

ANNUAL
REPORT

1989

NOMCB

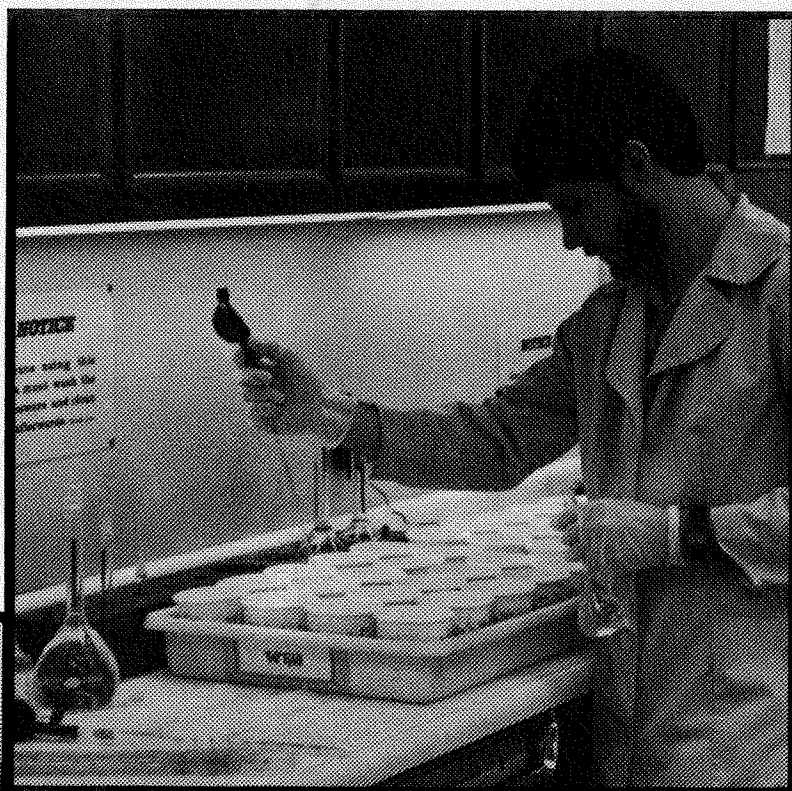
NEW ORLEANS MOSQUITO CONTROL BOARD

1964

25 years of service

1989

*Development
and
implementation
of safe,
economical
control methods*



*to provide the
best protection
from
mosquitoes
and diseases*

COVER STORY

The New Orleans Mosquito Control Board has always been committed to providing its citizens with the best year round protection from mosquitoes and the disease pathogens they may vector. Consequently, in 1989 we will continue the operational development and evaluation of our program. The goal is to provide the most practical, safe and economical surveillance and control methods for the suppression of mosquitoes.

Testing and screening of new or currently used insecticides is an important and indispensable function of a successful mosquito control program. Our standard larval susceptibility test (or modified WHO test) is one of our primary tools for obtaining baseline data to determine the insecticidal properties of new materials. It also allows us to determine changes in susceptibilities of field populations of the many mosquito species to insecticides applied by our trucks and airplanes. Subsequent tests with the wind tunnel and field cages, as well as routine calibration and droplet analysis, allow us to maintain the highest degree of proficiency.

Additionally, the NOMCB was granted a 1989-90 contract by the Centers for Disease Control to study the effects of aerial ultralow volume (ULV) spraying on populations of Aedes albopictus mosquitoes in New Orleans. Application methods of different insecticides will be evaluated individually to determine the control of adult populations in an urban environment. Extensive pretreatment and posttreatment surveillance methods will include the use of ovitraps, adult landing rate collections, adult resting collections and larval-pupal collections. Laboratory assessment of the insecticides will be done using standard larval susceptibility tests, wind tunnel tests and topical applications.

1989
ANNUAL REPORT

Director's Report

Control of Aedes albopictus was the main thrust of mosquito abatement activities in New Orleans during the past year. Three visiting scientists were funded by the Centers for Disease Control to work on the Ae. albopictus problem in Orleans Parish. Dr. Jerome Freier evaluated the use of aerial application to control Ae. albopictus in an urban environment. Dr. Gerry Marten identified seven species of cyclopoid copepods that could be used as biological control agents against Ae. albopictus. Dr. Wenyan Che worked on several projects that supported the work of both Dr. Freier and Dr. Marten. Dr. Freier conducted a series of field studies which focused on: assessment of Ae. albopictus population density; tests of the duplex cone trap for Ae. albopictus adults; assessment of the efficacy of ULV application of malathion; and survivorship of Ae. albopictus under natural conditions.

Dr. Gerry Marten's work on biological control of container breeding Ae. albopictus by using native "cyclops" proved to be very effective. Five species of cyclops were determined to be effective predators of first instar Ae. albopictus. Combinations of these predators were used to treat tire piles in the City and the results were astounding. Two weeks after the introductions there were no Ae. albopictus larvae left in any of the treated tires. Non-chemical control methodology is safe and effective, we should give biological control every opportunity to work.

To combat the buck moth infestation, NOMCB and the Parkway Commission have joined forces in developing a control strategy. This strategy consists of: (1) study of the basic biology and ecology of the insect; (2) determination of the most effective means of control; and (3) coordination of activities of city groups, state agencies, and pest control operators.

Encephalitis surveillance began in May and continued through September. The bird trapping operation included placement of 16 newly designed and constructed wild bird traps throughout the city. Bird blood samples were submitted to the State Laboratory for Hemagglutination Inhibition testing. Although some cases of Eastern Equine Encephalitis occurred in neighboring states and parishes, all blood samples submitted by Orleans Parish were negative. The program continued through September with 801 bird bloods submitted to the State Laboratory.

Our source reduction efforts this year focused primarily on clearing obstructive vegetation, re-ditching and cleaning of existing ditches. Areas of concentration were City Park, Gentilly, and Little Woods.

The main subjects of our public education program this year has been the buck moth caterpillar and integrated pest management. For this purpose, NOMCB produced two video tapes which include information on the buck moth caterpillar life cycle, feeding habits, and control methods and another tape that covered all aspects of mosquito biology and control. Copies of these videos were distributed to the Government Access and School Board cable channels. We also prepared Public Service Announcements (PSA) on these subjects. The PSAs were aired by the major television stations in the area.

In 1989 our training series for foreign visitors continued. The following list details these visitors:

| | | |
|------------------------------------|---------------------------------|----------------|
| German Guerrero | Health Ministry-Malaria Service | Aruba |
| Maria Lourdas Zegarra Nolasco | Vector Control Specialist | Bahru |
| Efrain Vallejo Castro | Chief Medic | Bolivia |
| Edgar Paradei Maldonado | Vector Control Specialist | Bolivia |
| Dr. Mario Villagra | Vector Control Specialist | Bolivia |
| Roberto Leon L. | Vector Control Specialist | Bolivia |
| Luis Eduardo Nunez Encinas | Vector Control Specialist | Bolivia |
| Rene E. Mollinedo Llave | Statistician | Bolivia |
| Abraham Gemio Alarico | Vector Control Specialist | Bolivia |
| Maria de los Angeles Valdez Laguna | Vector Control Specialist | Bolivia |
| Carlos Mario Toffen Rivero | Chief Medic/Malaria | Bolivia |
| Himbert Hurtado Gallardo | Health Medic | Bolivia |
| Maria Teresa Arandia | Vector Control Specialist | Bolivia |
| Quintanilla Luis | Vector Control Specialist | Bolivia |
| Gustave Marconi Ojeda | Medic | Bolivia |
| Jose Luis Herbas Gomez | District Director | Bolivia |
| Luisa Hurtado | Biochemistry/Pharmacy Teacher | Bolivia |
| Gustavo Quevedo | Chief Medic | Bolivia |
| Tito Luis Vargas Reque | Medic | Bolivia |
| Nyamah MD. Ali | Vector Control Specialist | Bolivia |
| Vincente Krozendijk | Public Health | Bolivia |
| Vilma Mekuria | Vector Control Specialist | Brazil |
| Kugenta Blackburn | Vector Control Specialist | Central Africa |
| Noebert L. Wills | Supervisor/Malaria Control | Colombia |
| Fernando Saume | Vector Control Specialist | Ecuador |
| Sandra Clark, M.D. | Vector Control Specialist | Ecuador |
| Eduardo Moreira | Vector Control Specialist | Ecuador |
| Tembeka Kuboni, R.N. | Registered Nurse | Ecuador |
| Mohamed Gedi Qayad, M.D. | Medical Doctor | Ecuador |
| Marcelo Castrillo, M.D. | Medical Doctor | Egypt |
| D. Beverly Barnett, M.D. | Medical Doctor | Ethiopia |
| Victor Coronado, M.D. | Medical Doctor | Guyana |
| Zacharias Itambi | Public Health | Guyana |
| Maria Barnett Herrera, M.D. | Medical Doctor | Honduras |
| Antoinette Korvah | Public Health | Honduras |
| Julio Yopez | Vector Control Specialist | Honduras |

Antoinette Korvah
 Julio Yepez
 Donald C. Kaminsky, M.D.
 Nahla Abdel Tawab
 Zohra Azlounk
 Carlos A. Javier-Zepeda, M.D.
 William Rojas M., M.D.
 Victor Reyes, M.D.
 Jose Adum, M.D.
 Arellano Felipe
 Enrique Granizo, M.D.

Public Health
 Vector Control Specialist
 Medical Doctor
 Public Health
 Student
 Medical Doctor
 Medical Doctor
 Medical Doctor
 Medical Doctor
 Senior Technical Advisor
 Vice-Minister of Health

Honduras
 Honduras
 Honduras
 Indonesia
 Jamaica
 Panama
 Peru
 Somalia
 Tunisia
 Venezuela
 West Africa

CDC REPORT - J. E. FREIER

A. Test of the Duplex Cone Trap for Aedes albopictus Adults

Tests of the modified duplex cone trap were conducted in New Orleans East at a site at the end of Michoud Blvd. This is a densely wooded 120 acre area with numerous tire piles located along the forest fringe near the roadway. Most tire piles contained between 100-500 casings. Aedes albopictus mosquitoes were abundant in the area. In preparation for sampling with the cone trap, 2 lbs. of dry ice were placed in a padded envelope and set on the ground. The inner cone of the trap was placed over the dry ice and the silver outer cone placed over the black one. A net bag was attached to the top of the updraft tube. The motor's lead wires were connected to a 6-volt lantern battery.

Ovitrap were used as a means of determining Ae. albopictus population density. Each ovitrap consisted of a single 16-oz black plastic cup. One strand of heavy gauge wire was placed across the cup opening to prevent water consumption by animals. In addition, an overflow hole was cut about 2.75-in above the base. The substrate for oviposition was a strip of red velour (1 in x 4.5 in). Ovistrips were attached to the inside of an ovitrap cup with a paper clip. In this study, 10 ovistrips were exchanged at daily intervals. Upon return to the laboratory, the number of Ae. albopictus and Ae. triseriatus eggs on each ovistrip was determined. The identity of these eggs was verified by hatching and identification of the larvae.

The results of this study, presented in Table 1, indicate that 12 species of mosquitoes were collected with the duplex cone trap. Eleven species were caught in the human-baited landing rate collections. Only Ae. albopictus eggs were collected in the ovitraps. Table 1 also shows that in order of rank, based on the total number of each species caught, that both the cone trap and the landing rate collections had the same species in the top 5 rankings. In both types of collections, Ae. albopictus mosquitoes were the most frequent species caught. Although this species accounted for only 27.4 percent of the total cone trap collection, Ae. albopictus females did result in 57.3 percent of the total landing rate collection. This difference is due to the large number of Culex salinarius and Coquillettidia perturbans mosquitoes caught in the cone trap. A comparison of capture rates shows that the mean number of mosquitoes

caught per hour in the cone trap and caught per minute in the landing rate collections were nearly equal for Ae. albopictus and Ae. triseriatus.

Table 1. Comparison of cone trap and human-baited landing rate collections from the Michoud study site between May 15 and June 29, 1989.

site between May 15 and June 29, 1989.

| Species | Cone trap collections | | | | Human-baited landing rate collections | | | |
|-----------------------------|-----------------------|--------------------|------------|------|---------------------------------------|---------------------|------------|------|
| | Total no. caught | Mean no. caught/hr | % of total | Rank | Total no. caught | Mean no. caught/min | % of total | Rank |
| <u>Ae.</u> | | | | | | | | |
| <u>albopictus</u> | 1459 | 45.6 | 27.4 | 1 | 1394 | 45 | 57.3 | 1 |
| <u>atlanticus</u> | 481 | 15 | 9 | 5 | 163 | 5.3 | 6.7 | 3 |
| <u>solicitans</u> | 12 | 0.4 | 0.2 | 11 | 11 | 0.4 | 0.5 | 9 |
| <u>taeniorhynchus</u> | 1 | <0.1 | <0.1 | 12 | | | | |
| <u>triseriatus</u> | 599 | 11.2 | 11.2 | 4 | 540 | 17.4 | 22.2 | 2 |
| <u>vexans</u> | 30 | 0.6 | 0.6 | 10 | 57 | 1.8 | 2 | 6 |
| <u>An.</u> | | | | | | | | |
| <u>crucians</u> | 282 | 5.3 | 5.3 | 6 | 24 | 0.8 | 1 | 8 |
| <u>punctipennis</u> | 48 | 0.9 | 0.9 | 9 | 2 | <0.1 | <0.1 | 11 |
| <u>Co.</u> | | | | | | | | |
| <u>perturbans</u> | 793 | 14.9 | 14.9 | 3 | 89 | 2.9 | 3.7 | 5 |
| <u>Cx. quinquefasciatus</u> | 143 | 2.7 | 2.7 | 7 | 7 | 0.2 | 0.2 | 10 |
| <u>salinarius</u> | 1327 | 24.9 | 24.9 | 2 | 119 | 3.8 | 4.9 | 4 |
| <u>Ps.</u> | | | | | | | | |
| <u>ferox</u> | 72 | 1.4 | 1.4 | 8 | 26 | 0.8 | 1.1 | 7 |

Figure 1 shows the daily population fluctuations for Ae. albopictus adults caught in the cone trap. Population peaks occurred at approximately 6 day intervals. Similar cycles of activity were observed in the human-baited landing rate collections, shown in Fig. 2. Figure 3 shows the results of the ovitrap collections. Previous studies have shown that, in areas with many tires, ovitraps are effective in collecting eggs of Ae. albopictus. The pattern of changes in egg density was similar to that observed for Ae. albopictus adults caught in the cone trap and landing rate collections. In some cases there was a 24 to 48-hour lag in the peak in relation to the adult collection peak.

Aedes albopictus Adults Caught in the Duplex Cone Trap

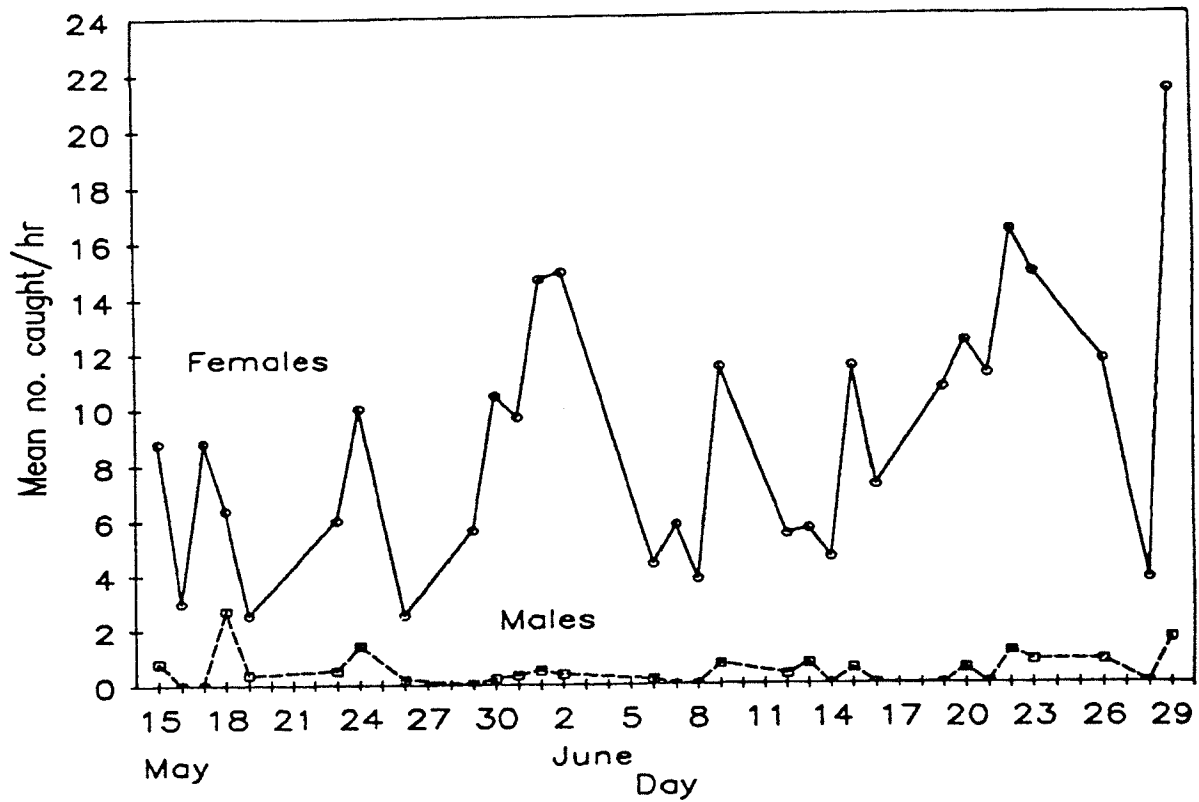


Figure 2.

Human-baited Landing Rate Collections from the Cone Trap Site

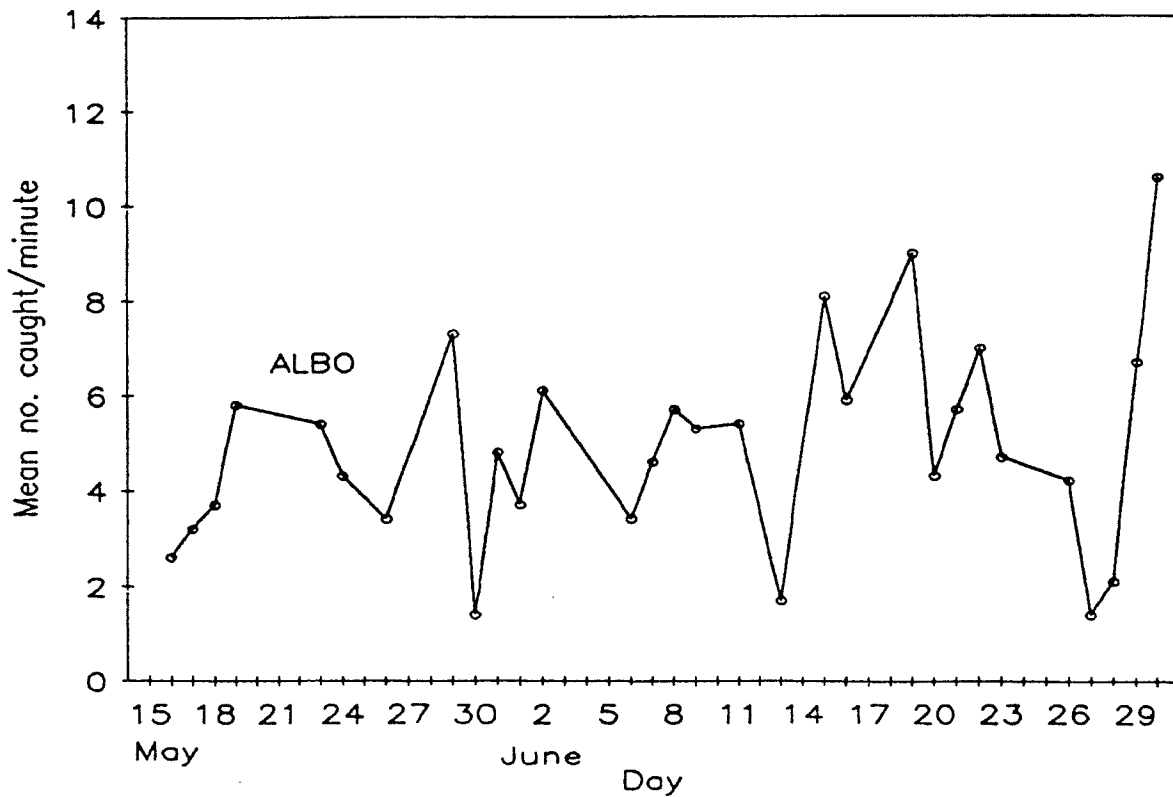
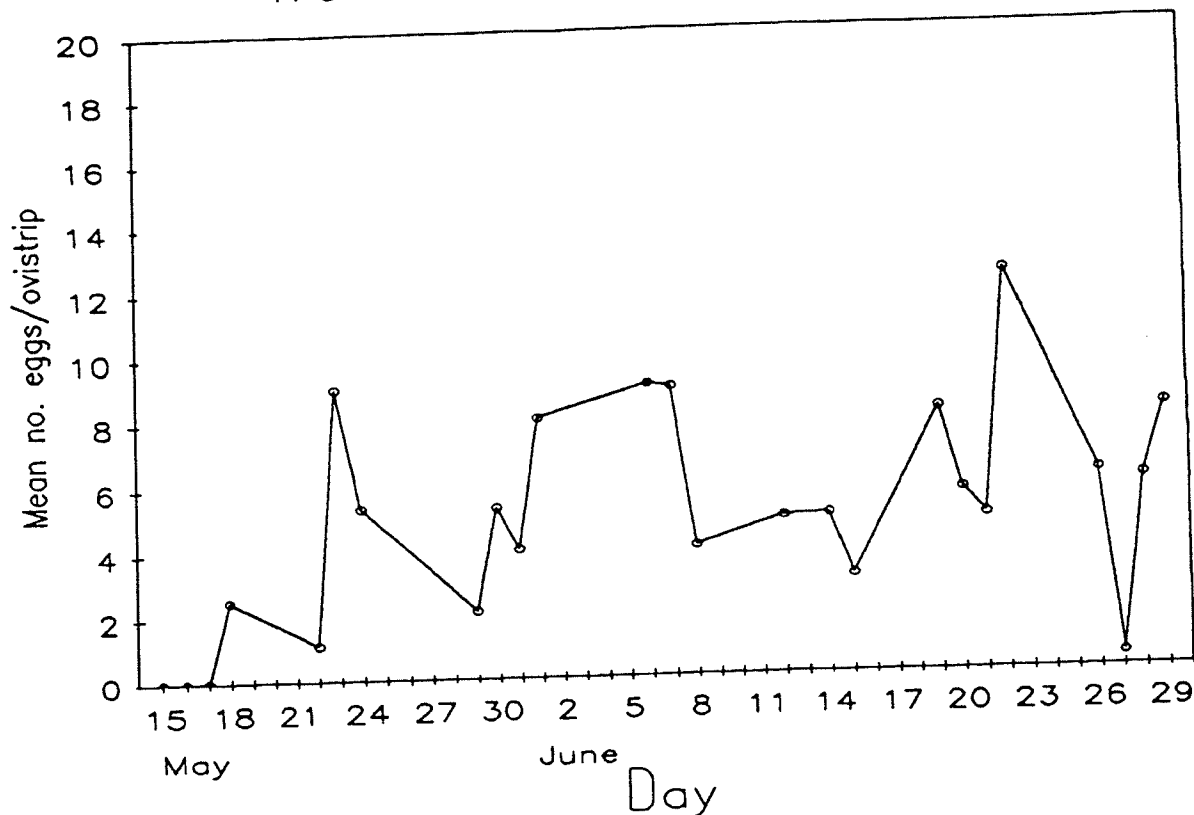


Figure 3.

