmitted by female *Aedes* mosquitoes. These mosquitoes acquire the virus when they feed on a person in the viremic phase of the disease. The virus then must incubate within the mosquito for 8 to 12 days (McBride and Bielefeldt-Ohmann 2000). During this time, the virus travels from the midgut to the salivary glands where it replicates. Once a mosquito carries dengue, it does so for its entire life and may even pass the virus to a small portion of its offspring transovarially (Rosen 1999).

Dengue virus particles have been found in several organs of the human body, including the lungs, spleen, liver, and lymph nodes (Rosen 1999). However, it is thought that the virus primarily replicates in the hepatocyte cells of the liver and the virus particles found in other organs were primarily degraded and were being inactivated (Rosen 1999).

Dengue hemorrhagic fever is a more severe manifestation of dengue. It shares many of the symptoms of dengue fever, but is accompanied by the loss of blood plasma (Rosen 1999). DHF patients often experience melena, epistaxis, hepatomegaly, hematemesis, and circulatory failure (Pan American Health Organization 1994). DHF is often fatal (World Health Organization 1998).

Currently there is no vaccine or cure for dengue. Because infection with one or two of the dengue viruses increases the chances for development of DHF, vaccine

development is hindered (World Health Organization 1998). Treatment for dengue involves closely monitored care. Patients should be watched for dehydration and should be encouraged to drink fluids. Intravenous delivery of fluids may be necessary. Therapy for pain and fever is often recommended, however aspirin should be avoided as it thins the blood. Patients should be carefully watched for signs of hemorrhage or shock (George and Lum 1997).

### ***Aedes aegypti* Mosquitoes**

*Aedes aegypti* mosquitoes are the primary vectors of the dengue virus serotypes in Central America. *Aedes aegypti* belongs to the family Culicidae and is characterized by a white line on the anterior surface of the mid-femur (Figure 1). The female has white scales on the sides of the clypeus. There is a crescent-shaped white marking on each side of the scutum in both sexes, and two white lines connecting these crescents to the lateral lobes of the scutellum (Christophers 1960).

The distribution of *Ae. aegypti* seems to center between approximately 40°N and 40°S latitudes (Kettle, ed. 1995). There are two subspecies of *Ae. aegypti*. *Ae. aegypti formosus* is a black subspecies that is limited to the tropics of Africa. This form feeds and breeds outdoors. *Ae. aegypti aegypti* is a brown or blackish form that is widely distributed and absent from the inland areas of the African tropics. It is domestic and feeds and breeds indoors (Mattingly 1957).

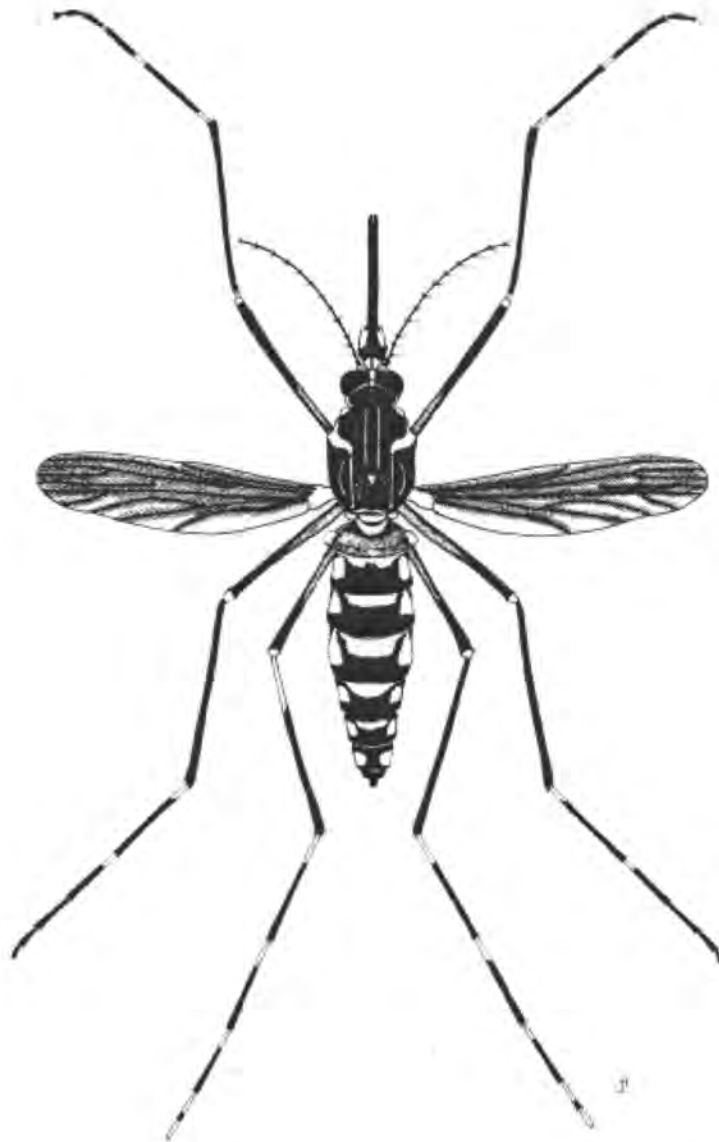


Figure 1: Female *Aedes aegypti* mosquito (From Kettle, ed. 1995).  
Reprinted with permission from CAB International.

The life cycle of *Ae. aegypti* begins with elongate eggs. These eggs have a hard shell that prevents the loss of water and allows for gas exchange (Nasci and Miller 1996). Eggs are laid on a moist substrate just above the surface of water. They may remain there, viable for up to 15 months until they are again exposed to water and hatch (Service 1993). Once they hatch, the larvae are legless, vermiform, and aquatic. There are four larval instars followed by an aquatic, mobile pupal stage. The adult mosquito then emerges from the pupal case at the surface of the water (Nasci and Miller 1996).

Male *Ae. aegypti* are unable to mate until their genitalia have rotated 180°. This may take as long as 24 hours (Service 1993). Generally, an adult female is not sexually receptive until approximately 2 days after ecdysis (Nasci and Miller 1996). The male detects a female by her wing beat frequency, which is unique to females of each species. The male then grabs the female from below using his hind legs. They then mate face to face in flight (Service 1993). Both male and female mosquitoes must feed on nectar for carbohydrates needed for flight. However, before laying eggs a female mosquito generally must take a blood meal. This provides the protein needed for egg development. When a blood meal is needed, the female locates a host by detection of carbon dioxide, lactic acid, and other attractants. As the female gets close to the host, she is attracted by the host's heat (Service 1993). To feed, the mosquito's labial sheath moves out of the way and the stylets beneath are inserted into the host. *Ae. aegypti* females prefer to feed on humans and other mammals and feed primarily during the day during mid-morning and mid- to late afternoon (Service 1993). The mosquito probes with these stylets until blood is detected. The mosquito then feeds until stretch receptors in the midgut promote

termination (Nasci and Miller 1996). Generally, an *Ae. aegypti* female needs approximately 4 mg of blood (Service 1993). The female lays eggs 2 to 7 days after a bloodmeal (Nasci and Miller 1996).

### **Control of *Aedes aegypti* and Dengue**

At the present time, a vaccine does not exist for the dengue virus. Because of this, the only means of dengue control lies in control of the vector mosquito. This is most efficiently done through integrated control methods.

Areas that have successfully eradicated the mosquitoes should implement surveillance against reinfestation by the vector. This may be done by monitoring seaports, airports, cemeteries, and tire facilities for possible sites of introduction of the mosquito (Pan American Health Organization 1994). This involves examining areas for larval infestation using three indices. These are the House Index which is the percentage of houses infested with larvae and/or pupae, the Container Index which is the percentage of containers with water that have immatures, and the Breteau Index which is the number of containers with immatures per 100 houses (Focks and Chadee 1997). Oviposition activity may also be monitored using oviposition traps, or ovitraps. A CDC ovitrap consists of a black glass or plastic container with a rough strip of paper against the inside (Fay and Eliason 1966). Mosquitoes prefer to oviposit on the strips as opposed to the smooth container surface. CDC enhanced ovitraps may also be used. These consist of two ovitraps side-by-side. One ovitrap contains hay infusion water and the other contains

a 10% formulation of this infusion (Reiter *et al.* 1991). The 100% infusion attracts the mosquitoes whereas they prefer to oviposit in the 10% solution.

Surveillance efforts such as these may be limited by many factors including rapid urban growth, limited government resources, poor management practices, inadequate training of field personnel, and insufficient public education (Reiter and Gubler 1994).

