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EVALUATION OF LAGENIDIUM GIGANTEUM FOR BIOCONTROL OF FLORIDA MANSONIA MOSQUITOES

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March 1995

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Florida Institute of Phosphate Research
1855 West Main Street
Bartow, Florida 33830
(863) 534-7 160
Fax: (863) 534-7165

EVALUATION OF *LAGENIDIUM GIGANTEUM* FOR BIOCONTROL OF FLORIDA *MANSONIA* MOSQUITOES

FINAL REPORT

J. P. Cuda, Ph.D. and Jonathan A. Hornby, Ph.D.
with
Brian Cotterill, M.S., Maria Cattell, B.S., and Dana Gosnell, B.S.

BOARD OF COUNTY COMMISSIONERS
Hillsborough County Road and Street Department
Mosquito and Aquatic Weed Control Unit
4220 Tampa Bay Boulevard
Tampa, Florida 33614

LEE COUNTY MOSQUITO CONTROL DISTRICT
15191 Homestead Road
Lehigh Acres, Florida 33971

Prepared for

FLORIDA INSTITUTE OF PHOSPHATE RESEARCH
1855 West Main Street
Bartow, Florida 33830

Contract Manager: Gordon D. Nifong, Ph.D.

March 1995

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PERSPECTIVE

Gordon D. Nifong, Ph. D.

Florida Institute of Phosphate Research

Over the years, the Florida phosphate industry has created many lakes and wetlands as a consequence of reclamation of mined lands. Lakes are of value in residential areas, recreational sites, and wildlife habitat. Mansonia mosquitos are a major pest species, not only in the lakes of the phosphate mining region, but elsewhere throughout the state. They are known to be vicious biters, and are believed to be second only to Culex as a potential vector for St. Louis encephalitis, a major concern to human health. Mansonia are rather unique, however, in that they do not develop on the surface of bodies of water. Rather, the larvae and pupae remain submerged, attaching themselves to the roots of floating aquatic plants in order to obtain oxygen. Favorite host species of vegetation are water hyacinth and water lettuce. Submersion makes the larvae more difficult to control through normal surface chemical spraying of insecticides or herbicides. Surface vegetation removal is about the only current option.

This research tested the feasibility of using a natural fungal pathogen, Lasenidium giganteum, as a biological control agent against the mosquito. The fungus directly attacks the mosquito during the breeding cycle. This project was a laboratory, or demonstration project, utilizing wading pools placed in the field near a phosphate lake, and into which lake water, vegetation, mosquitos, and fungus were introduced.

Results of this study were encouraging. In pools treated with the fungus, emergence of adult Mansonia mosquitos was reduced by more that 77%, as compared to untreated controls. It would appear that a next step would be an actual field test, in which L. giganteum would be added to an actual lake infested with vegetation and Mansonia. Any significant reduction in mosquito populations would be of benefit to the health and comfort of Florida citizens.

ACKNOWLEDGMENTS

We are indebted to Frank Sweat and David Sapp, Coronet Industries, Inc., and Lamar Willis, Consolidated Minerals, Inc., Plant City, Florida, for their cooperation and assistance without which this project could not have been undertaken. We thank Iris Sibal, Polk County Environmental Services, Bartow, Florida, for providing the emergence cages used in the field tests. We also thank Tom Loyless, Department of Agriculture and Consumer Services, Jacksonville, Florida, for the larval and adult Mansonia photographs. Finally, we gratefully acknowledge the administrative and technical support of the staff of the Hillsborough County Road and Street Department, Mosquito Control Unit, especially Dan Gorman, Joel Jacobson, Marianne Henson, Fred Boston, Dennis Boone, and Eddie Williams.

Current address of the senior author is as follows: Department of Entomology and Nematology, University of Florida, P.O. Box 110620, Gainesville, Florida 32611-0620.

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EXECUTIVE SUMMARY

Many unreclaimed pits and settling ponds created by the phosphate mining industry have become infested with waterhyacinth, Eichhornia crassipes (Mart.) Solms., and waterlettuce, Pistia stratiotes L. (Haeger 1979, Lounibos and O'Meara 1982, Morris et al. 1986). The prolific growth of waterhyacinth and waterlettuce associated with these excavations not only displaces more desirable native vegetation but the extensive mats formed by these floating plants produce enormous populations of mosquitoes which threaten the public health and comfort (Haeger 1979, Morris et al. 1986). Specifically, the immature stages of important disease-vectoring Mansonia mosquitoes are dependent upon these aquatic plants for their survival. Unlike other immature mosquitoes which breathe at the surface of the water and are susceptible to chemical controls, the larvae and pupae of Mansonia mosquitoes are unique because they remain attached to the roots of waterhyacinth and waterlettuce to presumably obtain oxygen (Wesenberg-Lund 1918) or escape predation (Van Den Assem 1958, Lounibos et al. 1992). Except for chemical or mechanical plant removal, no environmentally safe yet effective methods have been developed for controlling the cryptic aquatic stages of these pestiferous mosquitoes (Lounibos and O'Meara 1982).

Through a cooperative effort involving the U. S. Dept. of Agriculture, U. S. Army Corps of Engineers, Florida Dept. of Environmental Protection and the University of Florida, several host specific insects have been introduced into Florida from South America which provide substantial control of waterhyacinth and waterlettuce under certain conditions (Cofrancesco 1991). Although biological control of these plants may offer the most promising long-term solution to both the weed and Mansonia problems by reducing the density of the host plants upon which the mosquitoes breed, these weed biocontrol agents do not provide rapid control of the plants in all situations (Cofrancesco 1991). Consequently, control of waterhyacinth and waterlettuce in the mining area

of central Florida is still accomplished mainly with herbicides such as 2, 4-D, diquat and glyphosate. Unfortunately, there is increasing evidence to suggest that these herbicide applications provide only temporary control and may also be detrimental to weed biocontrol agent populations (Grodowitz and Cofrancesco 1990, Haag and Buckingham 1991). Furthermore, because the herbicides are applied directly to plants growing in the water the perception that these compounds are a health threat has been created by the fact that many surface water systems in central Florida are closely interconnected with the underlying ground water system through springs and sinkholes (Southwest Florida Water Management District, unpublished report). For example, the surficial aquifer is highly susceptible to ground water contamination due to the shallow depth to the water table and high recharge rate.