Aedes albopictus Eggs Collected in Ovitrap
from the Cone Trap Site



Dissections were performed on a sample of *Aedes albopictus* females caught in the duplex cone trap experiment. Of the 1,459 specimens caught, 337 were dissected. Each female was examined for body size (wing length), insemination, blood engorgement, and egg development. A comparison between body size and capture date is shown in Figure 4. The overall mean wing length for the dissected females was 2.73 ± 0.21 mm with a 1.81 to 3.28 mm range. Between May 15th and June 29th, relatively small fluctuations were observed for body size.

Figure 5 shows the relationship between body size and physiologic state. The number of specimens examined is shown within each bar. The physiological conditions evaluated were inseminated (I), not inseminated (NI), bloodfed (B), not bloodfed (NB), gravid (G), and not gravid (NG). Parous (P) and nulliparous (NP) conditions are currently being determined and data were not available for this figure. It can be seen in Figure 5 that the mean body size for each group was nearly identical.

Figure 4.

Body Size of *Aedes albopictus* Females
Caught in the Cone Trap in 1989

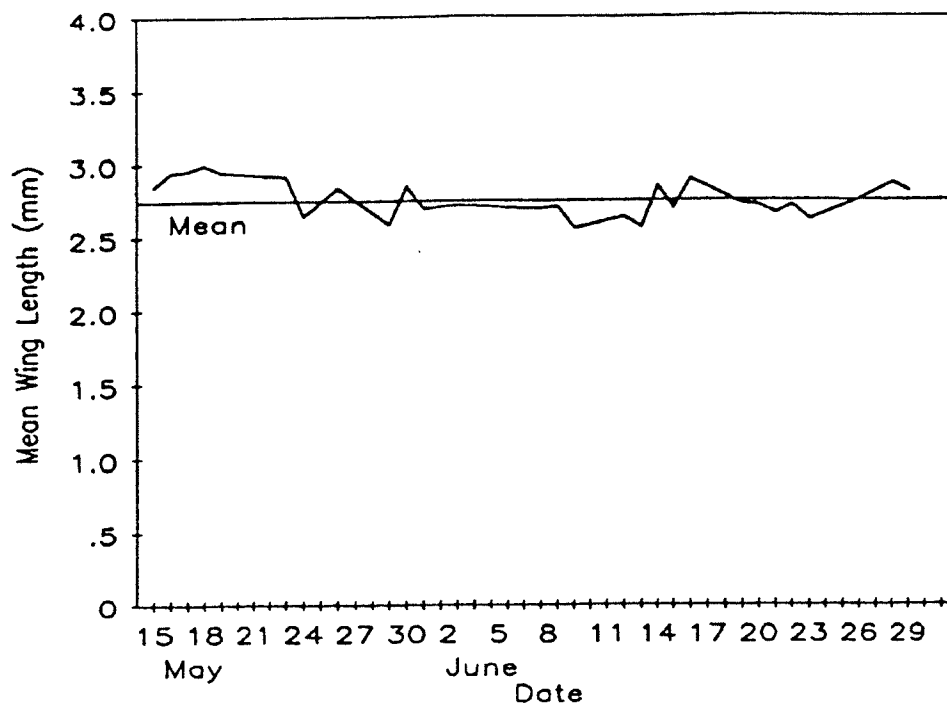
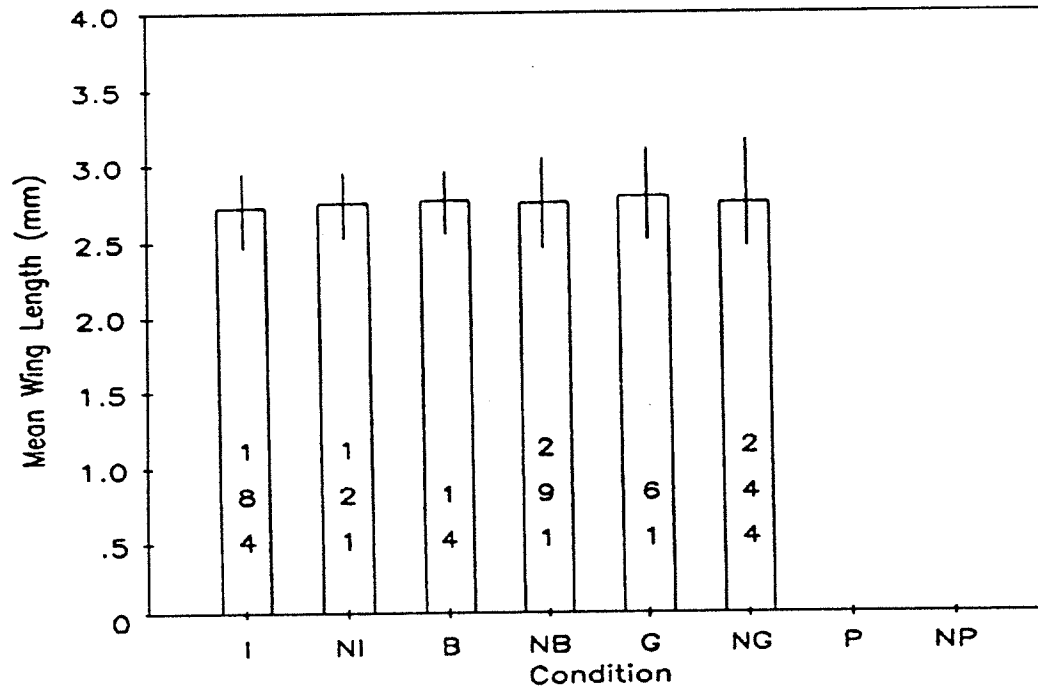


Figure 5.

Body Size of *Aedes albopictus* Females
in Relation to Physiologic State



The proportion of the population sample caught in the cone trap that displayed each physiologic condition is shown in Table 2. More than half of the females caught were inseminated, but only a small number were found engorged. Of the 14 bloodfed specimens caught, 9 were three-fourths or more engorged. Although 20 percent of the females examined were gravid, only a small number of eggs were found in most specimens. This may be due to autogenous development of eggs and may represent the frequency of occurrence in the natural population. Unfortunately, data on autogenous development of eggs in natural populations of Aedes albopictus in New Orleans is presently not available.

Table 2. Physiologic status of Aedes albopictus females caught in the duplex cone trap between May 15 and June 29, 1989.

| Physiologic condition | Number examined | Percentage positive |
|-----------------------|-----------------|---------------------|
| Inseminated | 305 | 60.3 |
| Bloodfed | 305 | 4.6 |
| Gravid | 305 | 20.0 |
| Parous | TBD* | TBD |

* To be determined.

B. ULV Spray Project

Since Ae. albopictus may become involved in the transmission of several important arboviruses causing human disease, it is critical to develop methods for the rapid suppression of adult mosquito populations. Recent evidence from field studies in New Orleans and Puerto Rico suggests that traditional methods of ground level ULV application of insecticides are not effective against another container breeding mosquito, Ae. aegypti. If this is the case with Ae. albopictus, other methods, such as aerial spraying, may be more successful. Information derived from this study will provide a basis for recommendations on the use of aerial application methods for controlling Ae. albopictus adults.

This summer, various experiments were conducted to determine the effects of aerial ultra low volume (ULV) spraying on populations of Aedes albopictus mosquitoes in New Orleans. Aerial ultra-low volume application treatments were evaluated individually. Malathion was evaluated in its ability to suppress adult populations of this mosquito species. Detailed pretreatment and posttreatment

surveys were conducted in two study sites using at least four different population assessment techniques. These sampling methods were used to evaluate levels of population reduction in relation to a particular control regimen and to assess the survival of certain mosquito sub-populations.

1. Study sites: Two study sites, Gentilly and Little Woods, were selected. Criteria for study site selection included: abundance of Ae. albopictus, accessibility for aerial treatments, and the presence of natural boundaries that might restrict the influx of this species from adjacent areas. The treatment area, Gentilly, is a suburban habitat encompassing a wide diversity of housing types and inhabitants of a broad range of socioeconomic levels. The Gentilly area is bordered by Paris Ave. and Press St. east and west sides. The north and south boundaries are Robert E. Lee Blvd. and Interstate-610. The untreated control site, the Little Woods section of New Orleans East, is located about 7 miles from the treatment area and is similar in habitat. This area is bordered on the east, west and south by Paris Road, Read Blvd., and Interstate-10, respectively. Southeasterly winds prevailed during the summer, so that the Little Woods control site was located upwind from the aerial application site and therefore did not receive accidental treatment through drift of aerosol droplets.

Each study site is square in shape and covers approximately 320 acres. A sampling grid was superimposed over the area, yielding 64 5-acre square-shaped plots. Each 5 acre plot is 460 feet on a side. In the center of this area, 16 plots (80 acres, 32 ha) in a 4x4 arrangement were utilized for detailed mosquito population and container surveys. The central sampling area was surrounded by a 2 plot-wide (920 ft.) peripheral zone which served as a buffer area. In this peripheral zone, eight plots were selected systematically for survey. These peripheral plots were located in each of the 4 corner and 4 midpoint plots situated along the outside edge of the buffer zone. Surveys in these perimeter plots were used to monitor mosquito immigration from adjacent areas that are outside of the treatment zone. Because the overall area of investigation within each study site was large in relation to the number of points that can be adequately sampled and because the distribution of Ae. albopictus was likely to be uneven, 4 of the 16 plots in the central sampling area were selected for an extensive survey. These 4 plots were arranged along a diagonal transect line extending from opposite corners of the 80 acre area. In this report, we will refer to these plots as the transect plots. In both treated and untreated sites the population densities of Ae. albopictus and Ae. aegypti were monitored 14 days before, during, and for at least 14 days after each malathion application.

2. Mosquito population survey methods: Four types of mosquito population survey methods were implemented: (a) ovitraps, (b) adult CO₂-enhanced landing rate collections, (c) adult resting collections, and (d) larval-pupal productivity surveys. The location and timing of mosquito surveys within each study site varied depending on the method employed.

a. Ovitraps: One ovitrap consisted of a single 16 oz black plastic cup supported by a plywood stand. A single strand of heavy gauge wire covered the cup opening to prevent consumption of the water by animals. The rough

surface of red velour paper strips (1" x 4.5") was used as a substrate for oviposition. At each study site 40 ovitraps were employed. Each of the 16 plots in the central sampling grid contained at least one ovitrap. 5 ovitraps were placed in each of the 4 transect plots. In addition, an ovitrap was placed in each of the 8 plots in the buffer zone. If only one ovitrap was placed in a plot, it was situated at the premise closest to the center of the study plot. In transect plots, ovitraps were arranged in a square pattern radiating around a central ovitrap. Oviposition strips were exchanged daily between May 1st and November 10th. After returning to the laboratory, eggs on ovistraps were conditioned by holding strips at high humidity for 7 days before hatching. Ovistraps to the laboratory, eggs were counted and classified as either Ae. triseriatus or those belonging to the subgenus Stegomyia. Since the morphology of Stegomyia eggs does not permit easy determination of species identity, ova were hatched after the completion of embryonic development. Larvae were allowed to develop to the 4th instar before specimens were killed in hot water and preserved in 70 percent ethanol.

b. Adult CO₂-enhanced landing rate collections. Daily landing rate collections were conducted at two stations located within each transect plot and at one station within each peripheral buffer zone plot. Stations were located in areas that appeared suitable for Ae. albopictus activity. Usually this was a small open area surrounded by vegetation. To avoid interfering with ovitrap operations, each landing rate station was at least 100 yards from any ovitrap located in the same plot. Two people were used for these collections; one served as bait, the other collected mosquitoes attracted to the bait. Before the beginning of a collection, field personnel dispersed CO₂ by blowing a current of air over dry ice for 1 minute. Mosquitoes were caught as they attempted to land on either the bait or the collector. The collector operated a battery powered aspirator for the collections. The collections lasted 5 minutes at each station and were conducted between 0800 and 1100 hours. Aliquots of specimens caught in the landing rate collections were dissected in the laboratory to determine proportions that are inseminated, previously blood fed, and parous. Also, wing lengths of both males and females were measured in an effort to correlate body size with survival after insecticide exposure.

c. Adult resting collections. Using a backpack aspirator, resting collections of adult mosquitoes were conducted at daily intervals. These collections were performed at 2 selected stations in each of the 4 transect sampling plots and from 1 selected station in each of the peripheral buffer zone plots. Containers and vegetation at each station were swept with the backpack aspirator for 15 minutes. Operators wore repellents to avoid sampling excessive numbers of host seeking adults. All collections were made at least 100 yards from ovitraps and CO₂ landing rate stations located in the same plot. Resting collections, too, were made between 0800 and 1100 hours. Each female specimen of Ae. albopictus and Ae. aegypti captured in the resting collections was examined to determine wing length, insemination, blood engorgement, and ovarian status.

d. Larval-pupal population surveys. Sentinel tires were selected to monitor the efficacy of these containers as sites for the development of aquatic stages. Two clean tires were placed in each of the 4 transect plots and one each in the 8 peripheral buffer zone plots. Tires were dispersed in a suitable

habitat within each plot and positioned at a 45° angle. The contents of each tire were examined twice a week, at 3 to 4 day intervals, for the presence of larvae and pupae. The number of larvae present as well as their instars were recorded for every survey tire. Pupae were removed, placed into emergence cages, and returned to the laboratory. The species, sex, and wing length of the emerging adults was assessed for each pupal specimen collected. In addition, the ovaries in Ae. albopictus females emerging from field collected pupae were dissected in effort to determine the proportion and extent of autogenous egg development in insecticide-stressed and unstressed populations. Water in survey tires was permitted to fluctuate naturally with the environmental conditions.

3. Container Survey: In preparation for the spray project, we conducted a container survey in the treatment and control areas. Every premise in each transect plot of the 16 plot central sampling area in each study site was surveyed for containers and the presence of container-breeding mosquitoes. This survey was conducted before the start of chemical treatments. We had intended to repeat the survey at the end of the spray season, but lack of manpower did not allow for this. In this investigation, a container was defined as any receptacle capable of holding water for several days. Natural containers, such as tree holes and other phytotelmata, were included in this survey. Some artificial containers, such as roof gutters, for example, were too difficult to survey and were excluded from the study. The following information was obtained from each premise: name of inhabitant, address, location code, date, time of day, number of buildings, type of premise, presence of vegetation, presence of animals, number of containers, types, sizes, colors, maximum water holding capacity, and breeding condition of each container. Information obtained from these surveys was entered into a data base for summarization and analysis. House, container, and Breteau indices were calculated and used for evaluation and comparison of treated and untreated areas.

4. Colonization: Field specimens of Ae. albopictus and Ae. aegypti mosquitoes, if they were present in the survey area, were collected for colonization. Colony founders consisted of approximately 100 females of each species that was obtained in separate collections from the Aurora Gardens and the Woodland Estates study sites. Captured specimens were blood fed in the laboratory and their eggs collected. Subsequent generations of progeny were used for insecticide susceptibility tests and as bioassay specimens during field trials. Since experimental studies need to be conducted with colonized material that is relevant to field populations, new colonies from treated and untreated sites were started in August, 1988, and May, 1989. All colonies were maintained during the project period for comparative studies.

5. Insecticide susceptibility tests: A variety of insecticides were evaluated in the laboratory to determine their efficacy as adulticides and larvicides. Adult Ae. albopictus and Ae. aegypti mosquitoes of both sexes were used in two types of susceptibility tests. Procedures for these tests were developed in accordance with the protocol established by the World Health

Organization. Insecticides that were tested included malathion, naled, and resmethrin. Tests were conducted with filial generations of the colonized material that are as close as possible to the field generations. Tests were performed with both 1988 and 1989 colonies. Both sexes were employed in these tests. Males were exposed to insecticides at 1, 5 and 10 days post-emergence. Four to 8 day-old females were tested in 4 different categories of ovarian state: nulliparous, bloodfed, gravid, and parous (uniparous). Mortality data obtained from topical and aerosol tests was plotted as a dosage mortality curve. The data was subjected to a probit analysis for the calculation of slopes, LD₅₀ and LD₉₀ values, and their corresponding fiducial limits.

For each aerosol susceptibility test, serial dilutions of insecticides were made in reagent grade acetone. The tests were performed using a wind tunnel consisting of a tube (16.3 cm dia.) through which ambient laboratory air will be blown at 1.8 M/sec.⁶ A volume of 0.5 ml of each concentration is atomized at a pressure of 30.3 kPa, 70 cm upwind from the exposure point. Mosquitoes were placed in disposable paper cages (5.4 cm x 8.6 cm, dia.) covered with a fine mesh (no. 16) screen. A test group consisted of 25 adult mosquitoes, 2-4 days old. Two cages of mosquitoes were tested per dilution, using 5-8 serial dilutions per test. In addition, 2 control cages were tested in which mosquitoes were exposed to acetone only. Each experimental test consisted of a minimum of 2 replicates. After exposure, mosquitoes were aspirated into clean holding cages and supplied water and a carbohydrate source. Mortality was determined at 24 hours after treatment. If the mortality rate among the controls exceeds 5%, then the test results were considered invalid. A probit analysis of the data was conducted according to the method described by Finney (Cambridge University Press: Probit analysis, 3rd edition, 1971).

Topical applications were based on a modification of the technique described by Khoo and Sutherland (Mosquito News, 41:802-804). The insecticides were applied with an ultra precision 0.25 ml micrometer driven syringe capable of dispensing 0.1 ul. For each experiment, 5-8 serial dilutions (expressed in ng of active ingredient) were applied, plus an acetone control. Each test included at least 2 replicates. Prior to exposure, 10 2-4-day old mosquitoes per concentration were anesthetized by chilling on ice. Individual mosquitoes were picked up by the legs, using microdissection forceps and given 0.5 ul of the insecticide-acetone solution. The insecticide was applied to the pleural area of the thorax. After exposure, treated and untreated mosquitoes were transferred to separate clean cages and provided with nutrient solutions. Mortality was determined after 24 hours.

The larval susceptibility test was based on a modification of the WHO technique. After dissolving a measured volume of insecticide (technical grade) in acetone (reagent grade), serial dilutions were prepared in either deionized water or acetone, depending on the solubility of the chemical under investigation. To 239 ml of deionized water in a 400 ml beaker, 1.0 ml of the final dilution was added. Twenty 4th instar larvae of either Ae. albopictus or Ae. aegypti were then added to each test beaker. Control beakers contained the same number of larvae; however, only 1.0 ml of either deionized water or acetone were added. Larval mortality was determined after 24 hours. If mortality in the untreated control was greater than 5%, or if more than 10% of

the larvae had pupated, the results were discarded and the test repeated.

The procedure for the larvicide tests involved exposing 20 early fourth instar larvae to a dilution of the material being tested. After exposure, larvae were held for 24 hours (27° C) in plastic coated paper cups before mortality was determined. If the mortality rate of larvae in the untreated controls exceeded 20 percent, or if 10 percent or more of the larvae pupated, a test was then considered invalid. Data from three or more successful tests, representing at least 240 larvae per concentration (20 larvae per replicate x 4 replicates per experiment x 3 experiments per larvicide evaluation) were combined for a probit analysis. The dosage-mortality regression line for each larvicide was plotted on logarithmic-probability paper. From this plot, the LC₅₀ and LC₉₀ values were determined. A probit analysis computer program was also employed to verify values obtained from the plots.

The relative toxicity of malathion to strains of Ae. albopictus and Ae. aegypti is shown in Table 3. In most cases, Ae. aegypti were more susceptible to malathion than Ae. albopictus. Malathion toxicity to strains of Ae. albopictus from Gentilly and Little Woods was similar to that observed for the GENTILLY strain of Ae. aegypti.

Table 4 shows the results of tests to measure the dose response of Ae. albopictus and Ae. aegypti to varying concentrations of naled. Similar levels of toxicity were observed with all of the mosquito species and strains tested.