In areas where the mosquito is already established, the main means of *Ae. aegypti* control is the elimination of breeding sites (Pan American Health Organization 1994). *Ae. aegypti* mosquitoes typically breed in man-made containers containing fairly clean water. This includes cemetery vases, cisterns, flower pots, tires, and other containers with water. Breeding sites may be eliminated either by an inspector or by the residents themselves, who may be encouraged by an inspector (Reiter and Gubler 1997). When containers cannot be controlled in any other way, larvicides may be used. Space sprays should be used only in emergency situations (Pan American Health Organization 1994).

Control activities may be promoted within different segments of the health sector, of other government sectors, and of the private sector. Public education is a very important part of *Ae. aegypti* control. Education may be accomplished through mass media, schools, community meetings, and fairs and contests (Pan American Health Organization 1994).

Other methods of control have been tested and have proven to be unsuccessful. These include sterile male releases and the introduction of *Ae. aegypti formosus*, which did not out-compete *Ae. aegypti aegypti* because of their differences in behavior. *Toxorhynchites* is a mosquito the larvae of which prey on other mosquito larvae.



However, it is very difficult and costly to rear. *Mesocyclops* is a copepod that is much easier and cheaper to rear and has been shown to effectively control *Aedes* larvae. It has yet to be seen whether this organism could be integrated into *Ae. aegypti* control tactics (Woodring and Davidson 1996).

### **Deltamethrin**

Deltamethrin is a pyrethroid that may be used in *Ae. aegypti* control. Pyrethroid insecticides are synthetic versions of pyrethrin. Pyrethrin is a broad-spectrum insecticide derived from the extract of dried and powdered flower heads of members of the *Chrysanthemum* genus, especially *Chrysanthemum cinerariaefolium* (Davies 1985). The insecticidal properties of pyrethrin were recognized by the middle of the 19<sup>th</sup> century when there was commercial sale of “insect powder”. This powder came from Dalmatian pyrethrum flower heads (Ray 1991). However, it is likely that prior to this time pyrethrin was used in the Caucasus-Iran region of Asia and in Dalmatia which is now part of the Adriatic coast of Yugoslavia (Davies 1985).

In 1949, allethrin became the first pyrethrin analogue to be developed with practical applications in insect control (Davies 1985). This was the first of the pyrethroids, more stable synthetic versions of the natural pyrethrins.

Pyrethroid insecticides are broad-spectrum and are relatively stable to light and air (Thomson 1994). They have high molecular weights and consequently low volatility (Davies 1985).

The mode of action of pyrethroids is similar to that of DDT. They have a high affinity for membrane sodium channels and change the kinetics of these channels (Ray 1991). They only affect open channels and cause sodium gates to stay open for longer periods of time resulting in hyperactivity (Bradbury and Coats 1989). In low doses, pyrethroids cause stable repetitive firing and at high doses they cause depolarization and conduction block (Ray 1991).

Deltamethrin is considered the most powerful of the pyrethroids, and often elicits repellency (Thomson 1994). It is about 1000 times as toxic to house flies as pyrethrins, however its effectiveness as an insecticide may be reduced at temperatures above 35°C (Thomson 1994). It is a contact and stomach poison insecticide that was developed by M. Elliot in 1974 and became available commercially in 1978 (Thomson 1994, Ray 1991). Its scientific name is S- $\alpha$ -cyano-3-phenoxybenzyl-(1R)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate (Ray 1991). Other names for deltamethrin include Decis® and K-Othrin® (Ware 1978). Its empirical formula is C<sub>22</sub>H<sub>19</sub>Br<sub>2</sub>NO<sub>3</sub> and its structure can be seen in Figure 2.

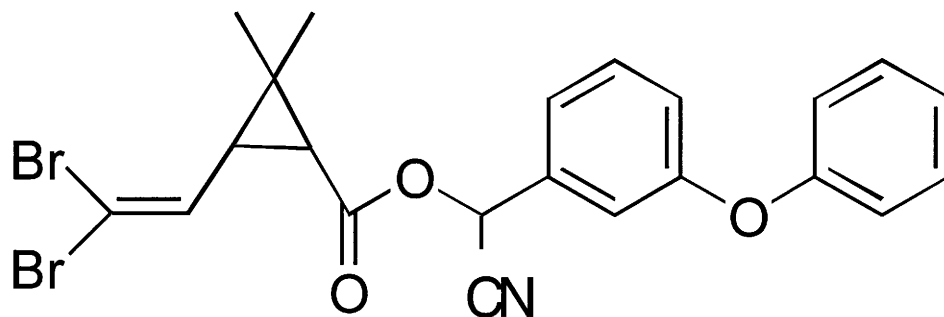


Figure 2: Structure of deltamethrin (Davies 1985).

Deltamethrin is a stable insecticide. Its molecular weight is 505.24 g and it melts between 98° and 101° C. Its vapor pressure is  $1.5 \times 10^{-8}$  mmHg at 25° C/g and its water solubility is less than 2 µg/L (Ray 1991).

Deltamethrin is low in mammalian toxicity. It is considered extremely low in toxicity to rabbits through dermal absorption (LD<sub>50</sub> of >2000 mg/kg), moderate in toxicity to rats through oral exposure (LD<sub>50</sub> of 128 mg/kg), and high in toxicity to rats when delivered intravenously (LD<sub>50</sub> of 2.3 mg/kg) (Ware 1978, Ray 1991).

Deltamethrin can acutely cause copious salivation and a writhing syndrome unique from other pyrethroids in rodents (Extension Toxicology Network 2001). In humans, acute exposure may cause a variety of symptoms including convulsions and muscle fibrillation and paralysis, dermatitis, headache, irritability, vomiting, and death due to respiratory failure. Chronic exposure may cause membrane irritation, prenatal damage, fluctuations of muscle tone ranging from hypotonic to hypertonic, and decreased blood pressure (Extension Toxicology Network 2001). Deltamethrin is metabolized by rapid ester cleavage and hydroxylation (Ray 1991).

The primary ecological effect of deltamethrin is a high toxicity to fish and other aquatic organisms, especially insects and crustaceans. However if used properly in the field, fish are not harmed (Thomson 1994, Extension Toxicology Network 2001).

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# **CHAPTER 3. EVALUATION OF A LETHAL OVITRAP FOR CONTROL OF *Aedes aegypti* (L.) (DIPTERA: CULICIDAE), THE VECTOR OF DENGUE IN COSTA RICA**

A paper to be submitted to the Journal of Medical Entomology

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## **INTRODUCTION**

*Aedes aegypti* mosquitoes are the primary vectors of the dengue virus in Central America, including Costa Rica. Currently there is not a vaccine or treatment for the diseases caused by this virus. Vector control is the only means of protection from these diseases. Breeding site reduction is the primary method of vector control (Pan American Health Organization 1994). However, this method is inadequate in many dengue-endemic nations. Alternative methods are desperately needed. Lethal ovitraps tested under laboratory conditions are highly effective at killing *Ae. aegypti* adults and larvae (Zeichner and Perich 1999). These traps consist of a black plastic cup with a strip of deltamethrin-treated paper clipped to the inside. The insecticide on the strips slowly leaches from the strips into 10% hay infusion water in the traps (Reiter *et al.* 1991).

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The traps attract gravid female *Ae. aegypti* that lay their eggs on the rough surface of the insecticide-treated paper. The mosquitoes die shortly after ovipositing and, more importantly, the first-instar larvae that hatch from the eggs die in the hay infusion water. These ovitraps show promise as a highly effective, inexpensive means of vector control that could be integrated with current control tactics.

The objective of this study was to determine if these lethal ovitraps could be effective in controlling *Ae. aegypti* in neighborhoods in highly endemic areas for dengue in Costa Rica. They have been shown to be effective for this purpose in Brazil (Perich *et al.* 2002). A second objective of this study was to examine the oviposition activity of *Ae. aegypti* in Costa Rica and determine whether they laid more eggs in traps placed inside houses or in traps located outdoors. By understanding the oviposition behavior of these mosquitoes, more effective ways to control them can be developed.