The toxicity of resmethrin to Ae. albopictus and Ae. aegypti larvae is shown in Table 5. As can be seen here, Ae. aegypti GENTILLY was more susceptible to resmethrin than either of the Ae. albopictus strains. Of the Ae. albopictus strains tested, the GENTILLY strain was slightly more susceptible than the one from LITTLE WOODS.

Table 3. Toxicity of malathion to strains of *Aedes albopictus* and *Aedes aegypti* larvae.

| Species | Strain | Generation | Lethal concentration in µg/ml A.I. | | | | Slope |
|-----------------------|-----------------|------------|------------------------------------|-----------------|-------|-----------------|-------|
| | | | LC50 | (95% F.L.) | LC90 | (95% F.L.) | |
| <u>Ae. albopictus</u> | GENTILLY | F5 | 0.105 | (0.101 - 0.109) | 0.249 | (0.235 - 0.264) | 3.4 |
| | LITTLE WOODS | F5 | 0.092 | (0.88 - 0.096) | 0.231 | (0.216 - 0.249) | 3.2 |
| | NEW ORLEANS-86* | F1 | 0.290 | (0.210 - 0.370) | 1.440 | (1.230 - 1.630) | N.A. |
| <u>Ae. aegypti</u> | GENTILLY | F5 | 0.090 | (0.073 - 0.105) | 0.197 | (0.165 - 0.255) | 3.8 |
| | NEW ORLEANS-86* | F1 | 0.160 | (0.110 - 0.023) | 0.330 | (0.279 - 0.353) | 4.4 |

*NOMCB data from experiments conducted in 1986.

Table 4. Toxicity of naled to strains of *Aedes albopictus* and *Aedes aegypti* larvae.

| Species | Strain | Generation | Lethal concentration in µg/ml A.I. | | | | Slope |
|-----------------------|-----------------|------------|------------------------------------|-----------------|-------|-----------------|-------|
| | | | LC50 | (95% F.L.) | LC90 | (95% F.L.) | |
| <i>Ae. albopictus</i> | GENTILLY | F5 | 0.115 | (0.108 - 0.122) | 0.177 | (0.162 - 0.200) | 6.9 |
| | LITTLE WOODS | F5 | 0.100 | (0.96 - 0.103) | 0.170 | (0.163 - 0.179) | 5.5 |
| | NEW ORLEANS-86* | F1 | N.A. | N.A. | N.A. | N.A. | N.A. |
| <i>Ae. aegypti</i> | GENTILLY | F5 | 0.130 | (0.127 - 0.133) | 0.200 | (0.191 - 0.255) | 6.9 |
| | NEW ORLEANS-86* | F1 | 0.115 | (0.111 - 0.120) | 0.246 | (0.234 - 0.252) | 3.9 |

*NOMCB data from experiments conducted in 1986.

Table 5. Toxicity of resmethrin to strains of *Aedes albopictus* and *Aedes aegypti* larvae.

| Species | Strain | Generation | Lethal concentration in µg/ml A.I. | | | | Slope |
|-----------------------|-----------------|------------|------------------------------------|---------------------|---------|---------------------|-------|
| | | | LC50 | (95% F.L.) | LC90 | (95% F.L.) | |
| <i>Ae. albopictus</i> | GENTILLY | F5 | 0.00267 | (0.00259 - 0.00276) | 0.00465 | (0.00411 - 0.00494) | 5.3 |
| | LITTLE WOODS | F5 | 0.00352 | (0.00271 - 0.00353) | 0.00634 | (0.00504 - 0.00989) | 4.0 |
| | NEW ORLEANS-86* | F1 | 0.01600 | (0.01300 - 0.01900) | 0.03700 | (0.02900 - 0.04300) | 4.1 |
| <i>Ae. aegypti</i> | GENTILLY | F5 | 0.00190 | (0.00181 - 0.00198) | 0.00368 | (0.00349 - 0.00391) | 4.5 |
| | NEW ORLEANS-86* | F1 | 0.01200 | (0.00900 - 0.01900) | 0.03500 | (0.03300 - 0.03900) | 4.0 |

*NOMCB data from experiments conducted in 1986.

6. ULV applications: Insecticide applications were conducted with a Britten-Norman Islander twin-engine aircraft equipped with 2 self-contained Micronair spray pod systems utilizing rotary atomizers. The aircraft was flown at a speed of 115 mph and at an altitude of 250 feet, resulting in a swath width of 500 feet. Treatments overlapped the study site by about 1,500 feet on all sides.

Aerial treatments began 30 minutes after official sunrise. At this time wind conditions were generally calm, humidity high, and temperature inversions are most frequent. Since the descent of droplets was impeded by rising warm air, there was less drift, as droplets were in suspension for a shorter period of time during temperature inversion conditions. Also, the high humidity during early morning hours was conducive to the survival of bioassay adults. Insecticide application over the study site was completed in less than 30 minutes.

The treatment schedule covered the entire season, June - October, 1989. Treatments were be delivered either as single applications in June and as paired treatments in July, August, and September. In the case of paired treatments, a second application was made 3 to 5 days after the first aerial treatment. Mosquito populations were monitored during the next 14 days. Afterwards, another single or paired treatments was initiated.

In the case of paired treatments, the timing of sequential applications was adjusted to account for new observations obtained from mosquito population surveys. For example, the length of the time interval between initial and follow-up treatments was shortened if we observed that a substantial proportion of bloodfed and gravid females survived the first application. The timing of the second treatment was also influenced by weather conditions.

Aerial spray equipment was calibrated before the first treatment and following every third treatment thereafter. Calibration was done for insecticide flow rate and droplet spectrum.

Swath width was determined at a location separate from the study site. This test was conducted on the crown of a levee located in eastern New Orleans. This test site is situated between an open marsh and Lake Pontchartrain. Employed in this test were oil sensitive dye cards which consisted of 2"x 3" strips coated with a white oil soluble film that produce a black dot where the film is hit by a droplet. Cards were placed horizontally along a straight line at 25 foot intervals over a 1,000 foot distance that is perpendicular to the prevailing wind direction. With rotary atomizer sprayer in operation, the aircraft passed the card line at a right angle, at an altitude of 250 feet above ground level. The cards were left undisturbed for 15 minutes before they were retrieved for examination. A density of 10 droplets per 6.5 cm² was considered the minimum outer limits of the swath width. Swath width determinations were conducted before aerial treatments commenced. Previous swath width determinations using the Britten-Norman Islander with malathion, naled, and resmethrin/piperonyl butoxide indicated an effective swath with of 500 feet.

Four monitoring stations will be setup in the transect plots. Each monitoring station was located near the center of each transect plot. Two stations were in open areas and two others in sequestered locations. Each station consisted of a spinner with teflon coated slides, vertically and horizontally arranged oil sensitive droplet cards, cups containing sentinel larvae, cups containing sentinel pupae, and 4 bioassay cages containing sentinel adults. During each spray mission, droplets were collected from the treatment sites. We calculated the density and size (length median diameters) of droplets according to the method described by Carroll and Bourg (Mosquito News, 30:645-655).

Two monitoring stations were setup in the untreated site. These were located in each of the two central transect plots. Each station had the same insecticide application monitoring and bioassay components as the treatment sites. The same number of sentinel larvae, pupae, and adults per station in the treated zone were used in the untreated area.

7. Bioassay of target and non-target species: In the open and sequestered stations within each treated and untreated site, bioassay of insecticidal action were performed with larvae, pupae, and adults of both Ae. albopictus and Ae. aegypti. Mosquito strains employed in each area were derived from that particular region. Larvae and pupae were included in the toxicity bioassay to measure the acute effect of these chemicals on the immature stages. For this bioassay, twenty laboratory reared larvae or pupae of each species were placed in pint-sized paper cups. After exposure in the field, cups containing larvae were returned to the laboratory for observation over the next 24 hours. Two similar groups of larvae were placed in each untreated zone for the purpose of comparison. The percentage mortality was determined for all groups.

Adult mosquitoes for bioassay were placed into pillow cages constructed from fine mesh screen wire. Each cage contained 10 males and 10 females between 2 to 4 days old. At every monitoring station in the treatment and control areas, there were two cages of each species. Mortality was determined at 1, 4, and 24 hours posttreatment.

Bioassay were also performed with the predaceous larval mosquito Toxorhynchites amboinensis. This species had been utilized as a biocontrol agent in a New Orleans pilot study. Toxorhynchites rutilus septentrionalis is the only naturally occurring Toxorhynchites species in Louisiana. It is frequently found in tree holes and tire casings in the sections of New Orleans where Aedes albopictus are abundant. This species is not adaptable to laboratory colonization. However, since the size and morphology of the two species are similar, it was hypothesized that Tx. amboinensis could be suitable in determining the toxicological effects of the aerial treatments.

Larvivorous fish were used in bioassay of nontarget organisms. One bioassay cup with adult larvivorous fish were placed at each open and sequestered sampling station in both the treated and untreated study sites. Each cup contained 10 unsexed adult Gambusia affinis, pot bellied minnows, which were subsequently monitored for mortality at 1, 4, and 24 hours posttreatment.

Predatory copepods were also employed in bioassay of nontarget organisms. There are four species of larvivorous copepods in the New Orleans area with significant potential for biological control of Aedes larvae: Acanthocyclops vernalis, Macrocyclus albidus, Mesocyclops sp., and Diacyclops navus. Each of these species virtually eliminates all Aedes larvae when it is present in a breeding container. Once introduced to a container, these copepods often persist for years. The location and abundance of these four species was assessed in each of the container survey sampling areas at the time the areas were surveyed for mosquito larvae. The survey included container habitats, ponds, swales, and other water where copepods might eliminate mosquito larvae. These four species of copepods were monitored throughout the project in the sentinel tires examined in the larval-pupal population surveys. In addition, copepods for bioassay during aerial treatments were bred from locally-derived laboratory colonies of the above listed species. The bioassay was implemented by placing twenty individuals of each species in pint-sized paper cups at each site where a bioassay of Aedes larvae and pupae is

conducted. Copepod mortality and reproduction was measured 24 hours and one week after each ULV application.

In each treatment zone, daily mean, maximum, and minimum air temperatures and relative humidity were measured throughout the season with an Omnidata hygrothermograph recorder. During the insecticide application procedure, air temperature, wind direction, and velocity at ground level, 50 feet, and 250 feet were recorded. Daily precipitation information was also obtained for each study site.

Data from samples to determine mosquito population densities, container survey results, and insecticide application monitoring observations were recorded on prepared data forms taken to study sites by field workers. Upon return to the laboratory, data sheets were reviewed daily by the field supervisor responsible for that section as well as by the data entry operator. Incorrect or inconsistent data was either corrected or deleted. Laboratory data was reviewed in similar fashion. The data were entered into individual databases using dBASE IV (Ashton-Tate Corporation) formatted entry screens. The accuracy of data entries was verified by the field supervisor. For statistical analysis the data files were converted to SAS data sets (SAS Institute).

Evaluation of effectiveness of aerial ULV malathion treatments:

Table 6 summarizes basic information about each treatment. Eight treatments were applied between June 15th and September 14th. The Micronair rotary atomizer spray system, used in the first 2 treatments, before it was replaced with a pressurized TeeJet system. The change in spray systems made it necessary to increase air speed to 150 mph. Weather conditions at the time of each spraying are shown in Table 7.

Table 6. Treatment data for aerial ULV applications of malathion to the Gentilly study site in 1989.

| Treatment no. | Date | Spray system | Treatment altitude (ft) | Air speed (mph) | Treatment rate (oz/a) | Flow rate (1/min) |
|---------------|------|--------------|-------------------------|-----------------|-----------------------|-------------------|
| 1 | 6-15 | Micronair | 120 | 115 | 1.8 | NA* |
| 2 | 6-29 | Micronair | 120 | 115 | 3.0 | 7.6 |
| 3 | 7-25 | TeeJet | 120 | 150 | 3.0 | 9.9 |
| 4 | 7-29 | TeeJet | 200 | 150 | 3.0 | 9.9 |
| 5 | 8-15 | TeeJet | 200 | 150 | 3.0 | 9.9 |
| 6 | 8-18 | TeeJet | 200 | 150 | 3.0 | 9.9 |
| 7 | 9-12 | TeeJet | 200 | 150 | 3.0 | 9.9 |
| 8 | 9-14 | TeeJet | 200 | 150 | 3.0 | 9.9 |

* Not available

Table 7. Meteorologic conditions for the 1989 aerial ULV spray treatments.

| Treatment no. | Sunrise | Application starting | Wind velocity | Air temperature (F°) | | Relative humidity (%) | Dew point (° F) |
|---------------|---------|----------------------|---------------|----------------------|-------|-------------------------|-------------------|
| | | | Surface 90° | Surface | 1000' | | |
| 1 | 7:15 | <4.0 | NA* | 76.1 | 68.0 | 90 | 72.0 |
| 2 | 6:10 | 3.0 | NA | 80.6 | 74.0 | 70 | 76.0 |
| 3 | 6:44 | 2.5 | NA | 77.0 | 72.0 | 95 | 72.0 |
| 4 | 6:35 | 0.0 | 3.0 | 76.1 | 77.0 | 87 | 75.0 |
| 5 | 6:38 | 3.8 | 4.0 | 75.0 | 75.0 | 80 | 70.0 |
| 6 | 6:47 | 0.0 | 0.0 | 75.2 | 75.0 | 86 | 71.0 |
| 7 | 7:04 | 3.0 | 5.0 | 77.0 | 75.0 | 91 | 70.0 |
| 8 | 6:56 | 0.0 | NA | 73.0 | 74.0 | 92 | 70.0 |

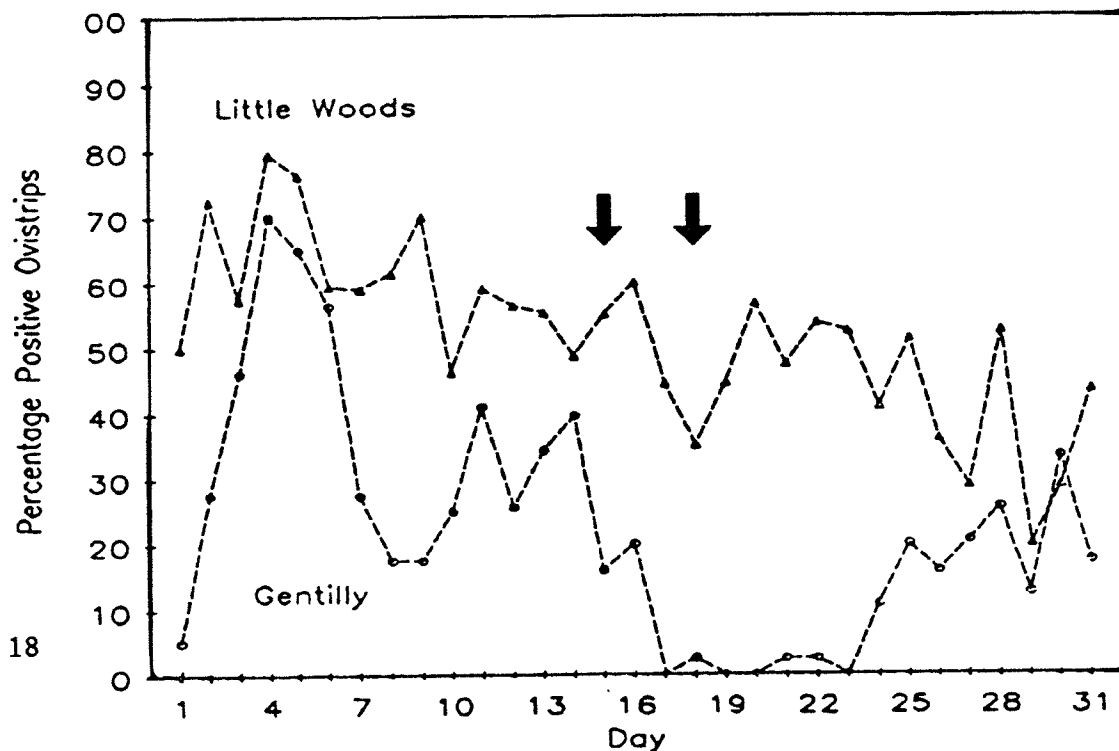
* Not available

Figure 6 shows the ovitrap results for August. Population density was expressed as a percentage of ovitraps that contained Stegomyia eggs. Preliminary egg hatching data indicates that the majority of Stegomyia eggs collected from the Gentilly treatment area are those of Ae. albopictus. Nearly all Stegomyia eggs from the Little Woods control area were identified as those of Ae. albopictus. Arrows in each figure represent the days when malathion treatments were applied to the Gentilly area.

The ovitrap results indicate that paired treatments of malathion succeeded in suppressing the population of Stegomyia mosquitoes for 7 or more days. Similar results were obtained for August and September; however, the general decline in the number of eggs collected in September was also influenced by dry weather conditions.

Figure 6.

Stegomyia Eggs Collected from the Treatment and Control Sites in August 1989



The density of droplets was measured with indicator cards placed in several locations in the Gentilly treatment area. Figures 7 and 8 are examples of the typical distribution of droplet densities among the monitoring stations located in the treatment area. Each number refers to the transect station where droplets were collected.

Figure 7.

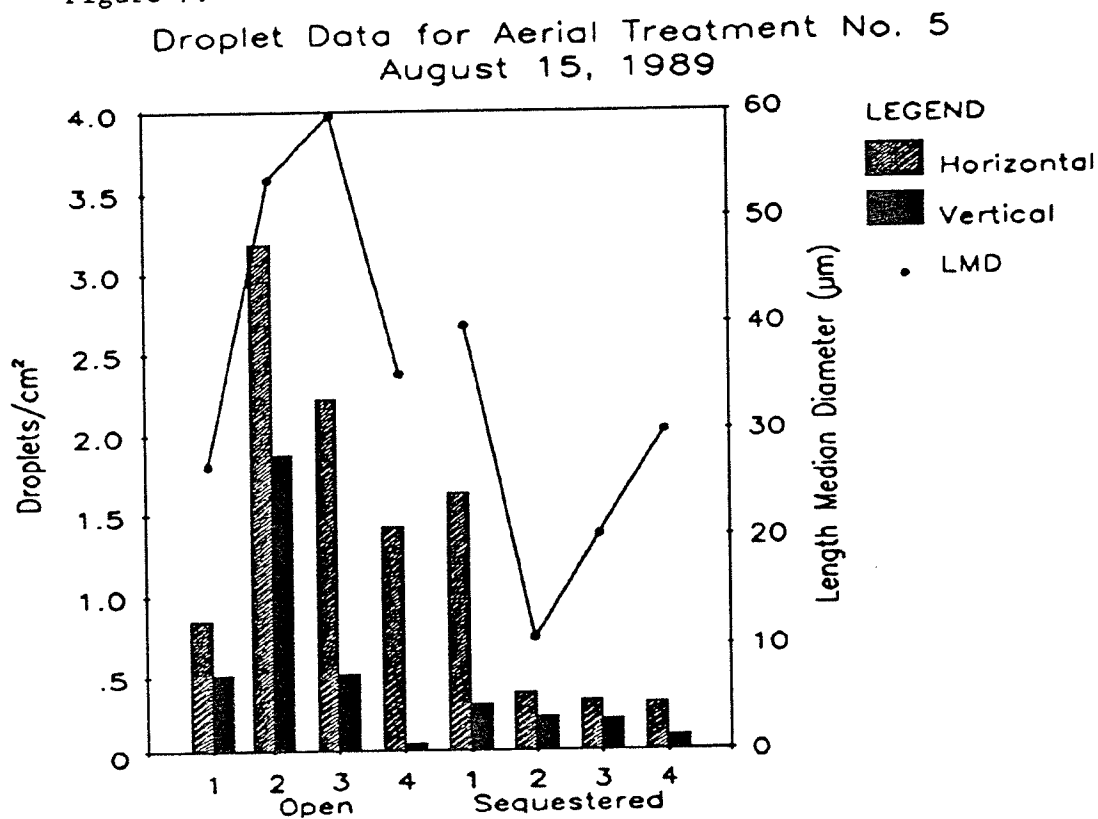
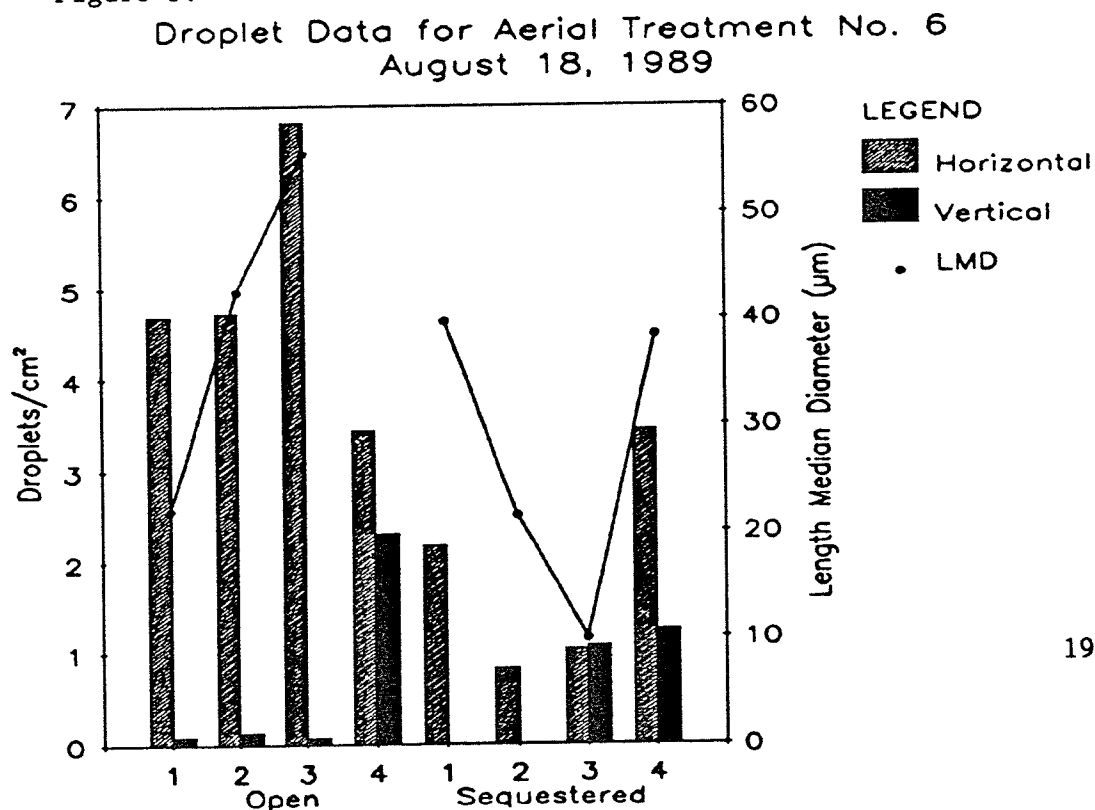


Figure 8.



The results of typical pre- and posttreatment human-baited landing rate collections are shown in Figures 9 and 10. Treatments applied on June 15 and June 29 were intended experimentally as single doses. The remaining treatments on July 25 and 27, August 15 and 18, and September 12 and 14 were designed to be delivered in pairs. In general, the results showed that the host-seeking Aedes albopictus population was suppressed to low levels by single and paired treatments. However, the duration of population suppression was greatest when treatments were applied in pairs. Single doses resulted in an average 5 day reduction in population. Paired treatments continued population suppression for an additional 2-3 days. The host-seeking Aedes aegypti population was generally suppressed for 6-7 days regardless of whether treatments were applied singly or in pairs.

Figure 9.

Aedes aegypti Females Caught in Human-baited Landing Rate Collections, August 1 - 31, 1989

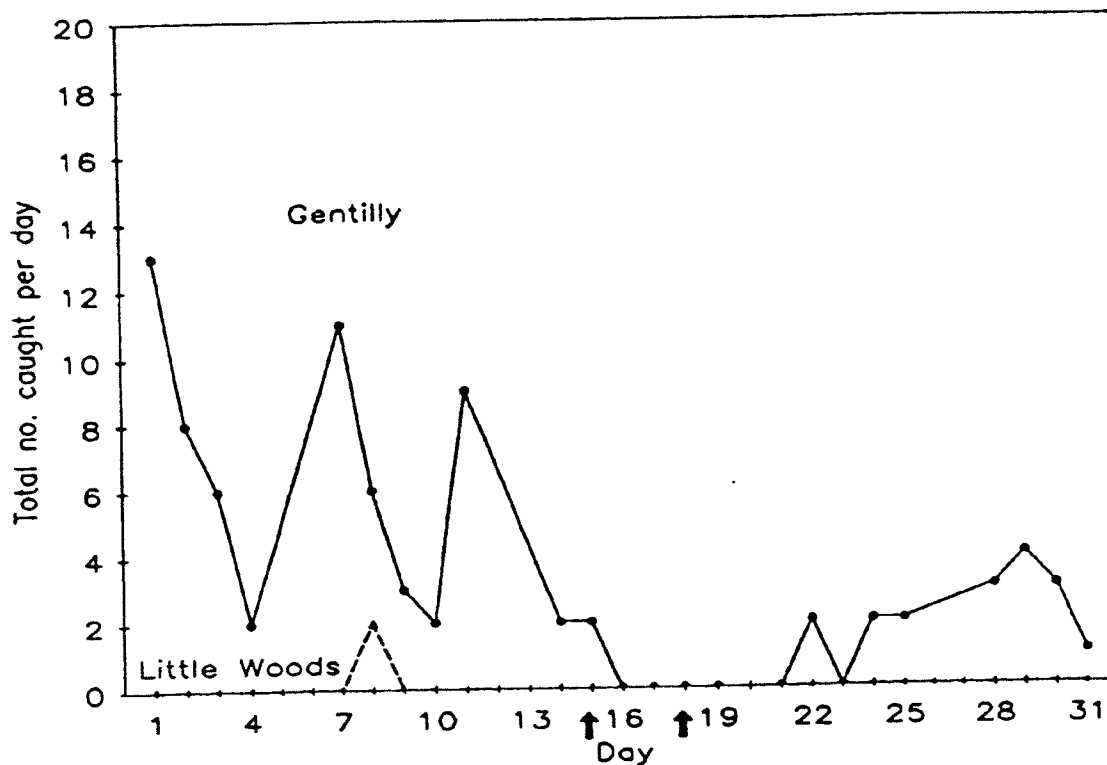
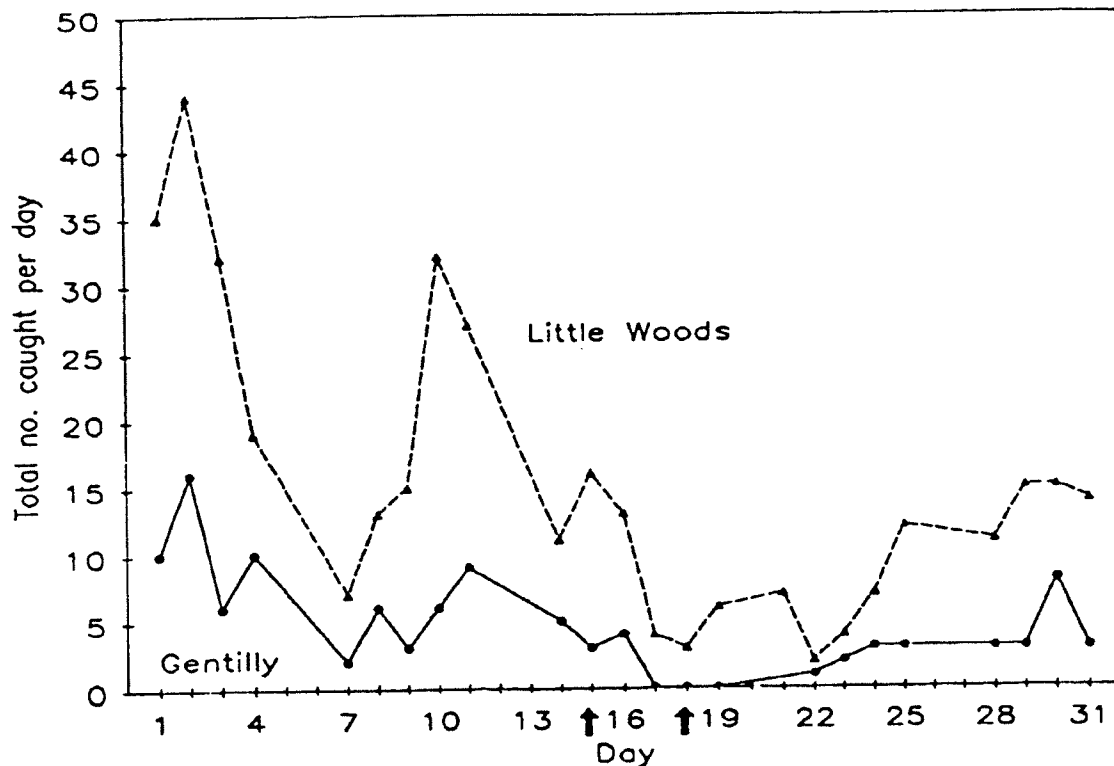


Figure 10.

Aedes albopictus Females Caught in Human-baited
Landing Rate Collections, August 1 - 31, 1989



C. Survivorship of *Aedes albopictus* under Natural Conditions:

As a part of life table studies on *Aedes albopictus*, experiments were performed to determine the survivorship of eggs. Because it is difficult to observe eggs under natural conditions, experiments were designed to measure changes in egg population density after exposure to one or more environmental conditions. Tires were selected as site of exposure, because they serve as important locations for larval development.

Experiments in this study attempt to answer the question of what happens to *Ae. albopictus* eggs after oviposition. Two types of events leading to the loss of eggs were considered: (1) intrinsic and (2) extrinsic events. An intrinsic egg loss event is caused by factors or conditions established by the parental female. Examples of intrinsic events would be unembryonated eggs that collapse or eggs that fall off because they are not properly attached to a substrate. Extrinsic egg loss events are due to external environmental factors such as predation, desiccation, or freezing. Through the use of appropriate controls, egg disappearance can be attributed indirectly to either type of event.

Eggs for these experiments were obtained from field sources. Field collected eggs were obtained from ovitraps located at the Grant Street site. Ovisrips were retrieved 7 days after placement. Eggs were collected on 1" x 5" fiberboard strips and stored in the insectary, under high humidity, between 5 and 7 days. Ovisrips were handled carefully to prevent accidental egg loss.

Ae. albopictus eggs were distinguished from those of Aedes triseriatus on the basis of luster. Aedes aegypti eggs were unlikely to be found on the ovistrips, as this species has not been found at the study site for at least 2 years.

The condition of each egg was recorded during the counting operation. Conditions scored were: intact, hatched, collapsed, and damaged. After counting, a boundary mark encircling the location of eggs on each ovistrip was drawn with a marking pen. Ovistrips were transferred to the Grant Street study site where they were attached to the inner sidewall of discarded tire casings. Eggs on ovistrips were returned to the laboratory for further analysis after 24 hours of exposure.

Two groups of tires were employed in these experiments. One set of 30 tires, referred to as the "dry tires", contained drain holes in the bottom to prevent the accumulation of water. Another group of "wet tires" was kept at maximum capacity by the daily addition of water to each tire. Each tire was inclined at a 60° angle against a wooden stake. Within a group, the daily pairing of ovistrips with tire casings was selected on a random basis. When ovistrips were placed in wet tires, they were attached either to a dry location along the inner side wall, or a portion of the ovistrip was immersed in the tire's water so that the sample population of eggs remained dry.

Ovistrips used as environmental controls were held in the insectary in a tightly sealed box. High humidity within the box was provided by a beaker containing 300 ml of a saturated solution of potassium sulfate. An ovistrip holding device held each strip vertically, reducing the chance of accidental egg loss through contact. Control ovistrips accompanied experimental ones on the trip to the field site, so that egg loss during transit would be reflected in the control egg counts.

Survivorship of field collected Ae. albopictus eggs placed in dry tires is shown in Fig. 11. Approximately 70 percent of the 376 intact eggs under investigation were lost during the first 24 hours. In contrast, only 5 percent of the control eggs were lost in the same time period. After the initial sharp loss of eggs from the experimental ovistrips, the rate of loss decreased greatly. Fig. 12, shows that when field collected eggs were placed in wet tires, the rate of egg loss was similar to that observed among ovistrips placed in dry tires. Partial immersion of an egg strip did not alter the rate of egg loss. After an initial 30 percent loss of eggs among the control ovistrips, a small decline was observed for 2 days followed by a rapid decline in the number of intact eggs. This decrease between days 3-7 was due in part to a nearly 3-fold increase in the number of collapsed eggs. Comparable proportions of collapsed eggs were not observed among the experimental group held under any of the conditions tested.

Figure 11.

SURVIVORSHIP OF *AEDES ALBOPICTUS* EGGS
WHEN EGGS DERIVED FROM FIELD SOURCES

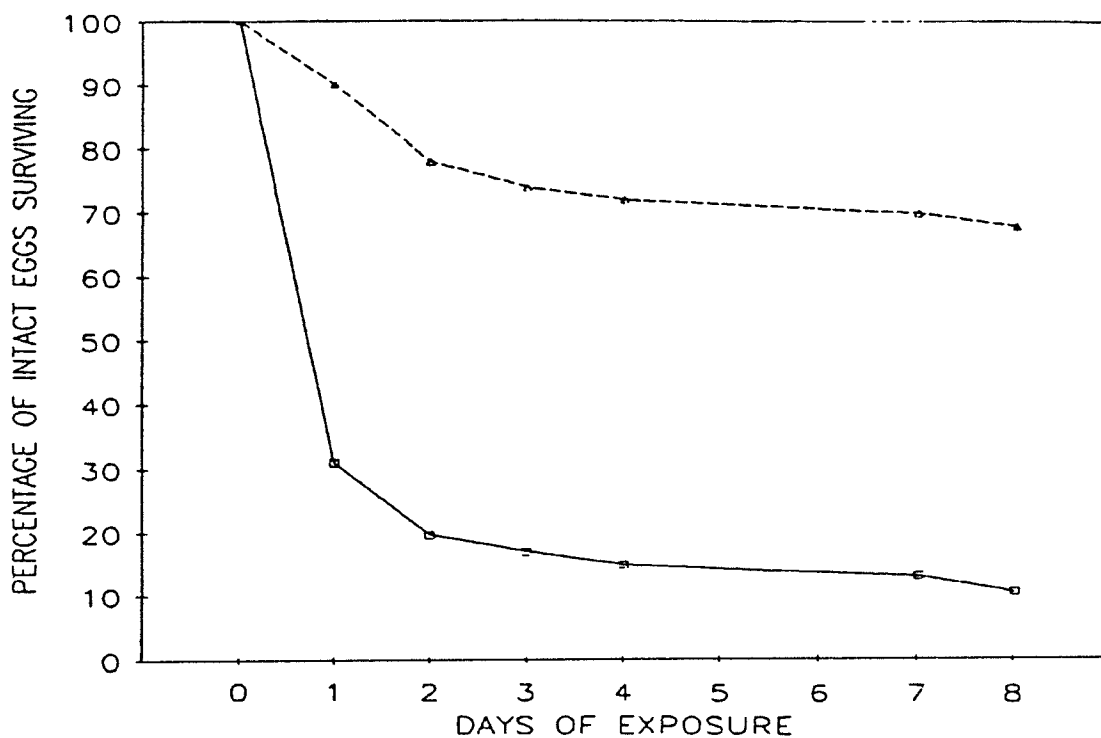
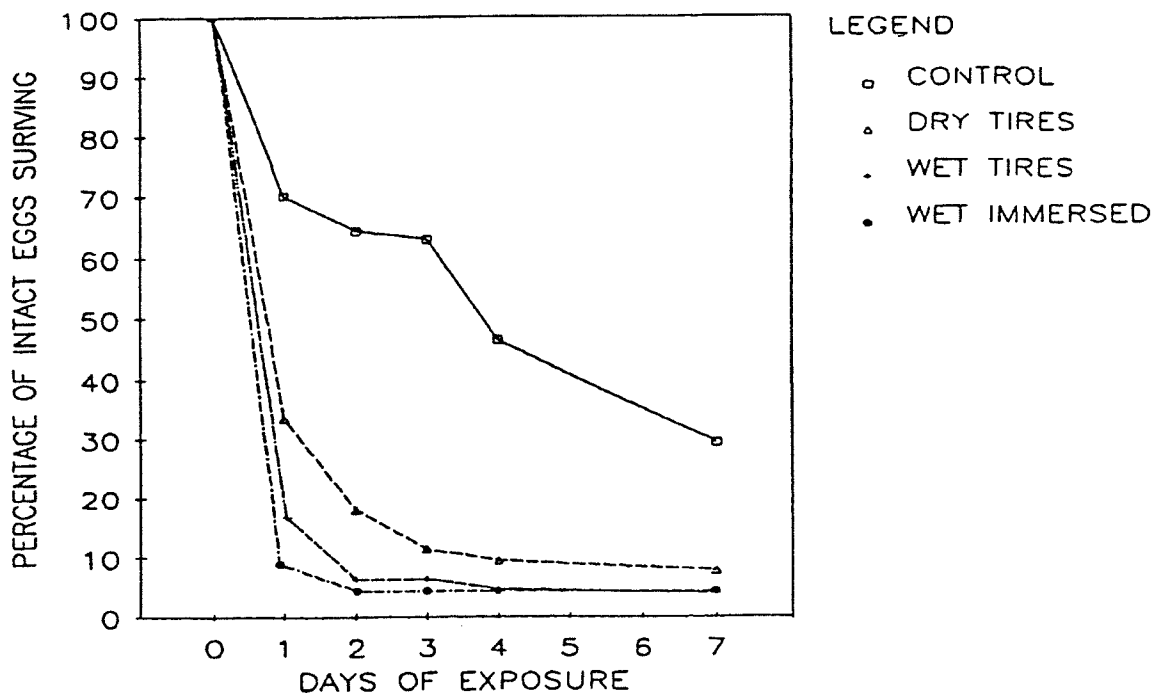


Figure 12.

SURVIVORSHIP OF *AEDES ALBOPICTUS* EGGS
WHEN FIELD COLLECTED EGGS ARE PLACED
UNDER DIFFERENT FIELD CONDITIONS



The relative distribution of egg conditions observed in the last experiment is shown in Figs. 13-16. The results indicate that intact eggs, regardless of placement in dry or wet tires, disappear initially at a rapid rate. However, after the initial egg loss, the rate shifts to a more gradual decline. It should be noted that the initial loss of eggs occurred when the mean number of intact eggs per ovistrip was approximately 50. However, when the mean number of intact eggs dropped to about 5 per ovistrip, the rate of egg loss was negligible. Although the precise nature of egg loss in these experiments is not known, it is presumed to be caused mainly by predation. It is also believed that a considerable portion of this predation is by arthropods such as arachnids, psocids, orthoperans, isopods and others. The data obtained thus far indicate that egg loss is dependent on density or patch size. A large patch size results in intense predation; however, as the patch size decreases, so does the rate of predation. When the patch size reaches a level of about 5 eggs per strip, little predation occurs. At this point, the predator may not readily detect eggs or the eggs are simply ignored because there are too few. Since *Ae. albopictus* females generally oviposit small numbers of eggs at a time, this reproductive behavior is likely to be a successful antipredation strategy.

The results of this study indicate that many of the eggs laid by *Ae. albopictus* females are lost to extrinsic events, most probably predation. However, species survival appears to be based on relatively small numbers of eggs that avoid predation by being widely spaced. Additional studies are needed to determine if deleterious factors can be brought to bear on the egg population that survives predation.

Figure 13.

CONDITION OF *Aedes albopictus* FIELD COLLECTED EGGS WHEN HELD UNDER CONTROL CONDITIONS

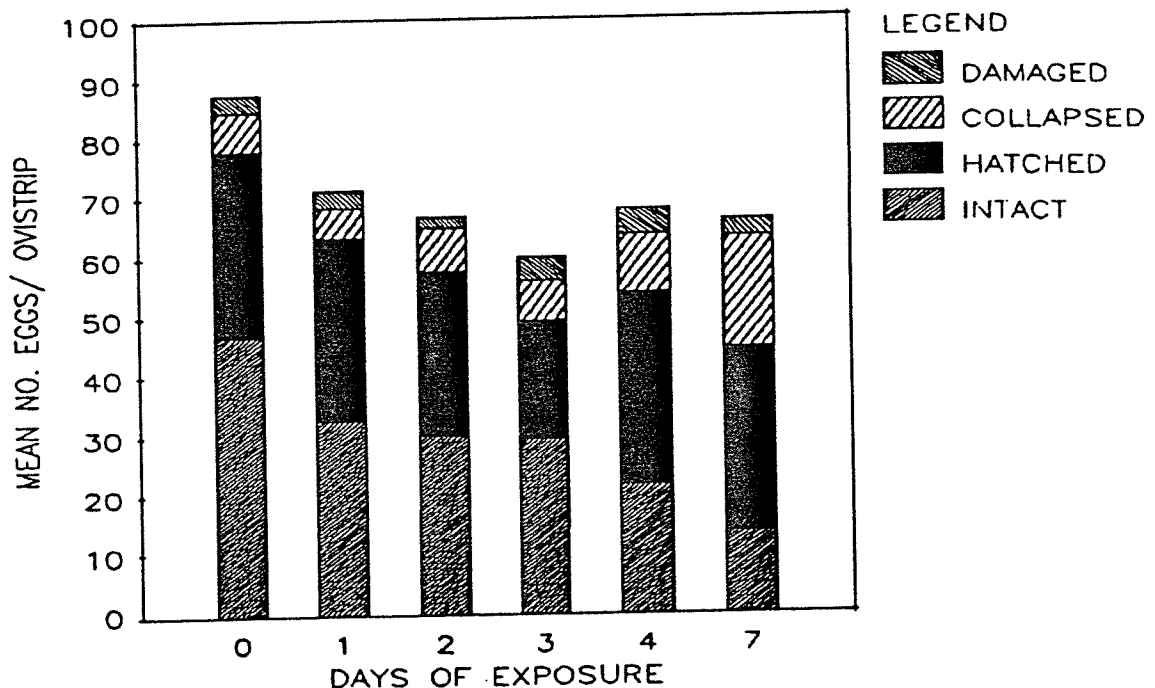


Figure 14.