## MATERIALS AND METHODS

**Study Locations.** Two cities in Costa Rica were selected for this study based on information from the Costa Rican Ministry of Health. One study site was in the Barranca barrio of the city of Puntarenas. Puntarenas (09°42'N, 84°50'W) is located on the west coast of Costa Rica in Puntarenas Province. During the study, the average temperature in Puntarenas was 33°C and the average relative humidity was 67%. The second study site was in Siquirres. Siquirres (10°06'N, 83°30'W) is a city in Limon Province. It is located in the east central highlands of Costa Rica. During the study, the average temperature in Siquirres was 32°C and the average relative humidity was 67%. In each city, a

neighborhood of sixty homes identified by the Costa Rican Ministry of Health as having a history of dengue fever was selected as a study site (Figures 3 and 4).

Residents living in selected homes were asked to participate in the study on a voluntary basis. Thirty homes were randomly selected as treatment houses. Thirty additional homes were designated control houses. Each house was a sampling unit. This included the indoor portion of the home as well as the front, sides, and back areas outside of the house. Houses were similar one-story concrete units. Each had a living area, a kitchen, a bathroom, approximately two or three bedrooms, and a small backyard with a back patio that serves as a laundry area. Houses were open, often with nothing or merely a sheet separating the rooms. Front and back doors were often kept open and a gap existed between the top of the outer walls and the roof for ventilation.

**Treatment.** Lethal ovitraps described by Zeichner and Perich in 1999 (U.S. patent number 5,983,557, 11 November 1999, international patents filed) were 473 ml black polyethylene cups (Larkin, St. Louis, MO, U.S.A.). Three holes 1 cm in diameter and 2 cm from the top were designed to allow slow drainage of rainwater and to keep residents from attempting to drink from the cups. Red velour strips 11 cm long and 2.5 cm wide of heavyweight paper (Beinfang no. 4006-Scarlet; Hunt Corporation, Statesville, NC, U.S.A.) were treated with approximately 1 mg/cm<sup>2</sup> deltamethrin (Suspend<sup>®</sup>, SC, DowElanco, Indianapolis, IN, U.S.A.). One treated strip was paperclipped to the inside of each trap with the rough side facing the inside of the cup. 200 ml of 10% hay infusion water (Reiter *et al.* 1991) was added to each ovitrap. Hay was obtained from the Department of Biology at the University of Costa Rica, San Jose, Costa Rica.

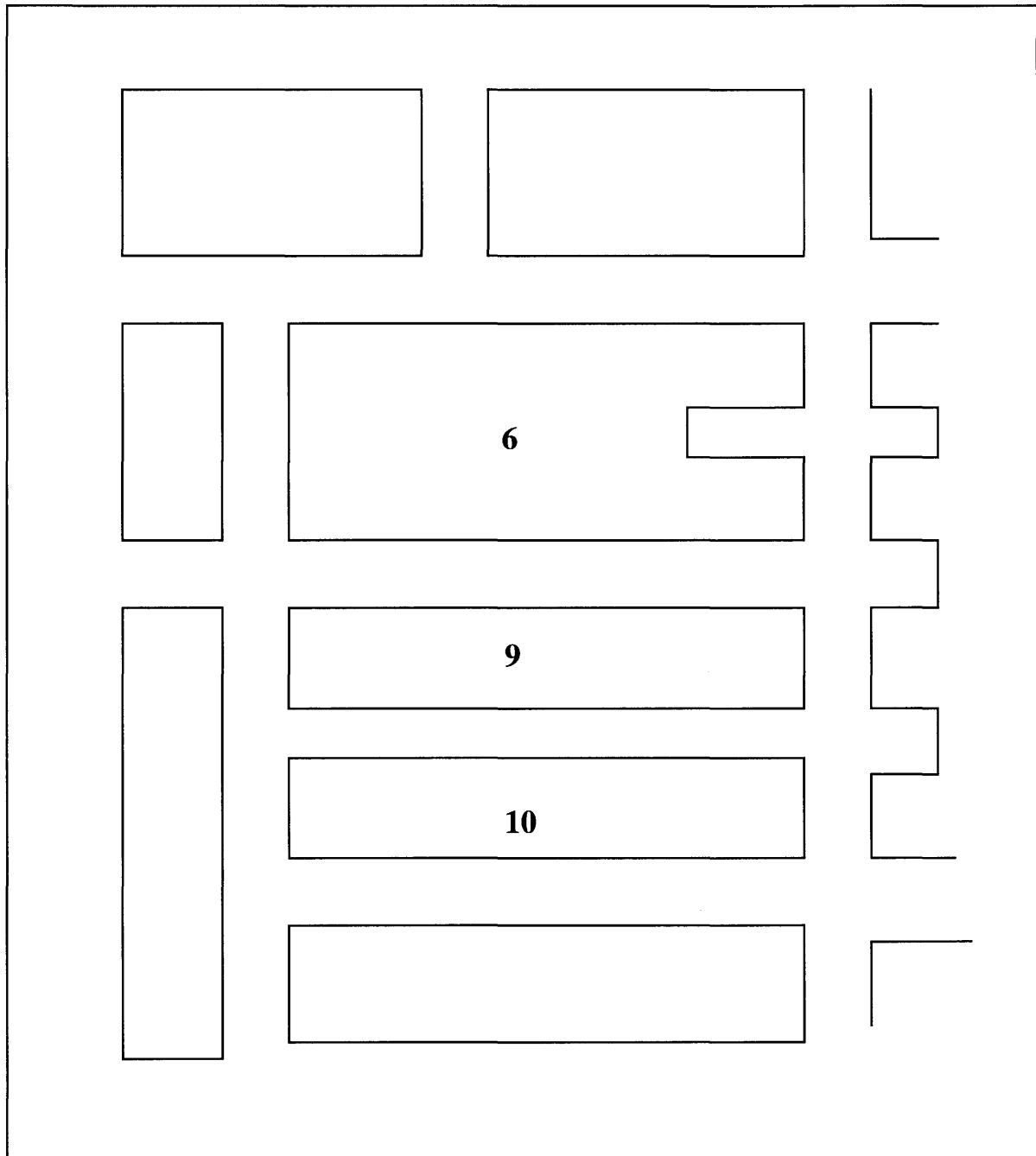


Figure 3: Map of Puntarenas study site in the Barranca barrio. Neighborhoods used in this study were designated by the Ministry of Health as 6, 9, and 10. Houses within these neighborhoods were randomly selected as treatment or control houses.