CONDITION OF *Aedes albopictus* FIELD COLLECTED EGGS WHEN HELD IN DRY TIRES

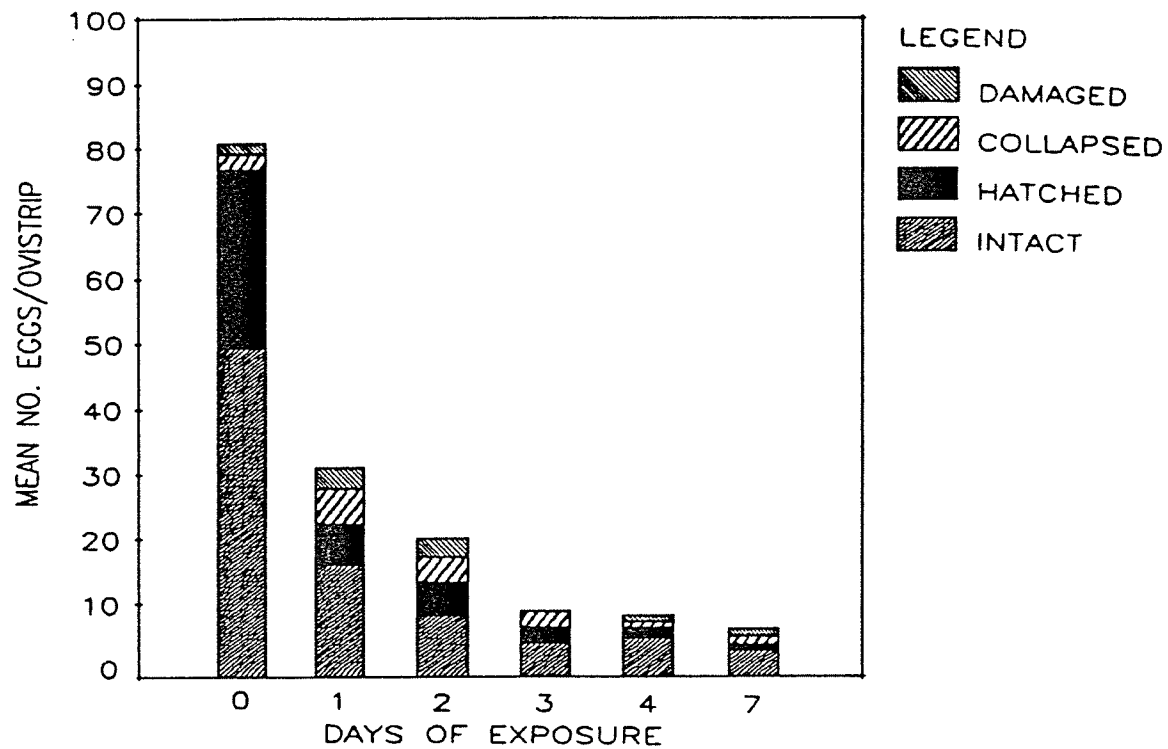
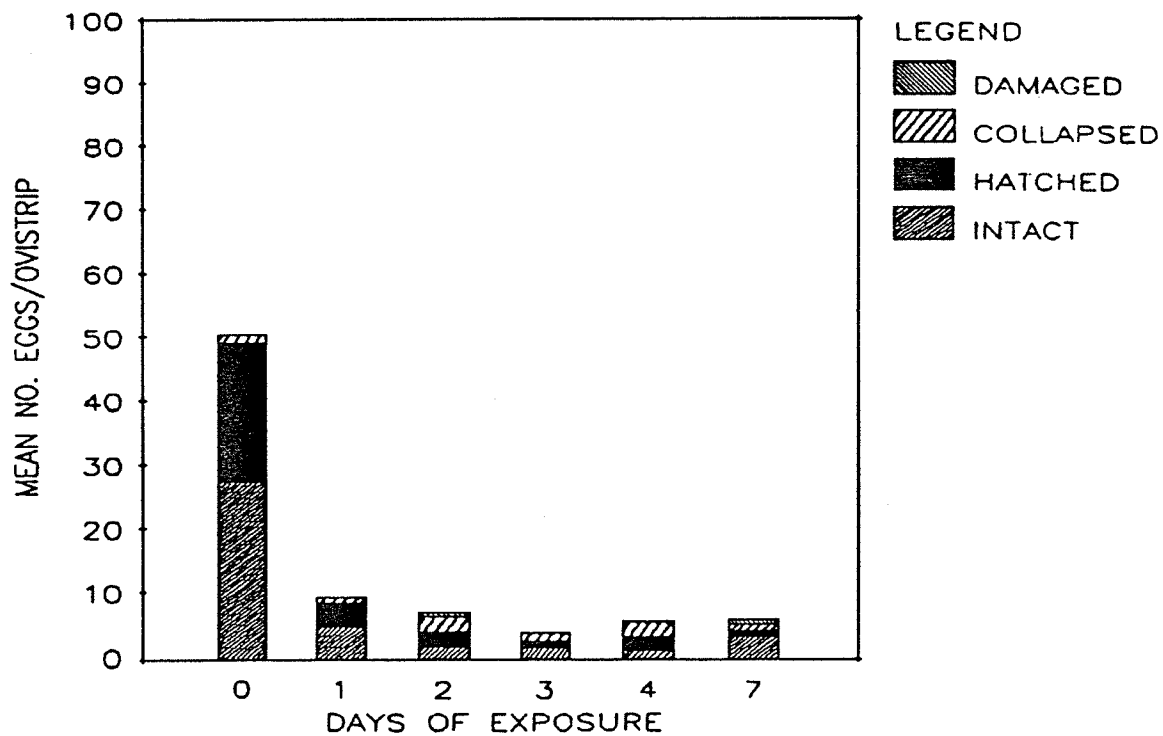
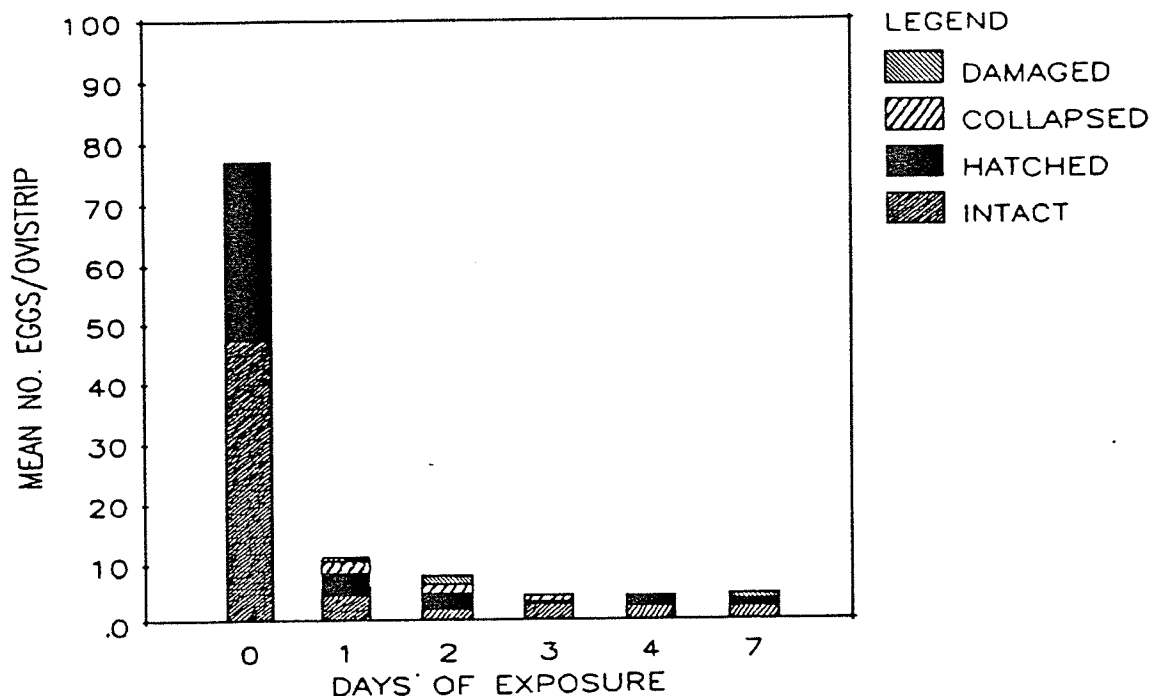


Figure 15.

CONDITION OF *Aedes albopictus* FIELD COLLECTED EGGS WHEN HELD IN WET TIRES



CONDITION OF *Aedes albopictus* FIELD COLLECTED EGGS WHEN OVISTRIPS ARE IMMERSED IN WET TIRES



D. Density fluctuations in *Aedes albopictus* populations:

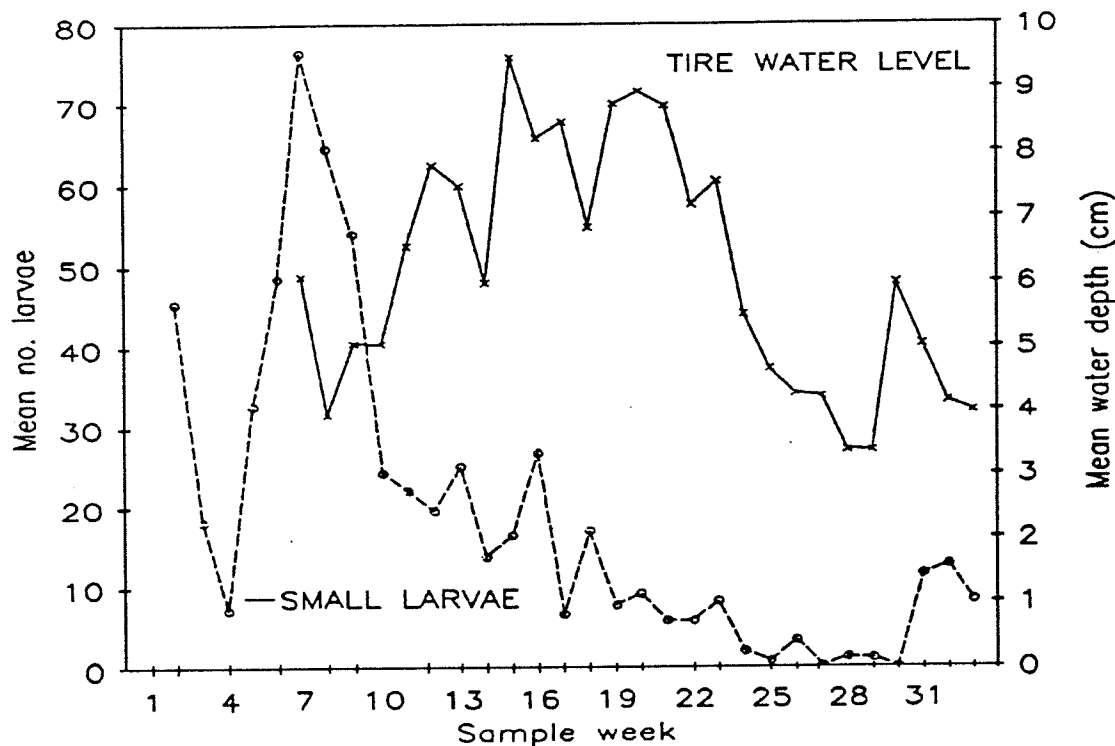
The purpose of this study was to examine fluctuations in the density of *Ae. albopictus* populations and to assess more precisely the events that cause these changes. Although *Ae. albopictus* is found in a wide variety of container habitats, this study was limited to the population dynamics of *Aedes albopictus* utilizing tires as micro habitats. The data for this investigation was gathered in 1987 and 1988. While the overall study site remained the same, different tires were employed in each year. Meteorological data were obtained from 2 U.S. Weather service reporting stations located at or near the study sites.

The first step in the experimental procedure for this investigation consisted of locating 200 discarded tires. These tires were then characterized according to habitat type, exposure to sunlight, and proximity to different types of vegetation. From these 200 tires, we randomly selected 31 for a detailed population census to be conducted twice each week. The survey procedure involved measuring the water depth followed by emptying the tire's contents into a large tray. The number of *Aedes* larvae were counted according to 2 size classes: small larvae, representing first and second instars, and large larvae, representing third and fourth instars. Pupae were removed by pipet and returned to the laboratory where adults were allowed to emerge. The contents of each tire were replaced until the next census. The tire contents were also examined for the presence and density of predators, such as *Toxorhynchites* mosquitoes and copepods. These predators were also returned to the tire habitat. After adult mosquitoes emerged from the field collected pupae, the species identity, sex, and wing length of each specimen was confirmed.

During periods of heavy rainfall the tire water level increased sharply. The average rainfall for New Orleans is about 1 inch of rain per week. It is possible that these smaller amounts of rainfall at frequent intervals are important in maintaining the water column at depths sufficient for larval development. The relationship between tire water level and the presence of newly hatched larvae is shown in Fig. 17. It should be noted that data are plotted as sample weeks. In both years, the first sample week began on the first full week of May. Tire water level measurements in 1988 did not begin until sample week 7, or the 3rd week of June; however, after this time, a rise in tire water level corresponded with an abrupt increase in the number of new larvae that were present.

Figure 17.

Relationship between Recently Hatched Larvae and Water Level in Tires in 1988



Once the eggs in rainwater-flooded tires hatch, this micro habitat is likely to remain wet for a length of time adequate for larval development. Figure 18 shows that the input of rainwater during the last week of May in 1988, followed by periodic showers, kept the 31 study tires in a breeding condition for nearly 5 months. Even under the drought-like conditions that began in mid-summer of 1987 more than half the tires under investigation contained enough water to support larval development.

Figure 18.

Proportion of Tires Containing Water for Larval Development

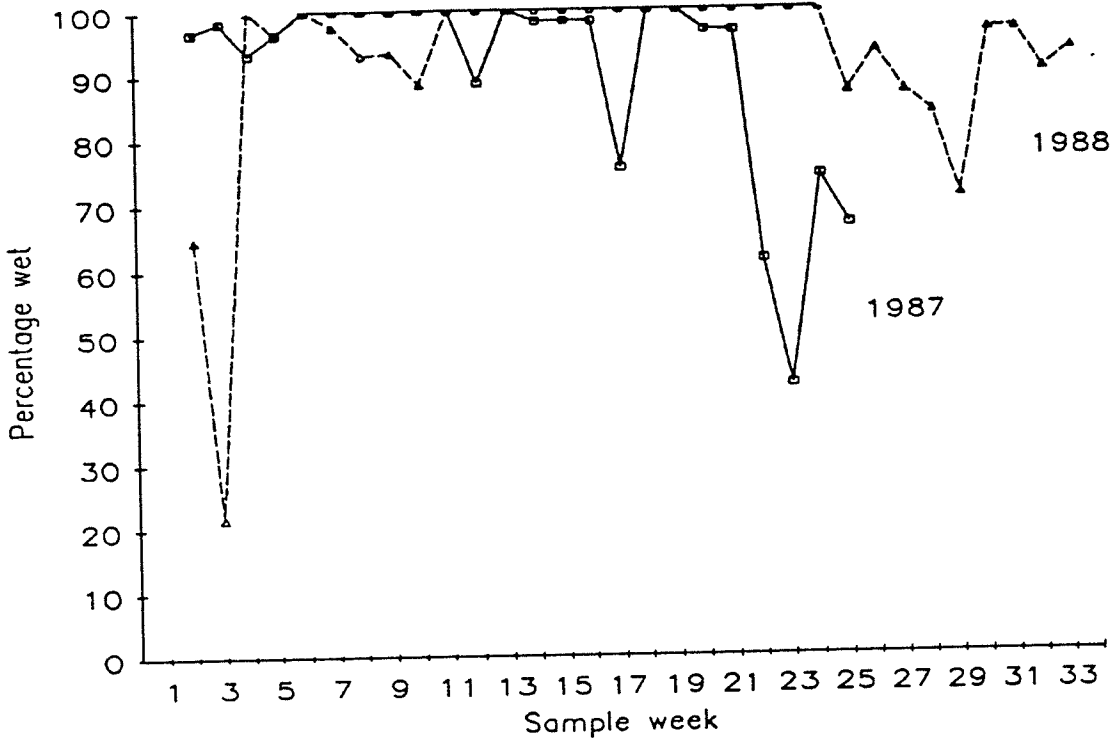
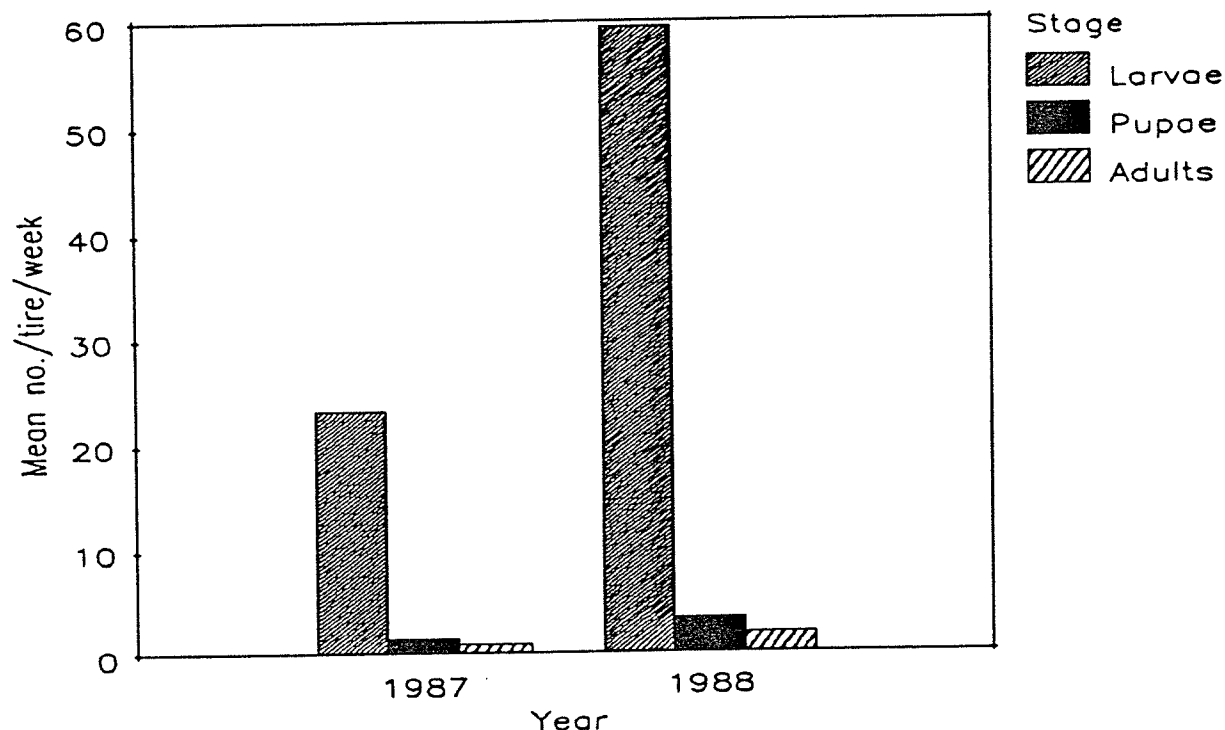


Figure 19 shows the seasonal average weekly population densities for Aedes larvae, pupae, and Ae. albopictus adults. Most of the larvae shown here are assumed to be Ae. albopictus, since 80 percent of all Aedes adults in 1987 and 96 percent of all Aedes adults in 1988 were Ae. albopictus. Regarding the weekly productivity of Ae. albopictus, this species was about two and a half times more abundant in tires in 1988 than in 1987. There was a 70 percent drop in Aedes triseriatus abundance between 1987 and 1988. Also, a comparison between the 2 most commonly encountered species showed that Ae. albopictus was 4 times more abundant than Ae. triseriatus in 1987, but the Asian tiger mosquito was 29 times more abundant than Ae. triseriatus in 1988. Although Ae. aegypti had once been common in the area of the study sites, this species was not found in 1987 and only rarely encountered in 1988. In the tires sampled in 1988, Ae. albopictus was 110 times more abundant than Ae. aegypti.

Figure 19.

Seasonal Average Weekly Population Densities for Larvae, Pupae, and *Aedes albopictus* Adults from Tires



Since each tire is a unique habitat in terms of nutrients, shade, water quality, presence of predators, and other intrinsic factors, the weekly production of *Ae. albopictus* adults was compared for each tire. The results presented in Fig. 20 indicate that individual tires as habitats for larval development vary tremendously in their ability to produce *Ae. albopictus*. The line on this figure represents the overall weekly mean per tire. Reasons for the high productivity of certain tires is currently under investigation.

The seasonal distribution of *Ae. albopictus* mosquitoes collected from wet tires is shown in Fig. 21. Although the number of larvae differ in magnitude from those of pupae and adults, the seasonal fluctuations show a similar pattern. Major oscillations in larval density are reflected in the pupal and adult populations. This can be seen between sample weeks 3 - 7. However, minor oscillations may not always show a perfect concordance among the life stages.

A comparison of the seasonal distribution of *Ae. albopictus* emerging adults with those of *Ae. aegypti* is presented in Fig 22. Approximately 7 abundance peaks were observed for *Ae. albopictus* between early May and early December. Even though few *Ae. aegypti* were found, 5 abundance peaks were observed between early May and mid-November.

Figure 20.

Aedes albopictus Population Density in Tires
in 1988 in Relation to Each Tire

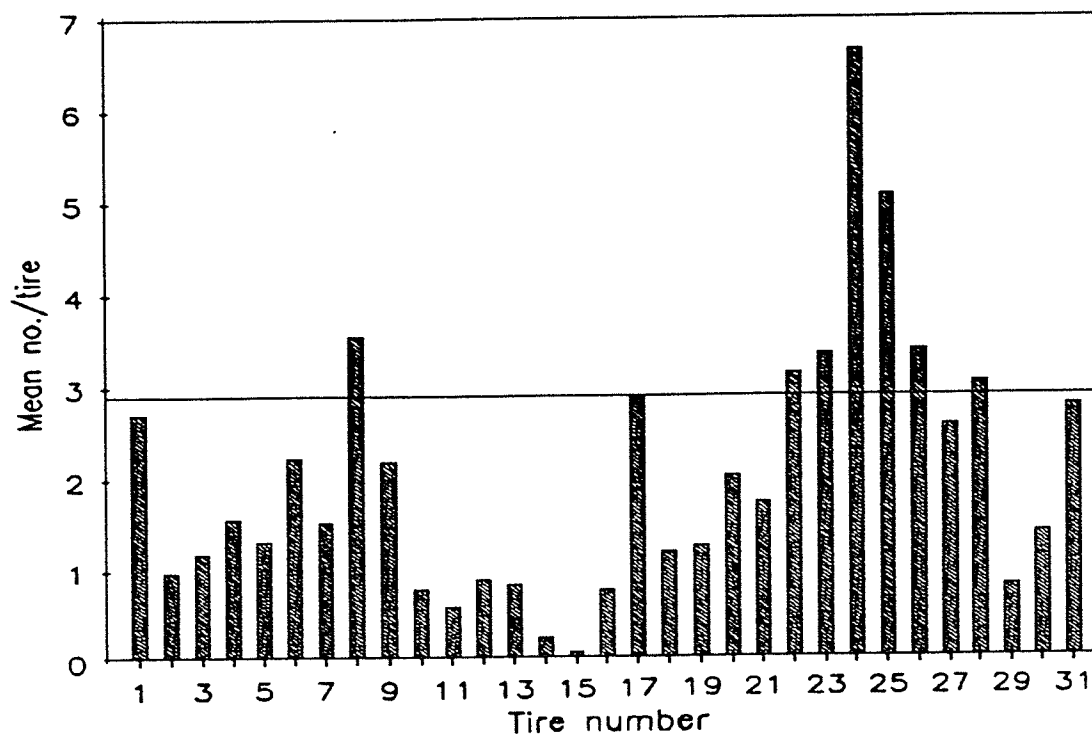


Figure 21.