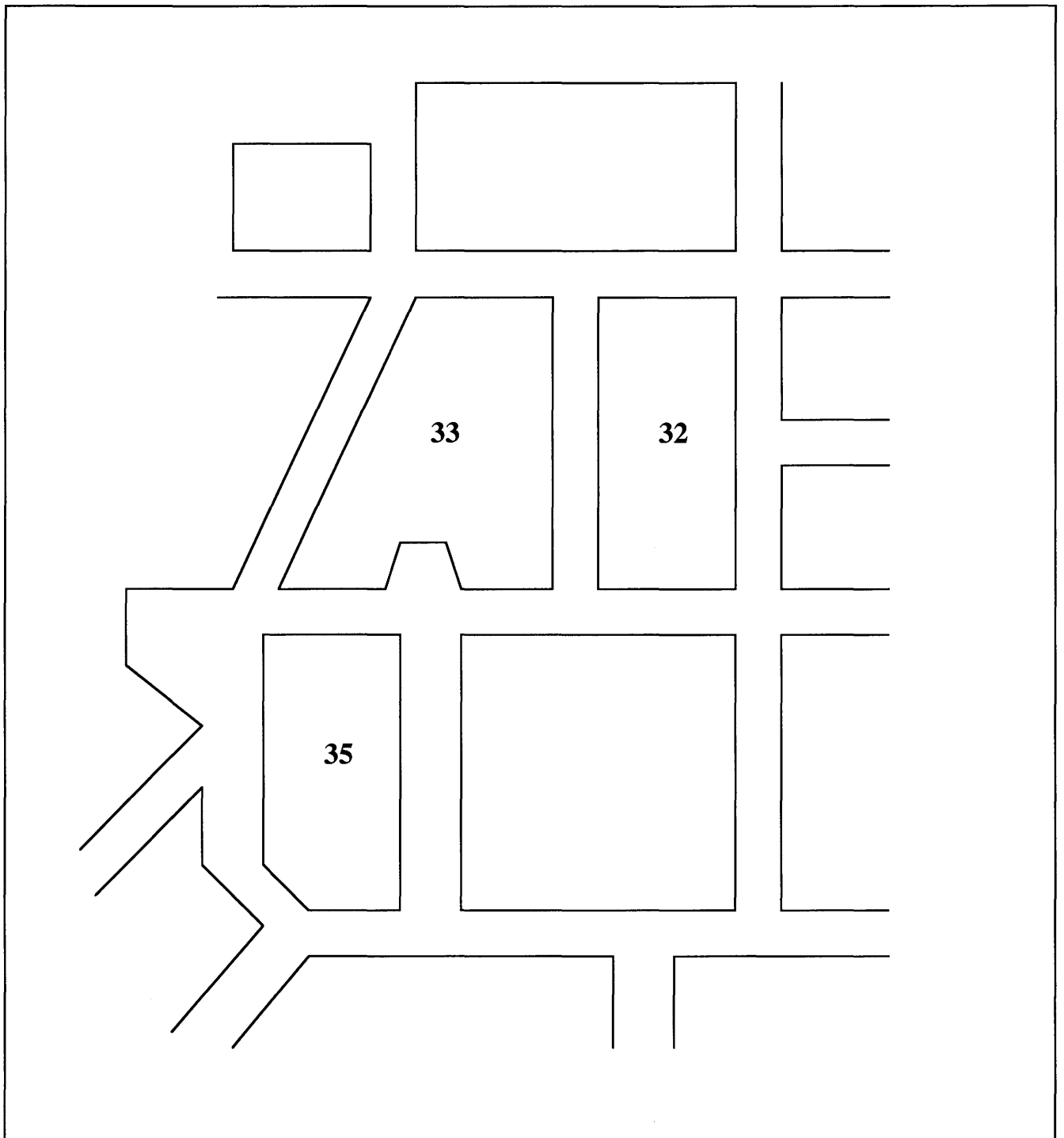


Figure 4: Map of Siquirres study site. Neighborhoods used in this study were designated by the Ministry of Health as 32, 33, and 35. Houses within these neighborhoods were randomly selected as treatment or control houses.

Five traps were placed inside each treatment house. An additional five traps were placed outdoors around the house. Traps in the houses were located in areas where *Ae. aegypti* are known to rest and where human disturbance would be minimal. Likely oviposition sites were under beds, on shelves in closets, behind toilets, below sinks, etc. Outdoor traps were placed where they were somewhat protected from the sun and rain and where human and animal disturbance would be minimal. Typically, traps were placed under outdoor sinks, in bushes, at the bases of trees, and on top of old refrigerators and other objects in the yards.

Traps were checked weekly to make sure the treated strips were submerged in water and that the water level was high enough for oviposition. If a strip was missing or a trap was damaged, they were replaced. Fresh hay infusion water was added to the traps as needed. After four weeks, the treated strips and hay infusion water were changed in all of the traps. At this time, it was noted that larvae of all four instars were present in most of the outdoor traps. As a result, treated strips and hay infusion water were changed at three-week intervals.

**Sampling.** Houses within each city were divided into three groups. Each group consisted of 10 treatment houses and 10 control houses. One group of houses was checked for mosquito abundance each week. Pretreatment sampling began three weeks before traps were placed in the houses. Sampling continued for 10 weeks afterwards (Table 1).

Three individuals checked each home. One person looked inside and outside around the house for containers with water. Of these, the number containing larvae

Table 1: Timing of pretreatment period and of each replicate of the study. Strips were replaced and fresh hay infusion water was added before each replicate.

<b>Sampling Period</b>	<b>Length of Time</b>
Pretreatment	3 Weeks
Replicate 1	4 Weeks
Replicate 2	3 Weeks
Replicate 3	3 Weeks

and/or pupae was recorded as was the total number of pupae found in the house. At the same time, two people from the local Ministry of Health office searched the inside of the house with hand-held flashlights and aspirated any mosquitoes they found with a battery powered hand-held mechanical aspirator (Hausherr's Machine Works, Toms River, NJ, U.S.A.). These mosquitoes were identified immediately and recorded as *Ae. aegypti* females, *Ae. aegypti* males, other females, or other males.

When the treated strips and hay infusion water were changed, the number of live larvae of each instar in each trap was recorded. Used oviposition strips were dried and taken back to Iowa State University where the number of *Ae. aegypti* eggs on each strip was counted using a dissecting microscope.

**Laboratory Trials.** In September 2000, 70 traps were placed behind the Insectary building and 70 traps were placed indoors in a laboratory in the Science II building at Iowa State University, Ames, IA, U.S.A. These traps were set up exactly as described in the "Field Trials" section. The hay used to make 10% hay infusion water was acquired from the horse stables on Pammel Road at Iowa State University. Every two days for a total of 28 days, 20 *Ae. aegypti* first instar larvae were placed in each of 10 traps, 5 from outside and 5 from inside. These larvae were from a laboratory-reared colony of a strain from Puntarenas, Costa Rica. After 24 hours, the number of live larvae in each trap was recorded. Larvae were counted for three consecutive days to ensure that any live larvae were counted.

A similar experiment was performed with used strips. Six used strips from each city were clipped into ovitraps that were filled with 200 ml distilled water. Two of these



strips were from the four-week changing regimen and four were from the three-week changing regimens. Half of the strips from each time period were from inside traps and half were from outside traps. The traps were allowed to stand for 24 hours so the insecticide had time to leach into the water. Fifty first-instar *Ae. aegypti* were placed in each of the traps. The number of larvae alive in each trap was recorded each following day.

**Deltamethrin Content of Strips.** The insecticide toxicology laboratory in the Department of Entomology at Iowa State University analyzed the deltamethrin content of unused and used strips from outdoor and indoor traps. Strips were cut into 2-cm pieces and placed in a 200-ml French-square bottle containing 25 ml of acetone. The bottles were shaken vigorously for three minutes and then were allowed to settle for 45 minutes. The extract was removed and analyzed without further treatment. Analysis was performed by gas chromatography (Varian 3400) equipped with a flame thermo-ionic detector (FTD – N and P selective). Carrier flow was helium at 5.2 ml per minute on a DB-5 column (30 m long, 0.32 mm ID, 0.5  $\mu$ m film thickness). The column program started at 250° C, was held for 2 min., increased 10° per minute to 300° and held for 7 min. Inlet temperature was 250° and the detector was 300° C. This analysis was done for 20 unused strips, 10 used strips from outdoor traps, and 10 used strips from indoor traps.

**Data Analysis.** Mosquito abundance for each house was calculated three different ways. First, the proportion of containers in each house that contained larvae and/or pupae was recorded. This is the Container Index (Focks and Chadee 1997). The

second measure of abundance was the proportion of houses positive for larvae and/or pupae, or the House Index (Focks and Chadee 1997). The third measure of mosquito abundance was the average number of *Ae. aegypti* mosquitoes captured per house. Each measure was averaged for treatment and control houses in each city during the pretreatment period and during each treatment replicate. The Container Index and the House Index for treatment and control houses for each time period for each city were compared using Statistical Analysis Systems' program JMP<sup>®</sup>, with a 2-tailed t-test for the Container Index and Chi-square tests for the House Index. Both of these tests were performed at the  $p \leq 0.05$  significance level. The average number of mosquitoes caught per house was compared in the treatment houses and in the control houses in each period in each city using a 2-tailed t-test with JMP<sup>®</sup> at  $p \leq 0.05$ .

The overall number of eggs per strip from the indoor traps was compared to the number per strip from the outdoor traps using a JMP<sup>®</sup> 2-tailed t-test at  $p \leq 0.05$ . The proportion of traps positive for eggs was compared using a Chi-square test.

The number of larvae of each instar in indoor traps was compared to the number in outdoor traps for each sampling period. The proportion of indoor traps positive for larvae was compared to that of outdoor traps using a Chi-square test at the  $p \leq 0.05$  level.

Deltamethrin levels from strips from indoor traps was compared to that of outdoor traps, also using a JMP<sup>®</sup> 2-tailed t-test at  $p \leq 0.05$ .

## RESULTS

**Mosquito Abundance.** Table 2 shows the mean Container Index for the control houses and for the treatment houses during each time period in each city. There was no statistically significant difference at the  $p \leq 0.05$  level between the Container Indices for the control houses and those for the treatment houses for each time period in each city.

Table 3 shows the House Index for the control houses and for the treatment houses during each time period in each city. When compared at the  $p \leq 0.05$  level, there was no statistically significant difference between the House Indices for control and treatment houses.

Figure 5 shows the average number of *Ae. aegypti* adults captured per house in control houses and in treatment houses before the treatment began and weekly during the study. At the  $p \leq 0.05$  level, there was no statistically significant difference between the average number of mosquitoes caught per house in control and treatment houses in either city either during the first 4 weeks before the traps were changed or during the period when the traps were changed every 3 weeks. Figure 6 shows the average number of *Ae. aegypti* captured per house for each of the three different time periods in each city. Again, at the  $p \leq 0.05$  level there was no statistically significant difference between the number of adult *Ae. aegypti* caught in control houses and treatment houses.

**Oviposition.** Figure 7 shows the average number of eggs found per trap in the inside traps versus the number found in the outside traps. At the  $p \leq 0.05$  level, there were significantly more eggs laid in the outside traps than were laid in inside traps. In Puntarenas, there were 80.5% fewer eggs in inside traps as in outside traps. In

Table 2: Mean Container Index (proportion of containers positive for immatures) for each treatment at each time interval.

City	Time Period	Trt/Ctrl	Mean	t-test <i>p</i> -value
Puntarenas	Pretreatment	Ctrl	0.008	0.902
		Trt	0.007	
	4 Weeks	Ctrl	0.025	0.310
		Trt	0.007	
	3 Weeks	Ctrl	0.006	0.248
		Trt	0.022	
Siquirres	Pretreatment	Ctrl	0.015	0.550
		Trt	0.007	
	4 Weeks	Ctrl	0.024	0.974
		Trt	0.024	
	3 Weeks	Ctrl	0.012	0.932
		Trt	0.011	

Table 3: House Index (proportion of houses with immatures) for each treatment at each time interval.

City	Time Period	Trt/Ctrl	Proportion	chi-square <i>p</i> -value
Puntarenas	Pretreatment	Ctrl	0.080	0.504
		Trt	0.037	
	4 Weeks	Ctrl	0.090	0.266
		Trt	0.028	
	3 Weeks	Ctrl	0.023	0.306
		Trt	0.069	
Siquirres	Pretreatment	Ctrl	0.107	0.306
		Trt	0.037	
	4 Weeks	Ctrl	0.108	0.748
		Trt	0.085	
	3 Weeks	Ctrl	0.043	0.593
		Trt	0.023	

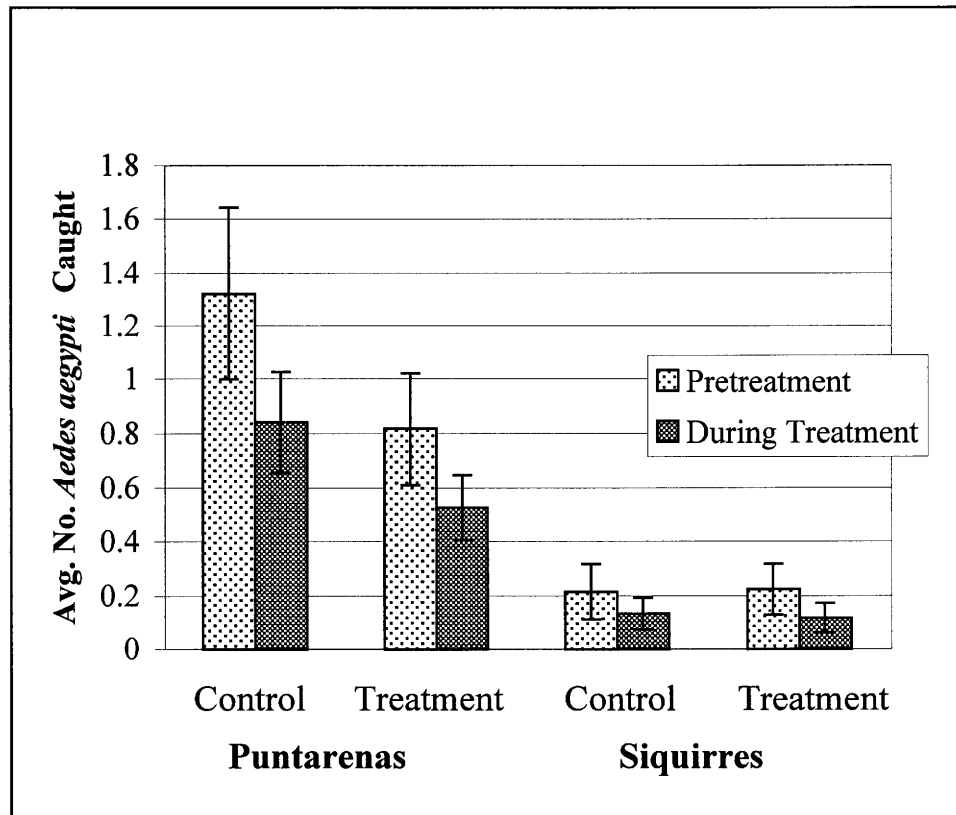


Figure 5: Average number of *Aedes aegypti* adults captured per home in the control houses and in the treatment houses in each city before traps were put out ("Pretreatment") and during the replicates of the study ("During Treatment").

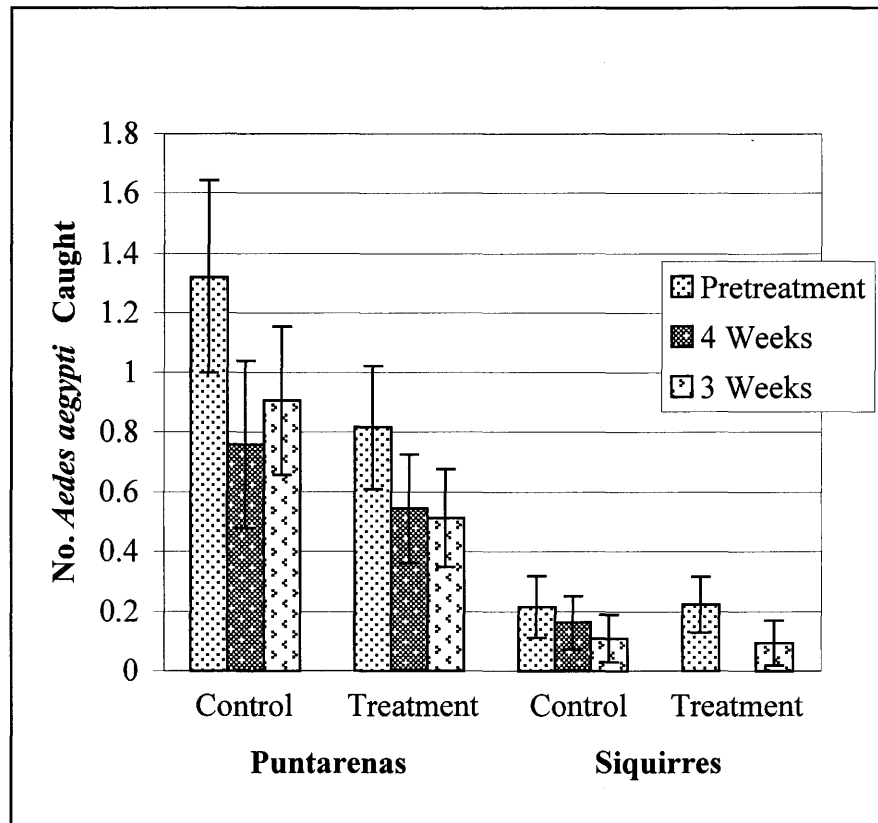


Figure 6: Average number of *Aedes aegypti* adults captured per home in the control houses and in the treatment houses in each city before the traps were put out ("Pretreatment") and during each replicate length of time ("4 Weeks" for replicate 1 and "3 Weeks" for replicates 2 and 3).

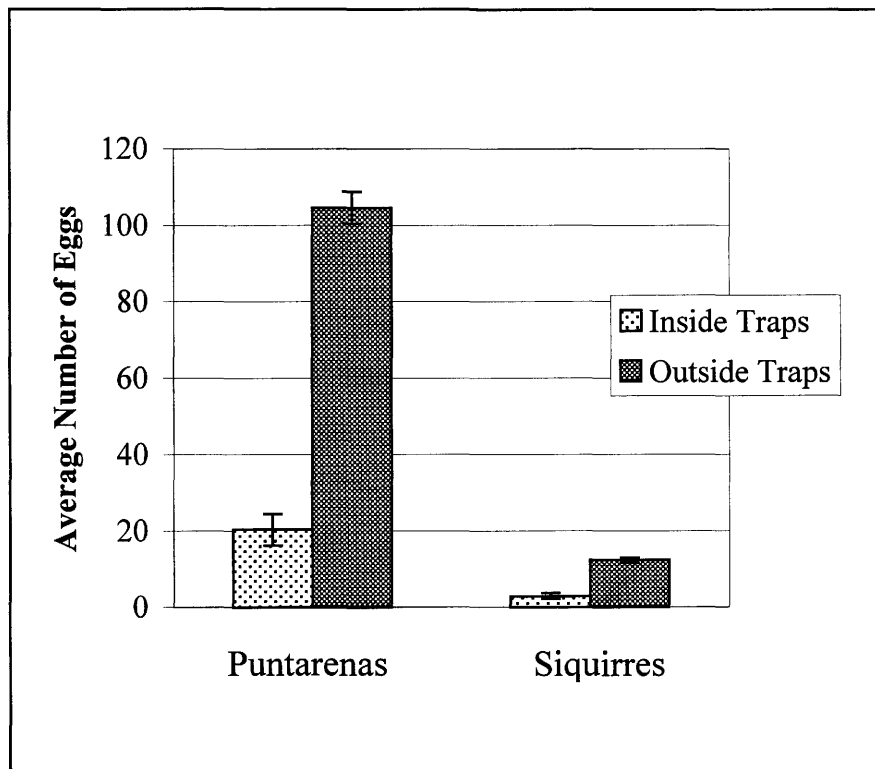


Figure 7: Average number of eggs on oviposition strips from inside traps and outside traps in each city.