Seasonal Distribution of Mosquitoes from
Wet Tires in 1988

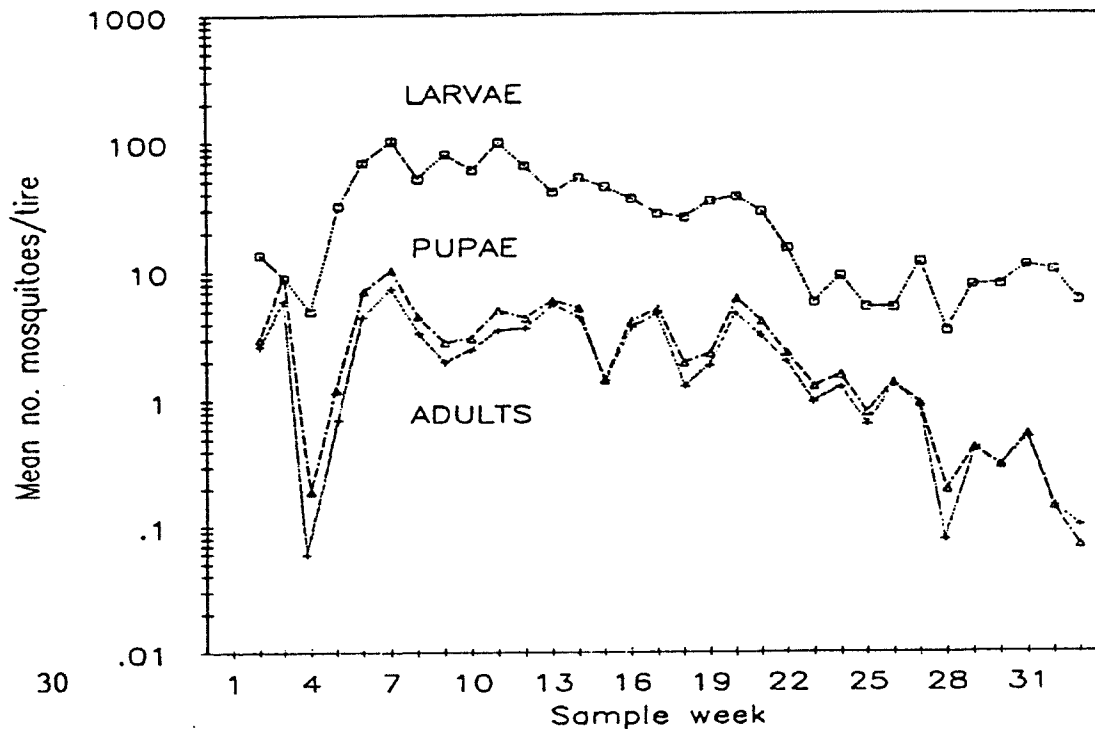
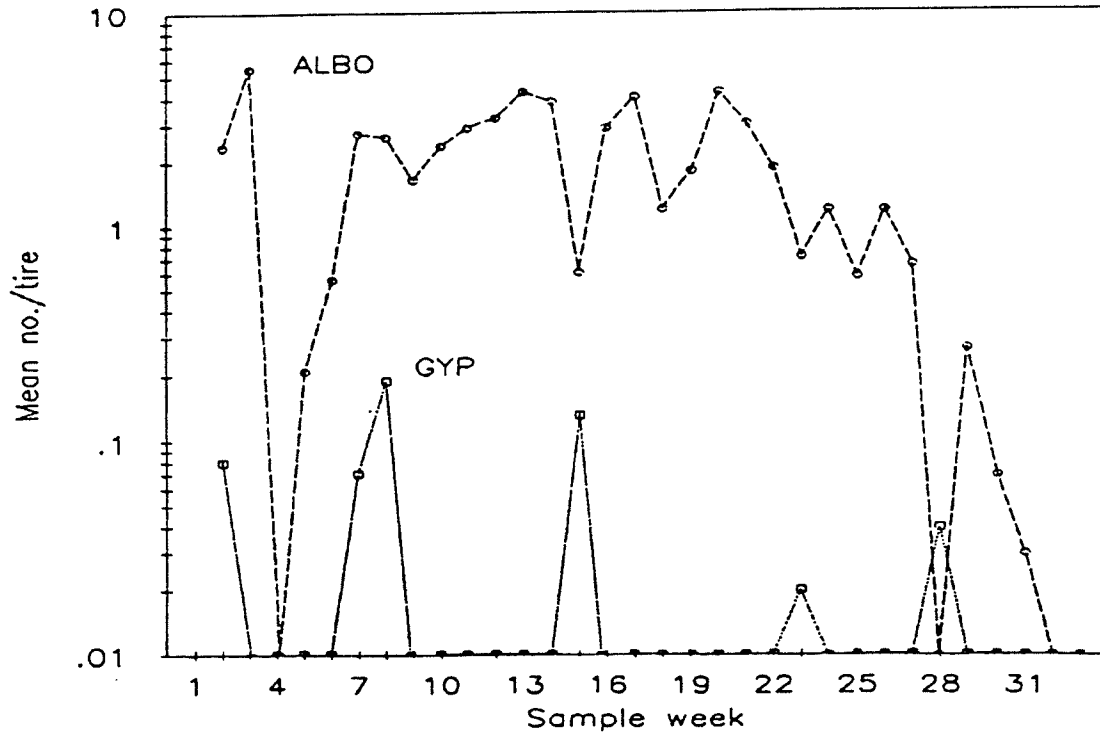


Figure 22.

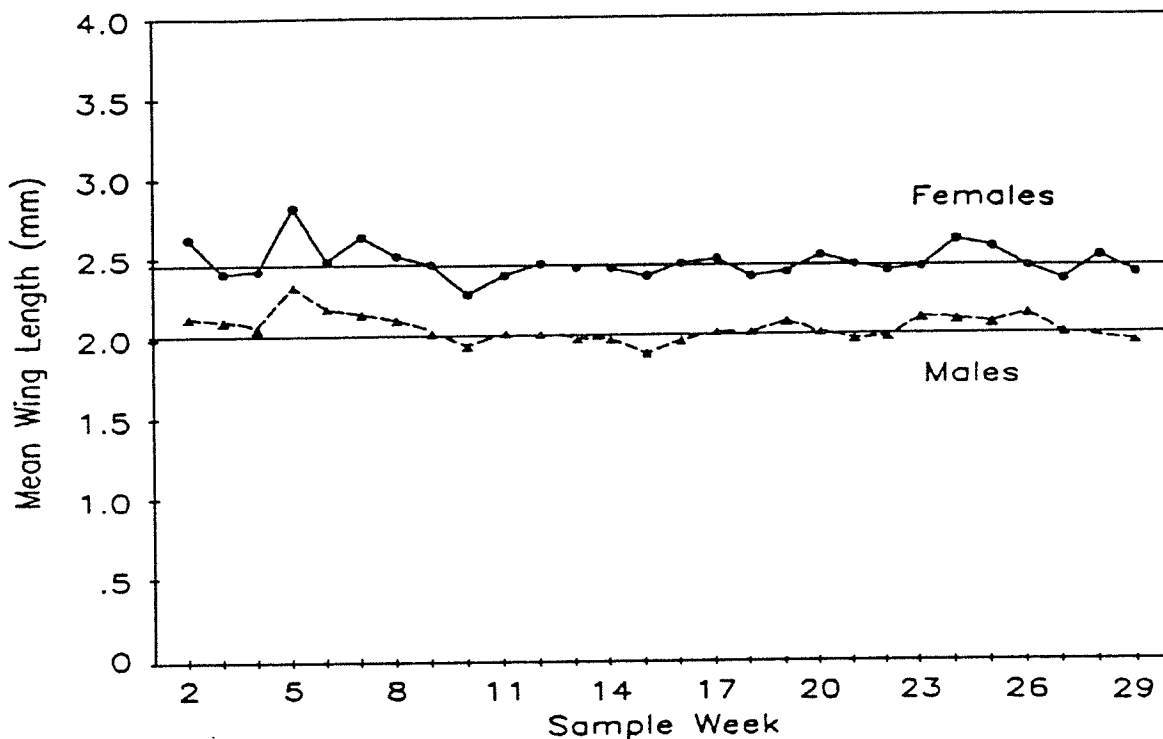
Seasonal Distribution of Container Breeding Mosquitoes in Tires in 1988



Lengths of wings from recently emerged adults were used to estimate adult body size. In 1988, approximately 1500 female wings and 1300 male wings were measured. The results for the season are shown in Fig. 23. As can be seen here, the lengths of female and male wings were relatively uniform throughout the season. The lines indicate the seasonal mean wing lengths for each sex.

Figure 23.

Seasonal Distribution of Wing Lengths of *Aedes albopictus* Mosquitoes Collected from Tires in 1988



In summary, it appears that while rainfall initiates a population cycle, the amplitude of population density for a generation is greatly affected by other factors. Our current work in New Orleans suggests that predation by *Toxorhynchites* and copepods plays a significant role in reducing *Ae. albopictus* populations, especially during July, August, and September.

BIOLOGICAL CONTROL - GERRY MARTEN

Seven species of cyclopoid copepods (henceforth called "cyclops") that occur naturally in New Orleans--*Acanthocyclops vernalis*, *Diacyclops navus*, *Macrocyclus albidus*, *megacyclops virdis*, *Mesocyclops edax*, *Mesocyclops longisetus*, and *Mesocyclops ruttneri*--were tested for biological control of *Aedes* larvae during 1989. All seven species killed large numbers of *Aedes* larvae when tested in the laboratory (Table 1). *Macrocyclus albidus* killed the most, averaging 45 larvae per day. (This was the number killed when fifty larvae were made available; *Macrocyclus* might have killed more if more were made available.)

Five of these cyclops species--Acanthocyclops vernalis, Diacyclops navus, Macrocyclus albidus, Mesocyclops edax, and Mesocyclops ruttneri--were tested in the laboratory in plastic containers that simulated conditions in tires where Ae. albopictus, Ae. aegypti, and Ae. triseriatus breed. (Megacyclops viridis and Mesocyclops longisetus were not available to the project in time for inclusion in this experiment, nor in the tire field trials reported below.) A month after introducing cyclops to the containers, first instar Ae. albopictus larvae were placed in the containers and counted after three days to ascertain their survival. Macrocyclus albidus was the most effective predator among the cyclops species that were tested. The Macrocyclus populations in the containers consistently killed all first instar larvae, even when several thousand larvae were placed in the container at the same time.

Table 1. Average number of *Aedes albopictus* larvae killed during 24 hours by individual cyclops in a standardized laboratory test.

| | Number of ¹ larvae killed | Sample ² size |
|--|---|-----------------------------|
| <i>Acanthocyclops vernalis</i> | 33.3 | 27 |
| <i>Diacyclops navus</i> | 9.5 | 17 |
| <i>Macrocyclus albidus</i> | 44.3 | 72 |
| <i>Megacyclops</i> (viridis sp. group) | 18.3 | 20 |
| <i>Mesocyclops edax</i> | 34.0 | 28 |
| <i>Mesocyclops longisetus</i> | 38.4 | 19 |
| <i>Mesocyclops ruttneri</i> | 40.6 | 18 |

¹ Average number of larvae killed, out of fifty larvae that were made available to each cyclops in the test.

² Number of cyclops tested.

The same five species of cyclops were tested in more than one thousand discarded tires under a broad range of ecological conditions in the field. Ten adult female cyclops were introduced to each tire in May.

All five species of cyclops reduced Ae. albopictus, Ae. aegypti and Ae. triseriatus larvae substantially compared to control tires (without cyclops), but Macrocyclus albidus was most effective by a wide margin (Table 2). Ninety-six percent of the Macrocyclus introductions were successful, and *Aedes* larvae and pupae were reduced 99.9% on average (compared to controls) in tires that contained Macrocyclus. Macrocyclus was still in 91% of the treated tires when they were examined six months after cyclops introduction.

Table 2. Performance of five species of cyclops when introduced to discarded tires in which Aedes albopictus was breeding.

| | Introduction success | 1 | Maintenance success | 2 | Reduction of larvae | 3 | Reduction of pupae | 4 | Sample size | 5 |
|--------------------------------|-------------------------|---|------------------------|---|------------------------|---|-----------------------|---|----------------|---|
| <u>Acanthocyclops vernalis</u> | 96 % | | 86 % | | 89 % | | 61 % | | 39 | |
| <u>Diacyclops navus</u> | 96 % | | 98 % | | 81 % | | 21 % | | 38 | |
| <u>Macrocyclus albidus</u> | 96 % | | 95 % | | 99 % | | >99 % | | 67 | |
| <u>Mesocyclops edax</u> | 83 % | | 75 % | | 95 % | | 86 % | | 42 | |
| <u>Mesocyclops ruttneri</u> | 89 % | | 89 % | | 95 % | | 82 % | | 62 | |

- 1 Percentage of tires that had a cyclops population six weeks after cyclops were introduced.
- 2 Percentage of tires to which cyclops were successfully introduced that still had cyclops population six months after introduction.
- 3 Reduction of Ae. albopictus larvae in tires containing cyclops six weeks after introduction, compared to control tires without cyclops. (Average number of larvae in control tires was 38 larvae/tire.)
- 4 Reduction of Ae. albopictus pupae in tires containing cyclops six weeks after introduction, compare to control tires without cyclops. (Average number of pupae in control tires was 2.4 pupae/tire.)
- 5 Number of tires.

Introducing a mixture of Macrocyclus albidus and Mesocyclops ruttneri was even more effective than Macrocyclus alone. One hundred percent of the introductions were successful, 100% of the tires still had Macrocyclus and/or M. ruttneri six months after introduction, and reduction Aedes larvae was 100% in all tires at all times. (The only exception was a lag of several weeks before 100% reduction took effect. Because cyclops prey only on first instar larvae, several weeks were required for second to fourth instar larvae that were in the tires at the time cyclops were introduced to finish their development and clear out of the tires.)

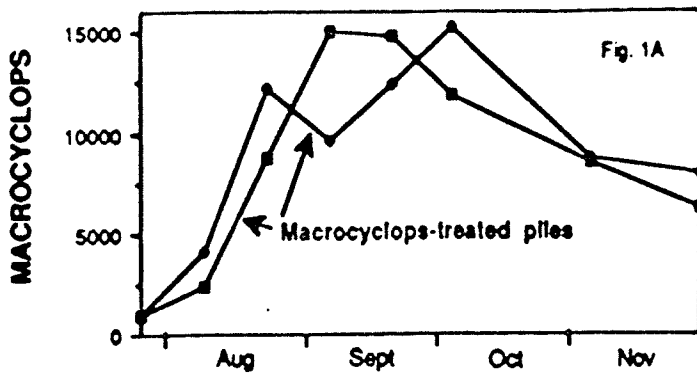
It is fortunate that Macrocyclus is so effective because laboratory experiments that we conducted on the relationship of adult mosquito production to the number of Ae. albopictus larvae in a container demonstrated that the production of adult mosquitoes is the same over quite a broad range of larval numbers. Aedes mosquitoes follow a strategy of loading containers with more larvae than food in the container can grow to adult mosquitoes. Incomplete predation may thin out overcrowded larvae without reducing the production of adult mosquitoes. This means the standard for biological control must be high. Predation must be nearly 100% to have significant impact on the production of adult mosquitoes.

To examine the impact of Macrocyclus treatment on adult mosquitoes, field trials were initiated with two isolated tire piles infested with Ae. albopictus. The piles, which contained 100 tires each, were located in a wooded area previously free of Ae. albopictus. They were treated at the end of July by introducing twenty-five adult female Macrocyclus to every tire. A third tire pile was not treated with Macrocyclus and served as a control. The number of Ae. albopictus larvae and pupae, landing rates of adult Ae. albopictus on human bait, and oviposition rates were monitored at all three tire piles until the end of the year.

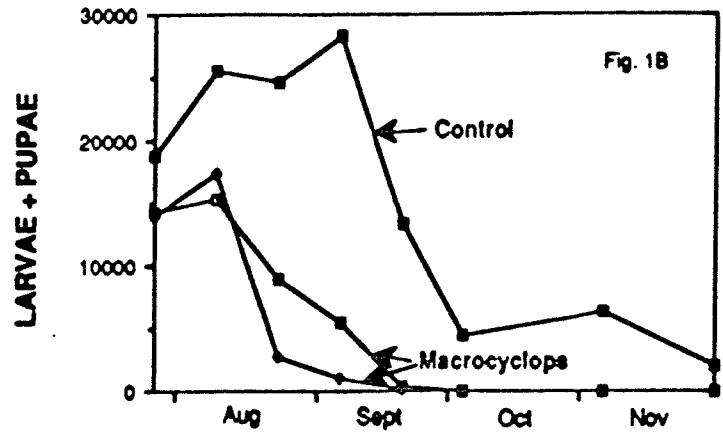
Aedes albopictus larvae started to diminish at the treated tire piles about two weeks after Macrocyclus introduction; there were no Ae. albopictus larvae left in any of the treated tires by two months after Macrocyclus introduction (Figure 1). Adult landings and oviposition reached zero at the treated piles by three months after treatment with Macrocyclus, and there was no sign of juvenile or adult Ae. albopictus at the treated tire piles after that.

There was a decline in Ae. albopictus at the control tire pile (Figure 1), but the decline at the control piles occurred later in the year, as a natural decline due to low temperatures and partial diapause of Ae. albopictus during the fall. Although the number of Ae. albopictus larvae and adults at the control pile was substantially less by the end of the year than it was during the summer, it was still large.

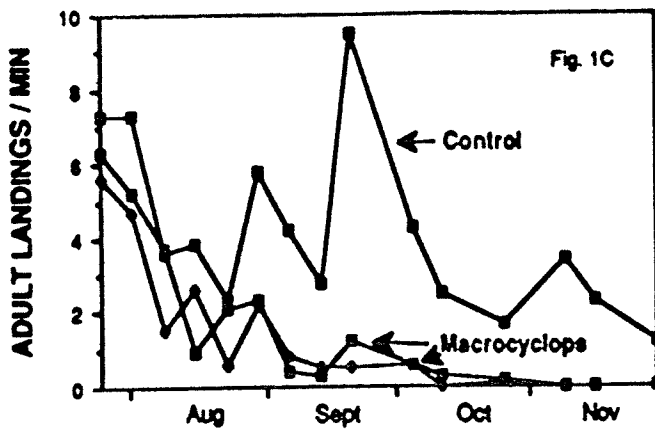
Figure 1. Aedes albopictus populations at tire piles after introducing Macrocyclus.



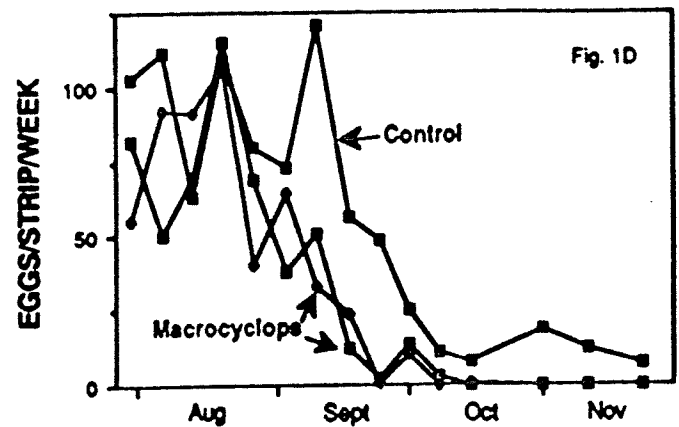
A. Number of Macrocyclus in the two tire piles to which they were introduced.



B. Number of Aedes albopictus larvae and pupae in each tire pile.



C. Landing rate of adult Aedes albopictus on human bait at each tire pile.



D. Oviposition of Aedes albopictus in ovitraps at each tire pile.

The time axis of the graphs starts on the date that Macrocyclus were introduced to the tires.

In summary, Macrocyclus virtually eliminated Ae. albopictus at the two treated piles, but it took several months to do it. This delay could be avoided by applying a larvicide at the same time tires are treated with Macrocyclus. The larvicide will kill all larvae immediately, and Macrocyclus will continue the treatment by killing all new larvae that hatch into the tires.

Laboratory studies conducted by Wenyan Che (visiting scientist) during 1989 demonstrated that all species of cyclops in our study are considerably less sensitive to the larvicides Bti, permethrin, and methoprene than Aedes larvae. Bti is the most promising larvicide for joint use with cyclops because the LD₅₀ of Bti is ten thousand times greater for cyclops than it is for Aedes larvae.

From the above results we can conclude that Macrocyclus holds considerable promise for operational mosquito control in tires and possibly other mosquito breeding habitats. However, we still must ask whether Macrocyclus can be produced and distributed in a form that is affordable and convenient for operational control. All indications so far are highly positive. During 1989 we developed simple and reliable culture techniques that can be used to produce Macrocyclus by the millions at a reasonably low cost. We have found that Macrocyclus can be applied to mosquito breeding sites using the same backpack sprayers that are used for larvicides.

ENTOMOLOGICAL REPORT - MICHAEL CARROLL

During 1989, mosquito pest and disease activity was the lowest in 23 years of data collection in New Orleans. Of the 104 New Jersey light trap nights, the 23 year average collection was exceeded only on two occasions.

Adulticiding did not begin until May, with spraying at a steady but low level throughout the remainder of the year.

The total number of Culex salinarius adults trapped was one third the number caught in 1988, and was 41% of the annual trap total. This was followed by Aedes vexans at 25%, Anopheles crucians at 25%, Uranotaenia sp., Culiseta inornata 3%, Aedes sollicitans 2%, Culex restuans 2%, Coquillettidia perturbans 2%, Psorophora columbiae 1%, Aedes taeniorhynchus 1%, others < 1% each.

Extensive biological and insecticidal studies were conducted throughout the year, which are reported elsewhere in this report.

LARVAL SURVEILLANCE - ED FREYTAG

The inspectors were involved in an extensive re-mapping and updating of swales and ditches, and the sizes of the larviciding zones were reduced to correlate to adulticiding zones. This allowed easier inspection and treatment of breeding areas. Additionally, all the larviciding rigs were re-designed to be skid-mounted, giving us several advantages over the permanent mounting system: it allows easier installation and removal, frees the truck beds when

larviciding is not needed, increases truck mileage, and allows us to store the rigs out of the weather.

Rain gauges were installed at all New Jersey light trap locations to more accurately pinpoint areas of rainfall. It had been noted by the inspectors assigned to different sectors of the city that on the average rainfall is usually localized, and not city-wide. By taking precipitation data twice weekly, the rain gauges allowed us to more accurately assign inspection and larviciding operations.

Larval inspections during the first three months of 1989 resulted in collections of mostly Culex salinarius, Cx. restuans, Cx. quinquefasciatus, and Culiseta inornata. No Aedes albopictus or Ae. aegypti were collected in container inspections during these cooler months, which received very little rainfall.

Aedes vexans was our most prolific floodwater mosquito breeder, comprising upwards of 70% of the larvae collected from swales and ditches during the rest of the year. Although 1989 was a relatively dry year, inspectors were kept busy larviciding Ae. vexans breeding areas for weeks after short but heavy showers. Positive sites were treated with methoprene and Bti liquid formulation, or the Florida oil mix in areas where pupations had already taken place. As noted by low light trap collection and few phone complaints, the larviciding campaign was very successful.

ADULTICIDING - ED FREYTAG

Very little adulticiding was needed by ground and by air in 1989 due to extremely low mosquito populations throughout most of the year. The few mosquito complaints received by telephone at NOMCB were in the later part of the year, and most of them were due to mosquitoes breeding in backyards in pots, boats, tires, and other water holding containers. In most cases, a visit by one of our inspectors solved the breeding problem and in effect negated the need for adulticiding.

As required by label directions, all the truck-mounted LECO ULV adulticiding units and the aircraft were calibrated and checked for correct droplet size. This is a very important maintenance step which is performed early in the year before the mosquito season begins. All the ULV adulticiding trucks used malathion (91%), and resmethrin/PBO (scourge) was used in the aerial ULV adulticiding.

A very successful larviciding campaign against Aedes vexans and other floodwater mosquitoes resulted in very little need for ground adulticiding missions.

SUSCEPTIBILITY STUDIES - ED FREYTAG

Hundreds of larval and wind tunnel susceptibility tests were conducted in the NOMCB laboratory for the CDC aerial spray contract. Strains of Aedes

albopictus from Little Woods and Gentilly and Ae. aegypti from Gentilly were tested for susceptibility levels against malathion, resmethrin, and naled. Data from these tests are presented in the CDC report. In order to accommodate the necessary number of mosquitoes and personnel needed for the CDC contract in the insectary, no other susceptibility studies were performed. The insectary was devoted primarily to rearing the three mosquito strains used by CDC personnel.

BUCK MOTH CATERPILLAR - STEVE SACKETT

A new challenge and responsibility was placed upon NOMCB in 1989, that of conducting operational research and control measures on the buck moth caterpillar, Hemileuca maia. The larvae of this insect does considerable damage to the foliage of oak trees (primarily live oak) and has the potential to stress many of our older trees to the point of permanent damage. In addition to the problem of defoliation of trees, the caterpillar also causes pain and sometimes severe allergic reactions in humans when the envenomated spines of the larvae are touched.

Populations of the buck moth have drastically increased over the past few years, and the city administration requested that we work with the Parks and Parkway Commission to draft a plan for the control of this pest. Having secured financial assistance from the U.S. Forest Service and met with a number of state and federal representatives, a 3-fold approach was formulated: (1) Study the basic biology and ecology of the insect (2) Determine the most effective means of control (3) Coordinate the activities of civic groups, city agencies, and pest control operators in a unified attack to effectively reduce populations of the buck moth caterpillar.

BIOLOGY

From late November to early January, adult buck moths emerge from their pupal cases which have been buried under shallow covering of leaf litter for the past 7-8 months. Males tend to be active flyers as they seek out a mate, while females "call" with their pheromones from a stationary position. Females are gravid when they emerge, and oviposition follows soon after insemination. Egg masses are wrapped around the distal ends of oak branches (2-6 mm in diameter) in close proximity to the site where new leaves will be produced. These grayish-colored eggs have a ceramic-hard covering which protects them from adverse conditions and potential predators. Egg masses typically vary in size from 60-300 eggs per cluster. The life span of the adult buck moth is short, with mortality generally occurring 2-3 weeks after emergence.

Egg hatch begins in late February or early March, coinciding with the time that new leaves or leaf buds begin to appear on the trees. Even within the same cluster of eggs, hatch is asynchronous, requiring 2-8 days for completion. Typical hatch success of egg masses we examined was ca. 95%. Hatch times within our locality probably cover 2-4 week period during a given season, with temperatures playing the most critical role in determining initial hatch dates. Although larvae become quite lethargic during periods of extreme

cold, it does not appear that die-off will occur even following several nights of sub-freezing temperatures.

Larvae progress through 5 instars, gradually changing in coloring from black (first instar) to a green/brown camouflage pattern that closely resembles the bark and lichen growing on the oak trees. First instars from an individual egg cluster tend to remain tightly grouped, and are many times located on the underside of a single leaf. As the larvae mature, the social unit dismantles (after third instar) and individuals disperse throughout the tree as they seek new feeding grounds. Following the fifth instar, the caterpillars pupate and lay dormant until winter.

LARVAL SUSCEPTIBILITY TESTS

Laboratory-reared caterpillars were subjected to four control products, Tempo 2, Orthene, Dipel, and Biobit, to determine if the insects (third instar larvae) were susceptible to the compounds at label rates. Tempo 2, a new pyrethroid on the market, demonstrated the quickest knockdown of the products tested, with 100% mortality observed within 30 minutes of applications. Using only 1 ounce/100 gallons of water, Tempo 2 proved to be a very safe and cost-effective product. Orthene, an organophosphate, gave 100% control within 24 hours using the label rate of 0.75 lb. a.i. per 100 gallons of water. Dipel and Biobit, both biological insecticides (B.t.k.) specific for caterpillars, were also 100% effective, but results were not immediate. Larvae ceased feeding soon after treatment and became sluggish, with mortality occurring 4-5 days post-treatment. These biologicals would be an excellent alternative to use in areas sensitive to standard insecticides.

FIELD TRIAL

To verify the efficacy of Tempo 2 against buck moth caterpillars under operational conditions, a field trial was conducted in a heavily-infested area of Banks Street in New Orleans. On April 4th, pre-treatment counts of caterpillars indicated an average of 12.4 larval masses per tree, with 89 large oaks examined. Trees were divided into 3 groups for treatment:

- Group 1 - treated at 1 oz/100 gal. water
- Group 2 - treated at 2 oz/100 gal. water (upper label limit)
- Group 3 - non-treated

On April 6th, 6:45AM, a 600 gallon John Bean sprayer was used to deliver the insecticide at ca. 500 psi, and an average of 16 gallons was required to adequately cover each tree. Beginning and ending temperatures and relative humidities were 62-70°F and 64-52%, respectively. Winds remained calm throughout the test.

Twenty-four hours after the application, post-treatment counts of larval masses indicated a 91% reduction in the "1 oz." group, a 93% reduction in the "2 oz." group, and a 24% reduction in the non-treated group. Additional surveys conducted 6 and 14 days after the spray indicated 100% control in the treatment area. Not a single living caterpillar was observed within the spray

zones, whereas every tree within the control area was still infested (average of 6.4 larval masses per tree).

OPERATIONAL CONTROL OF CATERPILLARS

Surveys of oak trees in Orleans Parish were initiated to determine the areas of highest infestation and to establish treatment priorities. In addition, a telephone hotline was installed to handle complaints, questions, and requests for treatments.

Treatments were initiated at the end of March and were completed by April 20th, with a total of 3,439 trees sprayed. Although the test results of the Tempo 2 field trial were relayed to the contractors, the majority of the trees were treated with Orthene 75 S. Post-treatment evaluations revealed that some re-treatment of trees was necessary, but this was probably due to inadequate coverage of the foliage and not insecticidal failure. Dipel 8 AF, a biological insecticide, was used by one contractor to treat 500 trees with excellent results. In addition to the trees treated by the contractors, 2000 trees were treated with Tempo 2 by NOMCB personnel, bringing the total number of trees sprayed to ca. 5,439.

Although it was impossible to locate and treat every infested tree in New Orleans, it would appear that our control efforts had a major impact upon reducing the overall population of buck moth caterpillars for 1989 and should reduce the potential for large-scale defoliation of oak trees in the future.

International Cooperative Studies - Wenyan Che

A. Copepod Survival in Dry Soil

Resistance to desiccation is an important factor if cyclops are to be an efficient mosquito control agent. Larvivorous copepods were tested for their ability to withstand desiccation. The species tested were Macrocyclus albidus, Acanthocyclops vernalis, Diacyclops navus, and Mesocyclops sp.

Ten adults and 10 copepodids of a species were placed in a 500 ml plastic food container with 30 grams of dry soil, 5 grams of leaves, and 40-90 ml of water extracted from tires. Six to ten containers were used for each species. The containers were held uncovered in the laboratory, allowing natural evaporation of moisture. Two methods were used to assess soil moisture during the desiccation process: (1) An ohmmeter measured the electrical resistance of the soil across the diameter of the container. (2) Assessing the ratio of water weight to dry soil weight. When the soil contained only 9% to 32% of moisture, 70 ml of water were added to the containers. Surviving cyclops were counted 10 minutes, 1 hour, and 24 hours after reflooding.

Late stage copepodids of all species survived better than nauplii and adult. However, among Acanthocyclops vernalis and Diacyclops navus, adults survived almost as well as the copepodids. These species also proved to be more resistant to desiccation than Macrocyclus albidus and Mesocyclops sp.

In this experiment, *Mesocyclops* sp. appeared to be least tolerant of desiccation.

The above results indicate that the differences between species could be enough to make the difference between extinction and survival in a temporary habitat. The species with the best survival rate were those that occur naturally in temporary habitats. This study suggests that after introduction into a habitat, cyclops are likely to survive for long periods under relatively dry conditions.

B. Susceptibility of Copepods to Insecticides

Adult stages of *Macrocyclus albidus*, *Mesocyclops* sp., and *Diacyclops navus* were tested for their susceptibility to permethrin and *Bacillus thuringiensis* H-14 (Bti), two larvicides commonly used in mosquito control. The tests followed modified WHO procedures for testing mosquito susceptibility to pesticides. For each species, 10-20 copepods were tested. Four replicates of each of five dosages plus a control (acetone only) were run in a test. The test cups were placed in the mosquito insectary at 25°C and 80% RH. For permethrin, mortality was assessed after 24 hours; and, for Bti, mortality was determined after 24, 48, and 72 hours. Each treatment was repeated three times. The results of each test were averaged together.

The test results indicate that copepods were much less susceptible to permethrin than *Ae. albopictus*. The LC₅₀ dosage of the insecticide for *Macrocyclus albidus* was nearly 100 times greater than that for *Ae. albopictus*. For *Mesocyclops* sp. and *Diacyclops navus* the LC₅₀ dosage was approximately 500 times greater than that for *Ae. albopictus*. When the Bti dosage was increased to 1279 ITU/ml, there was still no copepod mortality after 72 hours. This dosage is about 3000 times greater than the LC₉₉ dosage for *Ae. albopictus* and *Ae. aegypti* after 24 hours. These results support the feasibility of integrating copepods with Bti and permethrin applications in control strategies against container breeding mosquitoes.

Another experiment focused on the susceptibility of copepodids and nauplii of the above species to various concentrations of permethrin. The test procedure was similar to the one described for adults, but instead of determining mortality within 24 - 48 hours, copepod reproduction and survival was monitored for a six week period. We found that the nauplii stage is most sensitive to contact with permethrin. *Macrocyclus albidus* was the most susceptible of the three species tested. Nevertheless, the LC₉₀ dosage for *Ae. albopictus* was much lower than the dosage for the nauplii stage of *Macrocyclus albidus*.

C. Sugar Feeding by *Aedes albopictus*

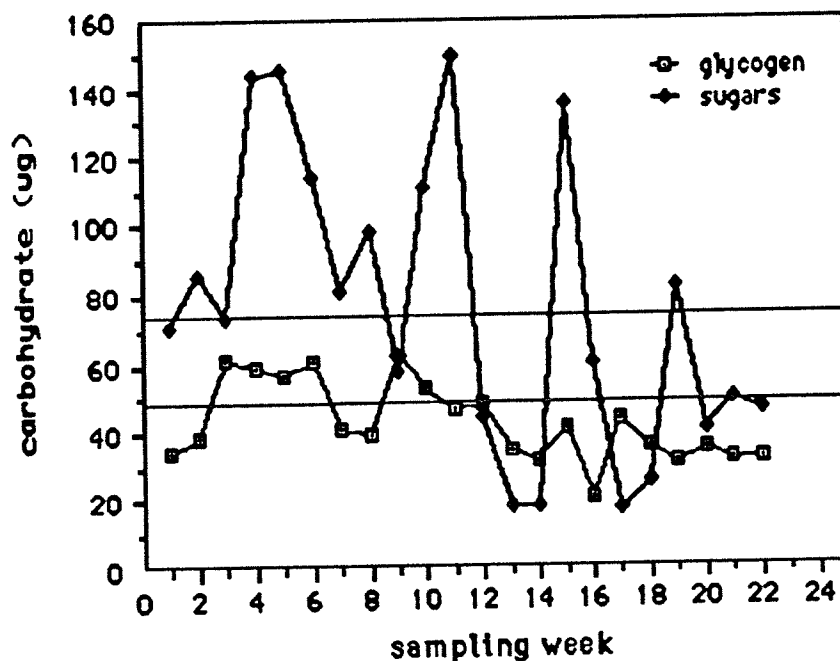
Carbohydrates play an important role in the survival, flight and reproduction of mosquitoes. Factors such as daily survival, dispersal and reproduction rates are important in determining the potential of a species to become a vector. To estimate the vector potential of *Aedes albopictus* in relation to the status of nutritional carbohydrates, a study was conducted to

determine quantitatively the level of different sugar types (Fructose, glycogen, and other sugars). Measurements of sugar concentrations in Aedes albopictus females were made throughout the season, in relation to body size, sex, daily activity, insemination, and parity.

The mosquitoes tested were collected with a backpack aspirator at the Rest Haven Cemetery and Clio Street study site. Collections were made twice a week. To slow enzymatic degradation of sugar enzymes, specimen were stored at 20°C within an hour after capture. The method for detecting fructose was based on Van Handel, (1972). The study results indicate that the percentage of mosquitoes with fructose gradually increased between April and June. Similar trends were observed for both Ae. albopictus and Ae. aegypti. There were no significant differences in feeding rates between males and females of either species.

The method for the detection of glycogen was based on Van Handel, (1985a). The details of the test procedure are described in NOMCB's July 1989 monthly report. Female Ae. albopictus contained significantly more sugar and stored significantly more glycogen than males of that species. These findings may help to explain why females Ae. albopictus are more resistant to starvation than males. Concentrations of glycogen and sugars were higher in the first 12 weeks and lower in the later 10 weeks of the study period (see Figure 1). It is likely that in May, June, and July, the abundance of flowering plants made more sites available for nectar feeding. Overall, sugar uptake was highly variable (from 18.1 to 148.9 ug), while glycogen uptake showed less variability. Usually, a low carbohydrate content suggests limited flight potential. A low sugar and high glycogen content indicated that most of the nectar has been digested and converted into glycogen. A high sugar content suggests a recent nectar meal. Absorption of sugars from crops is a very rapid process, especially when the sugar meal is small, thus levels of sugar uptake fluctuated greatly.

Fig 1 Seasonal change of glycogen and sugars of Ae. albopictus



For the daily uptake study, *Ae. albopictus* females were collected every three hours between 0700-19:30 hours from tire piles near Rest Haven Cemetery. This experiment was repeated three times and the results were averaged together. Figure 2 indicates that fructose content peaks in the morning between 0730-1030 hours. In the afternoon, a peak was observed between 1630-1930 hours. Figure 3 shows that the level of glycogen is highest at 0730 and 1930 hours.

Figure 2. Changes in nectar feeding rate in *Ae. albopictus* during the diel.

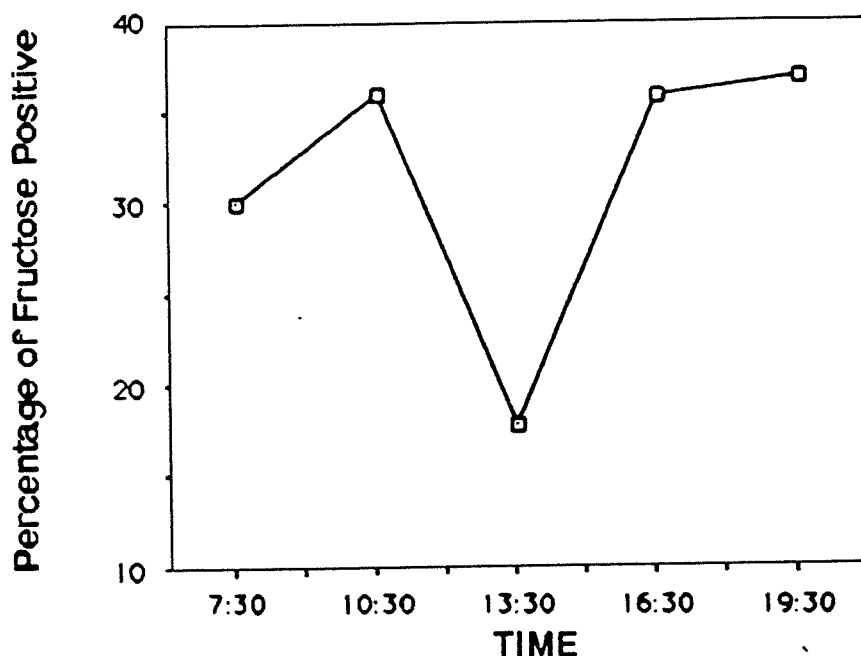
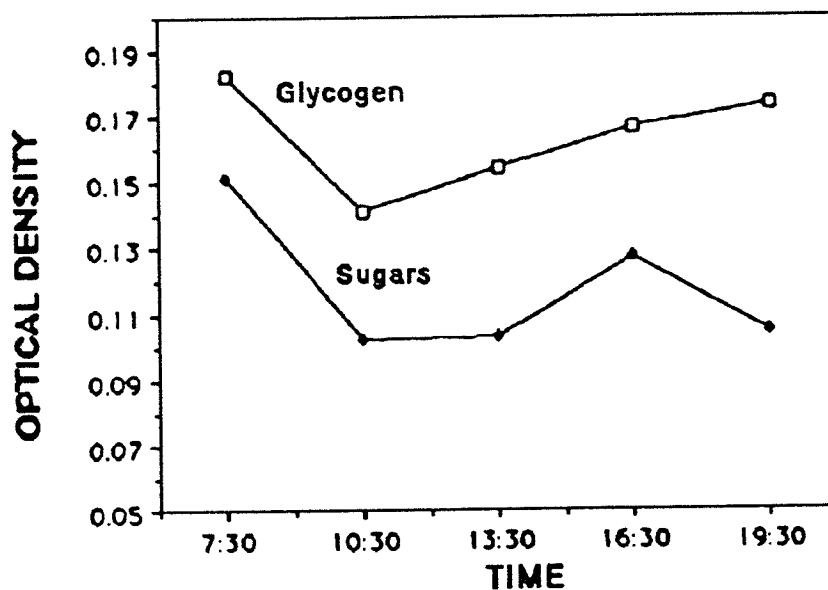


Figure 3. Changes in sugar uptake in *Ae. albopictus* during the diel.



In another experiment Ae. albopictus and Ae. aegypti were allowed to feed on 12 different species of plants that were collected from Rest Haven Cemetery and the Clio Street study site. The Ae. albopictus colony was derived from collections from Rest Haven Cemetery and Ae. aegypti colony from the Clio Street site. The details of this experiment are described in the NOMCB October 1989 monthly report. The results showed that the nectar feeding rate for Ae. albopictus on the plants from Rest Haven Cemetery were higher than those from Clio street. The opposite is true for Ae. aegypti. This indicates that both mosquitoes seem to prefer feeding on the plants from their own habitat. This could be one reason why Ae. albopictus is the dominant species in suburban areas and Ae. aegypti the predominant species in downtown New Orleans.