Siquirres, there were 77.4% fewer eggs in inside traps. There were also a significantly higher number of eggs laid in outside traps than in inside traps when the data are separated into the period of time before the traps were changed (Figure 8). In Puntarenas, after the first 4 weeks, there were 86% fewer eggs in inside traps as in outside traps. After 3 weeks, there were 74% fewer eggs in indoor traps. In Siquirres, after the first 4 weeks there were 82.5% fewer eggs in indoor traps as in outdoor traps. After 3 weeks, there were 70% fewer eggs in indoor traps. Figure 9 shows proportion of indoor and outdoor traps that contained eggs during each time period in each city.

Significantly more outdoor traps contained eggs than indoor traps. In Puntarenas, 38.3% more outside traps had eggs than inside traps when the traps were changed after 4 weeks. When the traps were changed after 3 weeks, 20% more outside traps had eggs. When the traps were changed after 4 weeks in Siquirres, 37.7% more outdoor traps had eggs than indoor traps. When the traps were changed after 3 weeks, 38.3% more outdoor traps had eggs.

**Larval Abundance.** Tables 4 and 5 show the number of larvae of each instar at each trap changing, expressed as a proportion of traps within each category. In Puntarenas, more larvae were in outside traps than in each corresponding group of inside traps. Figure 10 shows that proportion of inside and outside traps that contained larvae for each time period. At the  $p \leq 0.05$  level significantly more outdoor traps contained larvae than in indoor traps. In Puntarenas, after the first 4 weeks 38% more outside traps had larvae than inside traps. After 3 weeks, 26.3% more outside traps had larvae. After the first 4 weeks in Siquirres, 6.5% more outside traps had larvae. After 3 weeks,

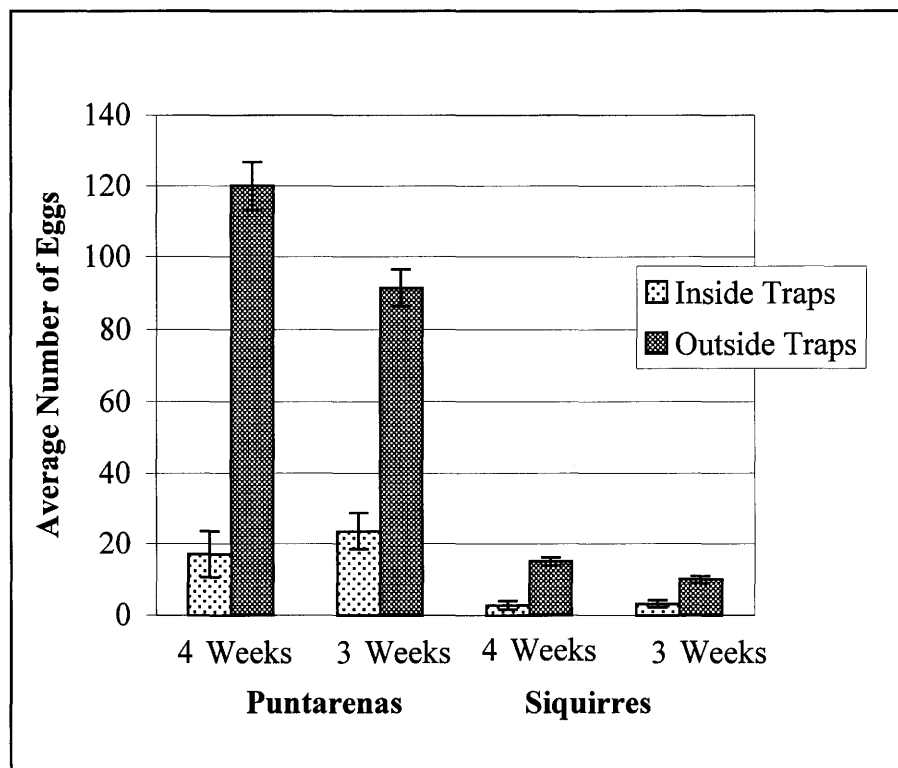


Figure 8: Average number of eggs on oviposition strips from inside and outside traps in each city when traps were changed after 4 and 3 week replacement times.

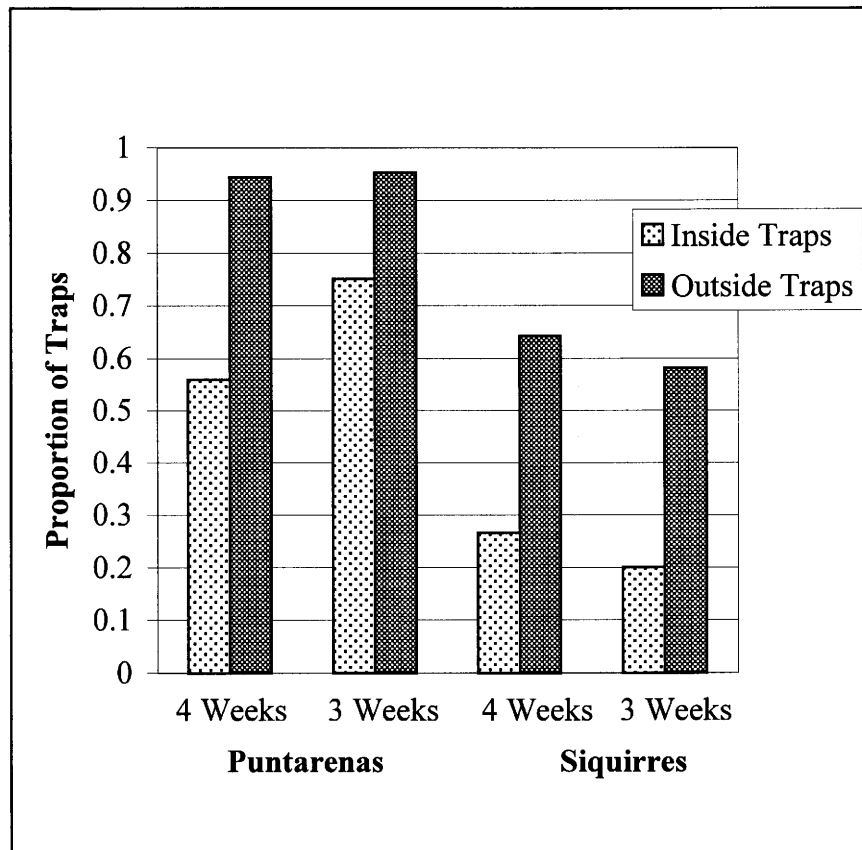


Figure 9: Proportion of inside and outside traps in each city that contained eggs after 4 week and 3 week replacement times.

Table 4: The number of larvae of each instar in Puntarenas at each trap changing expressed as a proportion of traps within each category.