Mosquito body weight has long been known to show a positive correlation with wing length. Larger mosquitoes usually store more nutrient reserves than smaller ones, and the glycogen reserves may be correlated with wing length. To test the glycogen measurement as a sensitive parameter for adult body size, we have applied the method of determining glycogen uptake in field collected Ae. albopictus. Wing length was measured with an optical micrometer from the notch where the posterior margin of the wing begins to expand the distal tip of the wing, not including the apical fringe. We found that glycogen content increased consistently with wing length. The variability in glycogen uptake is from 6.5 - 14.1% mean. Statistical analysis revealed a correlation coefficient (r) of 0.98, indicating that glycogen assay is a sensitive parameter for adult body size.

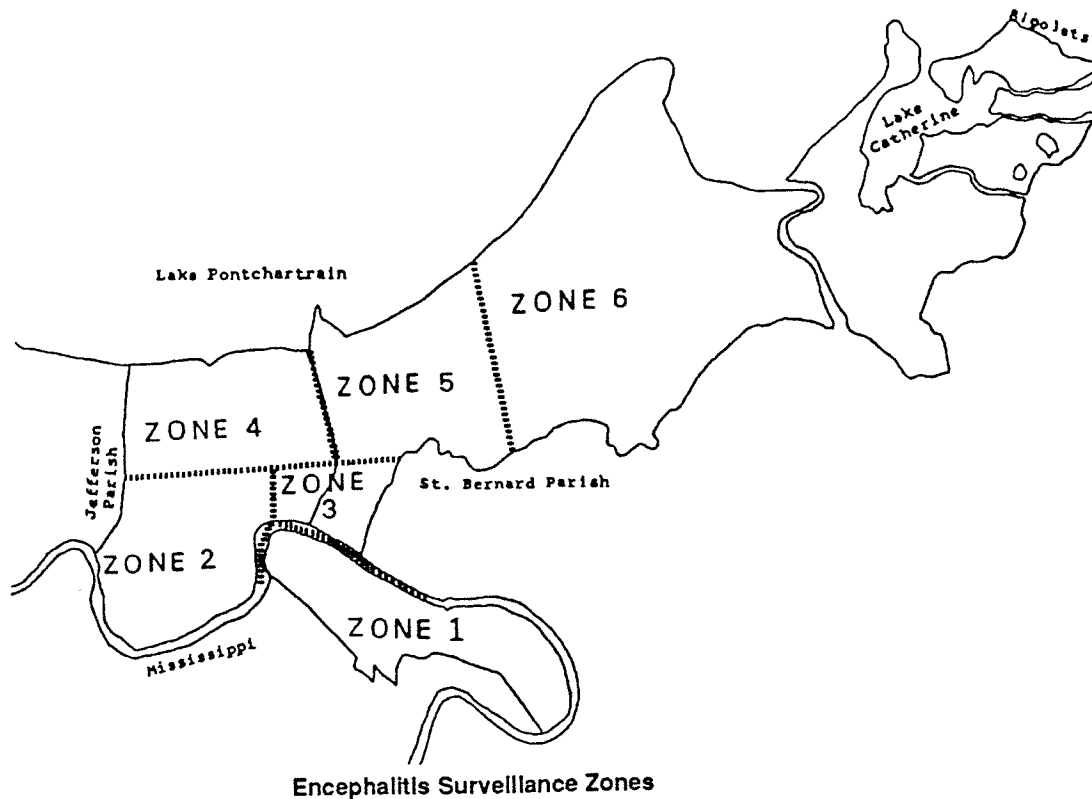
ENCEPHALITIS SURVEILLANCE - C.J. LEONARD

Encephalitis surveillance began in May, with the deployment of fifteen new elevator type bird traps that were designed and constructed at New Orleans Mosquito Control. The new traps worked very well and are capable of catching many different species of birds, such as, Red Wing Blackbirds, Cowbirds, Blue Jays, Starlings, Cardinals, Grackles, and Doves.

The old walk-in traps caught sparrows over 80% of the time. The new traps caught only 40%-50% sparrows. This greater variety of species should make our surveillance more likely to pick up viruses such as Eastern Equine Encephalitis.

Three positives were reported during the year. Two of these turned out to be an error, the third was EEE collected in Zone 2 on June 22nd. No further virus activity was reported. The program was terminated at the end of September with a total of 801 samples submitted.

Some initial problems with the State Laboratory eventually led to a close working relationship, we now receive results from our bird bloods in as little as three days. This makes the program an excellent early warning of virus activity.



ENCEPHALITIS SURVEILLANCE 1989

| | TOTAL | <u>SPARROWS</u> | | <u>OTHERS</u> | |
|------------------|-----------|-----------------|----------|---------------|----------|
| | | ADULT | IMMATURE | ADULT | IMMATURE |
| Zone - 1 | 51 | 16 | 5 | 22 | 8 |
| Zone - 2 | 1 / 88 | 23 | 1 / 25 | 35 | 5 |
| Zone - 3 | 97 | 33 | 36 | 27 | 1 |
| Zone - 4 | 68 | 24 | 10 | 30 | 4 |
| Zone - 5 | 203 | 23 | 44 | 96 | 40 |
| Zone - 6 | 294 | 65 | 98 | 91 | 40 |
| TOTAL | 1 / 801 | 184 | 218 | 301 | 98 |
| # POS / % POS | 1 / .12 % | 0 / 0 % | 1 / .4% | 0 / 0% | 0 / 0% |

AVIATION REPORT - P.A. ROCHE'

The required FAA Annual Inspection of the twin-engine "Islander" aircraft was completed in late December. Immediately following the inspection the spray system was removed in preparation for ferrying the aircraft to San Antonio, Texas for complete stripping and re-painting at Chaparral Aviation. Upon completion of painting in February the aircraft was picked up and returned to New Orleans. The Micronair Rotary Spray System was refitted to the Islander for special tests in conjunction with the Centers for Disease Control Aedes albopictus Aerial Spray Project. Shortly after this, blade failure on one of the rotary atomizers caused airframe damage at three locations necessitating removal of the aircraft from service for five days for inhouse repairs. This resulted in re-configuring the aircraft with Boom Mounted Tee-jet Atomizers to continue the spray program. Upon return to service, calibration was accomplished and the single-engine Ag-Cat aircraft was removed from service for its FAA Required Annual Inspection.

During the 1989 season, 22 flights were conducted with the single-engine aircraft, 19 of which were spray operations, 33 flights were made with the multi-engine aircraft, 28 of which were spray operations.

A recent rule by the FAA required purchase of a Beacon Transponder and Altitude Encoder Device which was installed in the Grumman Ag-Cat in our shop.

Upon completion of the installation of the radio equipment, both aircraft were brought to AERO Services Radio Shop for the required Bi-annual pitot-static system tests and Transponder frequency test.

The Tee-Jet spray system on the Islander was dismantled for cleaning and repair after dispensing "naled", while the system was open it was re-plumbed for higher flow rates.

A small portable chemical loading/unloading pump was fabricated in our shop for rapid pumping of chemicals at remote sites.

All of the paperwork for the Aircraft Hangar Project seems to be completed, and we are waiting on final approval from the FAA so that the Architectural Department can solicit bids for construction costs from the general contractors.

SOURCE REDUCTION - BROOKS HARTMAN

For most of 1989 the source reduction program was active with inner city projects, new ditch construction in New Orleans east with old ditch maintenance for added adult mosquito reduction for residents of surrounding residential areas.

Area S-21 (Alvar, Almonaster Road, France Road, Florida Avenue) was drained by installation of a 6" plastic drain pipe via subsurface drainage at Alvar. This will help reduce mosquito populations in this industrial and residential area.

Other areas around the city and in New Orleans east (S-21, U-13, U-9, V-6, U-15, V-15 and City Park) received new ditches or routine ditch maintenance by removal of dense obstructive vegetation.

The W-2 wetlands project has been placed on hold again for 1989 pending the institution of the Bayou Sauvage National Wildlife Refuge. Amphibious ditching of the "W" areas east of Paris Road was halted in 1986 until further notice. Ditching of these areas is important to New Orleans because of their close location to homes, schools and shopping areas.

| | BACKHOE 1 & 2 | CRAWLER 193 | DL-1 13 | DITCHER 4 |
|-------------------------------|------------------|----------------|------------|--------------|
| Total hours | 605 | | | |
| Digging hours | 352 | | | |
| Linear feet/ditch | 5535 | | | |
| Salary | \$13890 | \$ 3273 | \$ 253 | \$ 78 |
| Cost/fuel, oil maintenance | \$ 1278 | \$ 1112 | | |
| TOTAL COST | \$15168 | \$ 4385 | \$ 253 | \$ 78 |

| | |
|---|------------|
| Project inspection & evaluation | 839 hours |
| Miscellaneous shop duties & maintenance | 846 hours |
| | 1685 hours |

| Service support vehicles | Miles traveled | Cost |
|-----------------------------|-------------------|--------|
| S-89 | 4890 | \$ 855 |
| S-90 | 293 | 52 |
| DT-95 | 1350 | 298 |
| S-19 | 200 | 44 |
| S-3109 | 3212 | 601 |

PUBLIC EDUCATION - C.J. LEONARD

Two new video programs and four public service announcements were produced this year. "Integrated Pest Management" is a 14 minute program covering all aspects of mosquito biology and control. It is suitable for any adult group or for broadcast on cable.

Since the Mosquito Control Board has been involved with the control of the buck moth caterpillar; a video was produced explaining the caterpillar's life cycle and methods of control.

These video's are available to civic organizations, and are broadcast on the School Board and Government Access channels.

Four public service announcements were produced and sent to all the local broadcast stations. Although we have no control over when or how many times a station uses a public service announcement, several stations used them extensively, sometimes in prime time.

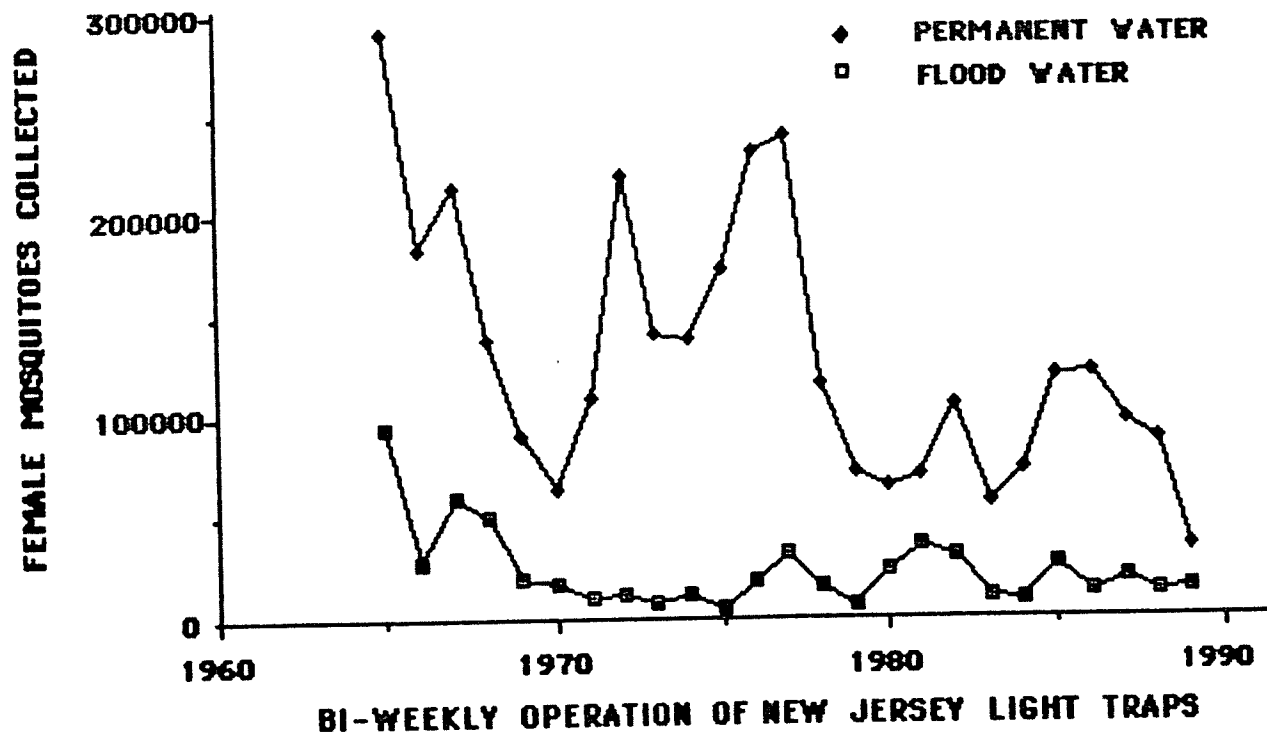
Lectures and slide or video presentations were give to classes at several local universities as well as to groups touring the New Orleans Mosquito Control facility.

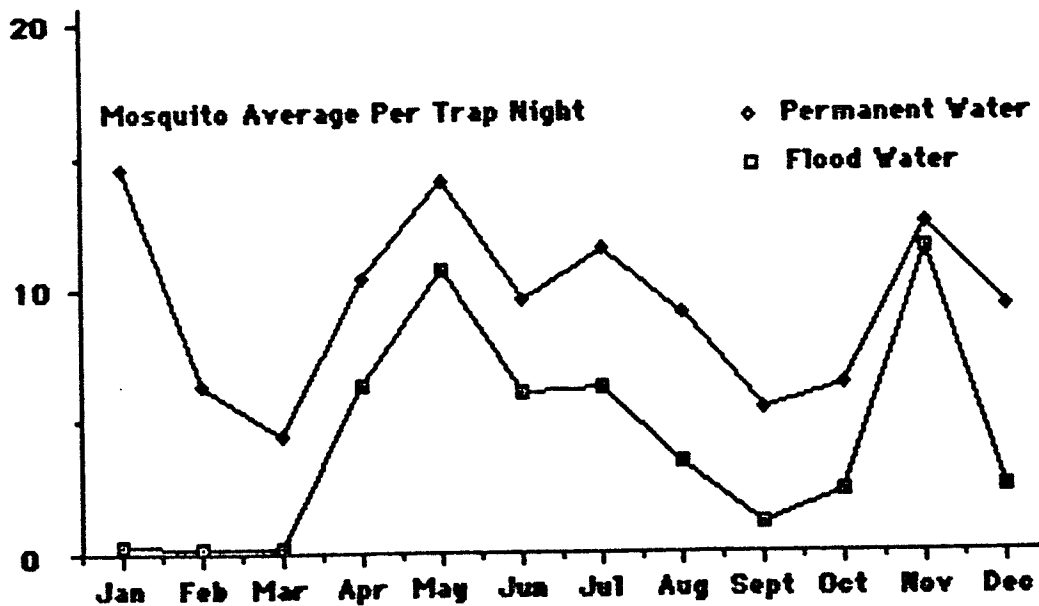
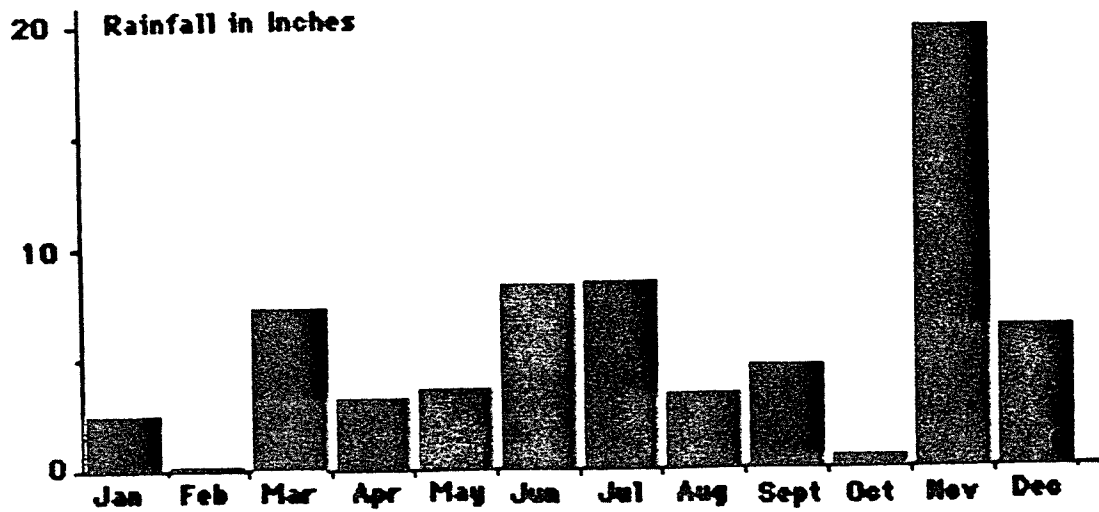
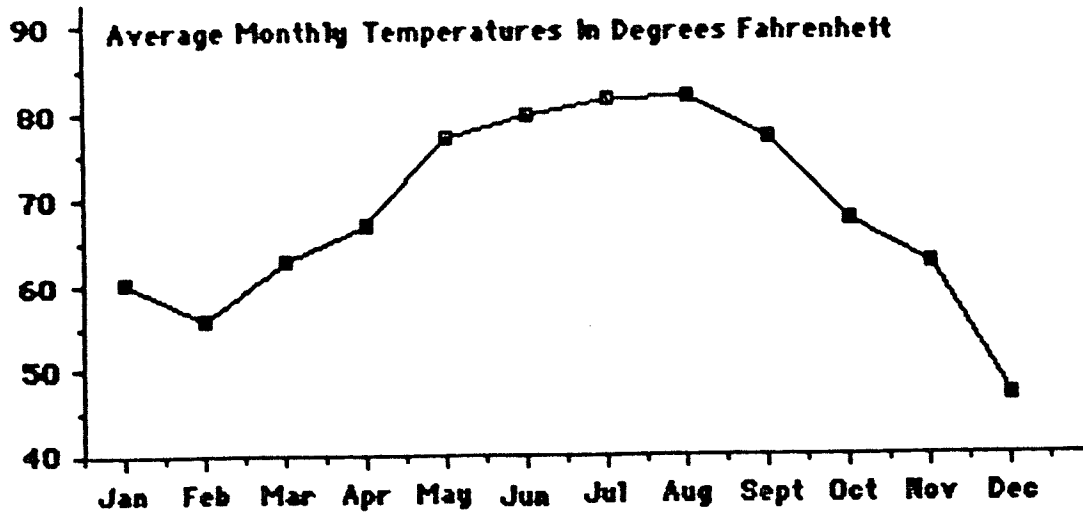
Video maintenance was performed as required, including a complete reviving of the audio system in the city's video van, which was improperly wired when it was built.

Additional video work was performed during the year for the City Public Information Department, on videos for the Fire Department, and the Art's and Entertainment Network's City Video competition. In addition, video was shot and sent to the Centers for Disease Control for use in their Aedes albopictus tape.

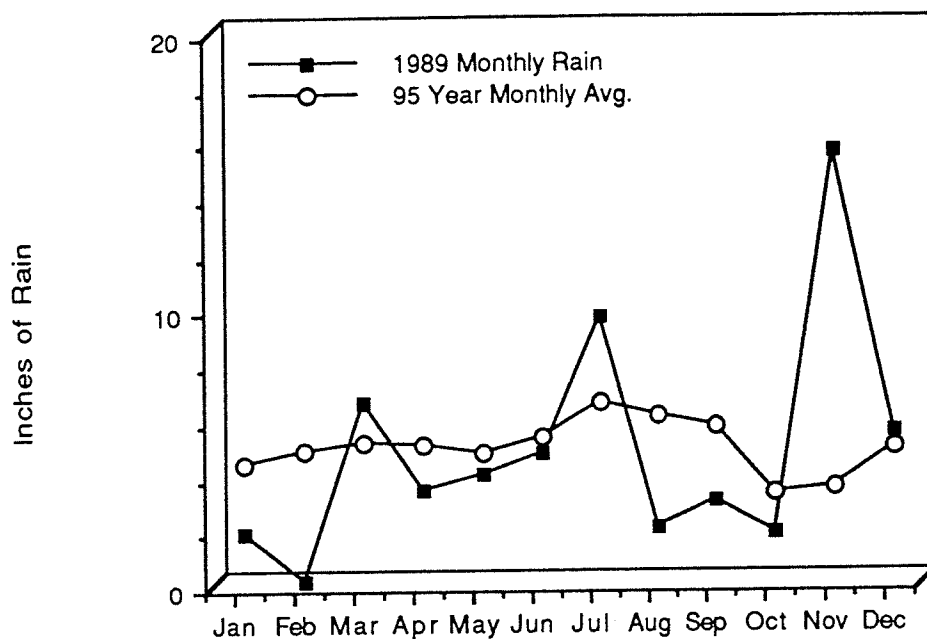
Aerial photography of the CDC study sites was done to produce aerial maps of the study areas.

* * *





1989 Annual Rain Report



| Month | Inches of Rain | 95 Yr. Average |
|-----------|----------------|----------------|
| January | 1.71 | 4.26 |
| February | .01 | 4.74 |
| March | 6.44 | 5.03 |
| April | 3.25 | 4.92 |
| May | 3.90 | 4.68 |
| June | 4.65 | 5.21 |
| July | 9.53 | 6.52 |
| August | 1.95 | 6.03 |
| September | 2.89 | 5.56 |
| October | 1.71 | 3.22 |
| November | 15.44 | 3.40 |
| December | 5.39 | 4.80 |

| | | | |
|----------------------------|---------|-------------------------------|--------|
| Average Rainfall for 1989: | 56.87" | 95 Year Accumulative Average: | 58.37" |
| Annual Deviation: | - 1.50" | | |

Measurable rain fell on 104 days during 1989. There were only 14 days on which the average rainfall for the entire city exceeded 1 inch.