Trap Change			No. of Larvae				
Group	Instar	In/Out	0	$\leq 5$	6-15	16-29	$\geq 30$
1	1	In	0.939	0.043	0	0	0.017
1	1	Out	0.672	0.032	0.112	0	0.184
1	2	In	0.896	0.061	0.017	0	0.026
1	2	Out	0.592	0.144	0.056	0	0.128
1	3	In	0.939	0.052	0	0	0.009
1	3	Out	0.664	0.152	0.112	0	0.072
1	4	In	0.983	0.009	0.009	0	0
1	4	Out	0.712	0.184	0.024	0.008	0.072
2	1	In	0.919	0.054	0	0	0.027
2	1	Out	0.845	0.060	0.017	0	0.078
2	2	In	0.991	0.072	0	0	0.018
2	2	Out	0.741	0.190	0.017	0	0.052
2	3	In	0.901	0.081	0.009	0	0.009
2	3	Out	0.681	0.250	0.043	0	0.026
2	4	In	0.901	0.081	0.009	0	0.009
2	4	Out	0.638	0.293	0.052	0	0.017
3	1	In	0.895	0.019	0.029	0	0.057
3	1	Out	0.655	0.118	0.059	0.025	0.118
3	2	In	0.810	0.152	0.019	0	0.019
3	2	Out	0.605	0.269	0.050	0.008	0.067
3	3	In	0.800	0.181	0.010	0.010	0
3	3	Out	0.571	0.303	0.101	0	0.025
3	4	In	0.752	0.219	0.019	0	0.010
3	4	Out	0.639	0.277	0.084	0	0

Table 5: The number of larvae of each instar in Siquirres at each trap changing expressed as a proportion of traps within each category.

Trap Change			No. of Larvae				
Group	Instar	In/Out	0	$\leq 5$	6-15	16-29	$\geq 30$
1	1	In	1	0	0	0	0
1	1	Out	1	0	0	0	0
1	2	In	1	0	0	0	0
1	2	Out	0.979	0.021	0	0	0
1	3	In	0.991	0.009	0	0	0
1	3	Out	0.984	0.016	0	0	0
1	4	In	0.991	0.009	0	0	0
1	4	Out	0.979	0.021	0	0	0
2	1	In	1	0	0	0	0
2	1	Out	0.975	0.025	0	0	0
2	2	In	0.929	0.048	0.024	0	0
2	2	Out	0.975	0.025	0	0	0
2	3	In	0.952	0.048	0	0	0
2	3	Out	0.975	0.025	0	0	0
2	4	In	0.952	0.048	0	0	0
2	4	Out	0.975	0.025	0	0	0
3	1	In	0.992	0	0	0	0.008
3	1	Out	1	0	0	0	0
3	2	In	0.969	0.023	0	0	0.008
3	2	Out	0.983	0.017	0	0	0
3	3	In	0.985	0.008	0	0.008	0
3	3	Out	0.992	0	0	0	0.008
3	4	In	0.977	0.023	0	0	0
3	4	Out	0.959	0.025	0.008	0	0.008

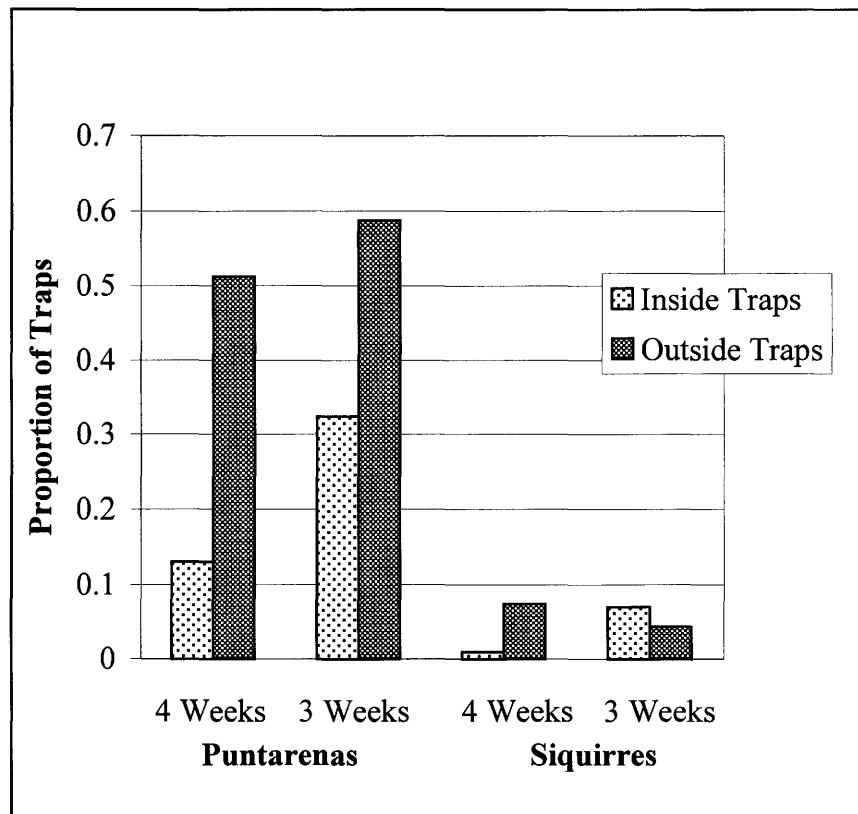


Figure 10: Proportion of inside and outside traps in each city with larvae after 4 week and 3 week replacement times.

however, there was no statistically significant difference between the number of eggs laid in outside traps and the number in inside traps.

Table 6 is a side-by-side comparison of the proportion of traps positive for eggs and the proportion positive for larvae inside and outside for each time period. Overall, a significantly higher proportion of the traps contained eggs than contained larvae.

**Laboratory Trials.** When traps with unused strips were tested in Ames, Iowa, no *Ae. aegypti* larvae survived for the first 27 days. On day 28, a single larva was found alive in 1 ovitrap. When the used strips were tested, the Siquirres strips killed all larvae within 48 hr. The Puntarenas strips killed all but one larva within 72 hr. and the last larva was dead at 96 hr.

Unused strips contained the amount of deltamethrin predicted. The average deltamethrin content was  $0.95 \pm 0.15$  mg per strip. Overall, used indoor strips contained an average of  $122.3 \pm 46.2$   $\mu$ g deltamethrin per strip while used outdoor strips contained an average of  $123.5 \pm 38.0$   $\mu$ g per strip. These deltamethrin levels were not significantly different at the  $p \leq 0.05$  level (Figure 11).

## DISCUSSION

**Mosquito Abundance.** Lethal ovitraps did not have an effect on the number of immature mosquitoes found per house. The Container and House Indices were similar in both the treatment houses and control houses before the traps were put out and during the

Table 6: Proportion of traps outside and inside containing eggs and proportion containing larvae when traps were changed after 4-week and 3-week replicate times.

<b>City</b>	<b>Time</b>	<b>In/Out</b>	<b>Proportion Positive for Eggs</b>	<b>Proportion Positive for Larvae</b>
Puntarenas	4 weeks	In	0.559	0.130
		Out	0.943	0.512
	3 weeks	In	0.751	0.324
		Out	0.951	0.587
Siquirres	4 weeks	In	0.265	0.008
		Out	0.642	0.074
	3 weeks	In	0.199	0.069
		Out	0.582	0.043



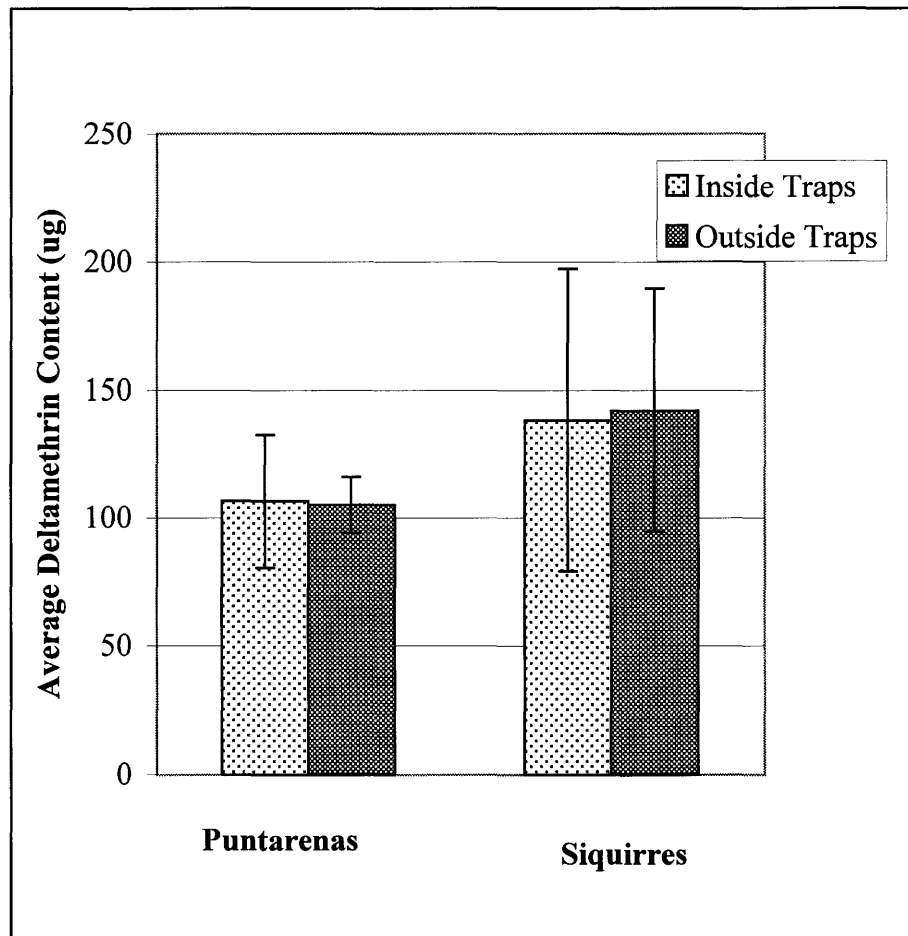


Figure 11: Average amount of deltamethrin extracted from strips from inside and outside traps in each city.

time when the traps were out. Thus, there was no difference in the number of containers that contained immatures or the proportion of houses that contained immatures. This was the case in both treatment and control houses.

Lethal ovitraps failed to reduce the number of *Ae. aegypti* adults captured during the study. There was not a significant difference in the number captured before and during the treatment in either the treatment or the control houses. As the rainy season progressed, the *Ae. aegypti* population should have increased. Thus, the number caught in control homes should have increased while, if the traps were effective, the number caught in treatment homes should have decreased. However, there was no increase in mosquito numbers in either treatment or control houses. The fact that adult numbers did not increase could be a function of less rainfall than normal or effective ongoing mosquito control measures conducted by the local Ministry of Health.

**Oviposition.** Up to 600% more eggs were laid in traps situated outside around the homes as inside the houses. Also, as many as 38% more outside traps contained eggs than did inside traps. Thus, the mosquitoes laid eggs more in outdoor traps. The deltamethrin in the ovitrap was expected to kill adult females subsequent to oviposition and the resulting first-instar larvae. Minimal difference in insecticide content between outside and inside traps should not have had an effect on the number of eggs laid or on which traps the mosquitoes preferred. At high doses, however, pyrethroid insecticides have been shown to repel mosquitoes (Charlwood and Graves 1987, Lindsay *et al.* 1989).

**Larval Abundance.** Traps located outside but near the houses contained more larvae than those inside. Other factors could have influenced where the mosquitoes laid

their eggs. Torres-Estrada *et al.* (2000) found that *Ae. aegypti* laid more eggs in water containing copepods. This mosquito may have been attracted to different microorganisms present in outside traps. More organic debris found its way into the hay infusion in outdoor traps in some cases. Water in the outdoor traps was cleaner because of a difference in the rate of evaporation or a dilution of the hay infusion because of rain water getting into traps.

To determine insecticide content of the outside strips as compared to the inside strips, used strips were tested for deltamethrin content. There was not any difference in the amount of insecticide on any of the strips. Therefore, differences in numbers of larvae and the number of eggs laid in outside and inside traps could not have been due to a difference in insecticide content on the strips.

**Laboratory Trials.** Lethal ovitraps similar to those used in the Costa Rican field trials were 100% effective in killing first instar *Ae. aegypti* larvae for up to 28 days in Ames, Iowa. Costa Rican ovitraps did not limit larval production. Thus, there must be a difference in the deltamethrin content in the water or in the environmental conditions.

In the laboratory, there was not a loss in efficacy in the lethal ovitraps after being flooded in a manner similar to extensive rainfall or after being exposed to aging and evaporation for one month (Zeichner and Perich 1999). The insecticide content of the unused strips was the same as that in the laboratory experiments. Because deltamethrin is photostable (Ray 1991), sunlight should not have played a role on the loss of efficacy of the traps. It is possible, however, that in Costa Rica the insecticide was degraded by

microorganisms in the hay infusion not present in the Ames study. Such microorganisms might have been present in the water, in the hay, or even in the air.

The mosquitoes used in the Iowa State University laboratory portion of this study were from a laboratory-reared colony established from *Ae. aegypti* eggs collected in Puntarenas, Costa Rica one year prior to the study. The colony-reared mosquitoes were not resistant to the insecticide. In 1998, *Ae. aegypti* eggs collected in Costa Rica were sent to the Centers for Disease Control and Prevention (CDC) in Atlanta and tested for deltamethrin resistance. None was found. Thus, the Costa Rican mosquitoes would have had to develop a resistance within one year or the colony would have had to lose previously established resistance during that same year. When used strips were placed in traps and first-instar *Ae. aegypti* larvae were added, however, the strips were highly effective in killing laboratory-reared first-instar larvae.

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## CHAPTER 4. GENERAL CONCLUSIONS

### Summary of Experimental Results

The presence of lethal ovitraps did not have a statistically significant effect on the House Index, the Container Index, or the number of adults captured per house (Focks and Chadee 1997). Thus, unlike in a similar study in Brazil (Perich *et al.* in press), the lethal ovitraps failed to reduce mosquito abundance as expected.

More outside traps contained eggs than inside traps. Also, the outside traps contained a higher number of eggs than the inside traps. This indicates that *Ae. aegypti* mosquitoes oviposited more in the outdoor traps. More outdoor traps contained *Ae. aegypti* larvae than indoor traps. This further supports outdoor oviposition.

New strips and used strips were tested for insecticide content. The new strips contained the amount of deltamethrin expected. The used strips were tested to compare outdoor strips to indoor strips. There was no statistically significant difference in the insecticide content of these strips.

New strips were tested with hay infusion made in Ames, Iowa and first-instar *Ae. aegypti* from a Puntarenas strain laboratory-reared colony. In this trial, the ovitraps were 100% effective in killing the first-instar larvae for 27 days. This indicates that it is unlikely that the mosquitoes in the Costa Rican study were resistant to the insecticide. However, used strips were also effective in killing first-instar laboratory-reared *Ae. aegypti*. Therefore, either a resistance existed in the Costa Rican mosquitoes during the field study and not the laboratory-reared mosquitoes, or the water in the traps had an

effect on the efficacy of the insecticide. It is possible that the hay infusion used in Costa Rica contained more suspended organic matter to which the deltamethrin may have bound.

### **Recommendations for Future Research**

To determine why the lethal ovitraps failed to control *Ae. aegypti* mosquitoes, more studies should be performed. Different hay infusion formulations should be tested to determine if the type of hay used had an effect on the insecticide. Traps without hay infusion, using only clean water, should also be tested for this purpose.

Oviposition behavior of *Ae. aegypti* mosquitoes in Costa Rica could be examined by placing non-lethal oviposition traps inside and outside of homes and comparing the oviposition activity. This would help to determine if the insecticide had an effect on the activity of the mosquitoes in the study. Also, lethal ovitraps could be placed inside and outside of homes as they were in the study, and water samples could be taken from the traps to determine if there was a difference in contents in the indoor and outdoor traps such as the amount of insecticide, amount of microorganisms, and amount of suspended organic matter.

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## ACKNOWLEDGEMENTS

This research would not have been possible without the contributions of many people. First, thanks to the members of my committee, Dr. Wayne Rowley, Dr. Michael Perich, Dr. Kenneth Platt, and Dr. Joel Coats for their advice and assistance with this project. I also thank individuals from the Ministry of Health of Costa Rica for their cooperation and help with the research. In particular, I thank Douglas Perez Cortes and Fernando Vega Campas as well as the rest of the staff at the local Ministry of Health offices in Puntarenas and Siquirres for diligently searching for mosquitoes and putting out ovitraps, but mostly for obeying my poorly spoken Spanish commands. Thank you to the University of Costa Rica, Dr. Oscar Rocha, and in particular Luis Castro for their assistance with the project. From Iowa State University, I thank Ann Peters for her month of service to the project and Julie Coan for her help in the final week. Thank you to the toxicology lab of the Entomology Department at Iowa State University for testing the deltamethrin content of the strips and especially to Chris Peterson for helping me brainstorm. A wealth of gratitude goes to Mary Beth Penisten, Mandi Lingren, Sara Erickson, and Dave Dorhout, the undergraduate laboratory assistants in Dr. Rowley's lab, for their many hours of counting eggs and entering data sets. Most importantly, I sincerely thank the residents of Puntarenas and Siquirres for welcoming us into their homes and cooperating with the project.