Faced with society's growing concern over pesticide contamination in surface and ground water supplies, it is imperative that innovative management strategies for Mansonia mosquitoes be developed which contribute minimally to the water quality problem, are cost effective and are also compatible with the biological agents purposefully introduced into Florida to control waterhyacinth and waterlettuce.

Lagenidium giganteum Couch is a naturally occurring entomopathogenic fungus that attacks a broad spectrum of mosquito species and appears to be restricted to this group (McCray 1985, Hornby et al. 1992). The non-discriminating host range within the insect family Culicidae, or mosquitoes, results from the ability of the fungus to differentiate between the cuticle of mosquito larvae and other aquatic animals (Kerwin et al. 1991). Upon application to a breeding site, the fungus is activated in water where motile biflagellate zoopores are produced. Unlike bacterial larvicides which must penetrate the foliage and be ingested by the larvae, the free-swimming zoospore actively seeks out its larval mosquito host and infects resident larvae. One to three days after infection by L. giganteum, the mosquito larvae die, new spores are produced and larva-to-larva transmission continues.

We experimentally tested the effect of inoculative releases of the mosquito-specific fungus Lagenidium giganteum Couch on a population of Mansonia dyari during a typical breeding season at one site in eastern Hillsborough County. Mansonia dyari was selected as the target species for this investigation because it is the most abundant species on waterlettuce (Slaff and Haefner 1985), which formed almost a pure stand on the surface of the unreclaimed phosphate pit at the project site.

The temporal distribution of Ma. dyari in the central Florida phosphate mining region has been described in detail elsewhere (Lounibos and Escher 1985, Slaff and Haefner 1985) but is briefly summarized here. There is a peak emergence of adults in the spring and another in the late summer and early fall. Adult emergence ceases during the winter months due to the cooler water temperatures and plant mortality. In general, the fall emergence is greater than in the spring. Unlike other mosquitoes which have a relatively short larval/pupal period, the aquatic stages of Ma. dyari have a protracted life cycle. Data on the closely related Ma. titillans (Haeger 1960) indicates development from egg to adult occurs in approximately six weeks.

We introduced L. giganteum (California isolate), cultured in the laboratory on yeast extract, dextrose and egg yolk into outdoor caged replicated test pools containing water lettuce and larvae of Ma. dyari collected from an inactive phosphate pit. We added susceptible larvae (first and second instars) twice a week between August and December 1993 to simulate natural oviposition by Ma. dyari. We also pumped fresh water into the test pools from the phosphate pit twice a week (August 1993 to March 1994) to prevent stagnation and replenish nutrients extracted by the water lettuce plants. We collected data on weather at the project site, water temperature in one of the test pools, and water quality in the test pools and inactive phosphate pit.

The data from the emergence trap samples and sentinel larvae indicated that Ma. dyari was highly susceptible to L. giganteum at the field application rates of 400 and 800 ppm. Adult emergence was reduced by more than 77 % in comparison to untreated pools. Sentinel larvae provided evidence of recycling of the fungus approximately 14 days post-treatment. We also observed continuous mortality of larval Ma. dyari for a period of 46 days after the second pool inoculation in November 1993 when the water temperature in the test pools did not exceed 38°C, which is lethal to the zoospores. Except for the high water temperature we observed in the test pools prior to the first inoculation (September), our data showed water quality in the test pools and phosphate pit was suitable for fungal growth and zoosporogenesis for the remainder of the study period.

INTRODUCTION

Mansonia mosquitoes are important pests worldwide because they are aggressive biters and are capable of transmitting diseases (King et al. 1960, McDonald 1973, Lounibos and Escher 1985, Lord and Fukuda 1990). North American representatives of the genus, *Mansonia dyari* Belken, Heinemann & Page and *Ma. titillans* Walker (Figure 1), occur in subtropical areas of Florida, Texas and occasionally Georgia (King et al. 1960, Darsie and Ward 1981). These mosquitoes are potential vectors of dangerous human arboviruses such as St. Louis and Eastern Equine Encephalitis (Lounibos and Escher 1985, Lounibos et al. 1990) as well as dog heartworm (Nayar 1990). In central and south Florida, *Ma. dyari* and *Ma. titillans* exhibit three to four generations per year with the potential for year-round occurrence depending upon environmental conditions (Bidlingmayer 1968, Provost 1976, Lounibos and Escher 1985). Both species deposit egg masses on the leaves of the floating aquatic macrophytes waterhyacinth, *Eichhornia crassipes* (Mart.) Solms. and waterlettuce, *Pistia stratiotes* L., (Figure 2). The larvae (Figure 3) and pupae attach to the roots to obtain oxygen and possibly avoid predation (Slaff and Haefner 1985, Lounibos and Linley 1987, Lounibos and Dewald 1989, Lounibos et al. 1992). Because of this habit, the larvae can be controlled effectively only by plant removal (Haeger 1979, Lounibos and O'Meara 1982). However, *Mansonia* mosquitoes continue to plague Florida residents despite aggressive weed control efforts. Faced with the possibility of decreased federal and state funds for aquatic weed control (Department of Natural Resources, unpublished report), development of alternative control strategies of *Mansonia* spp. is essential.

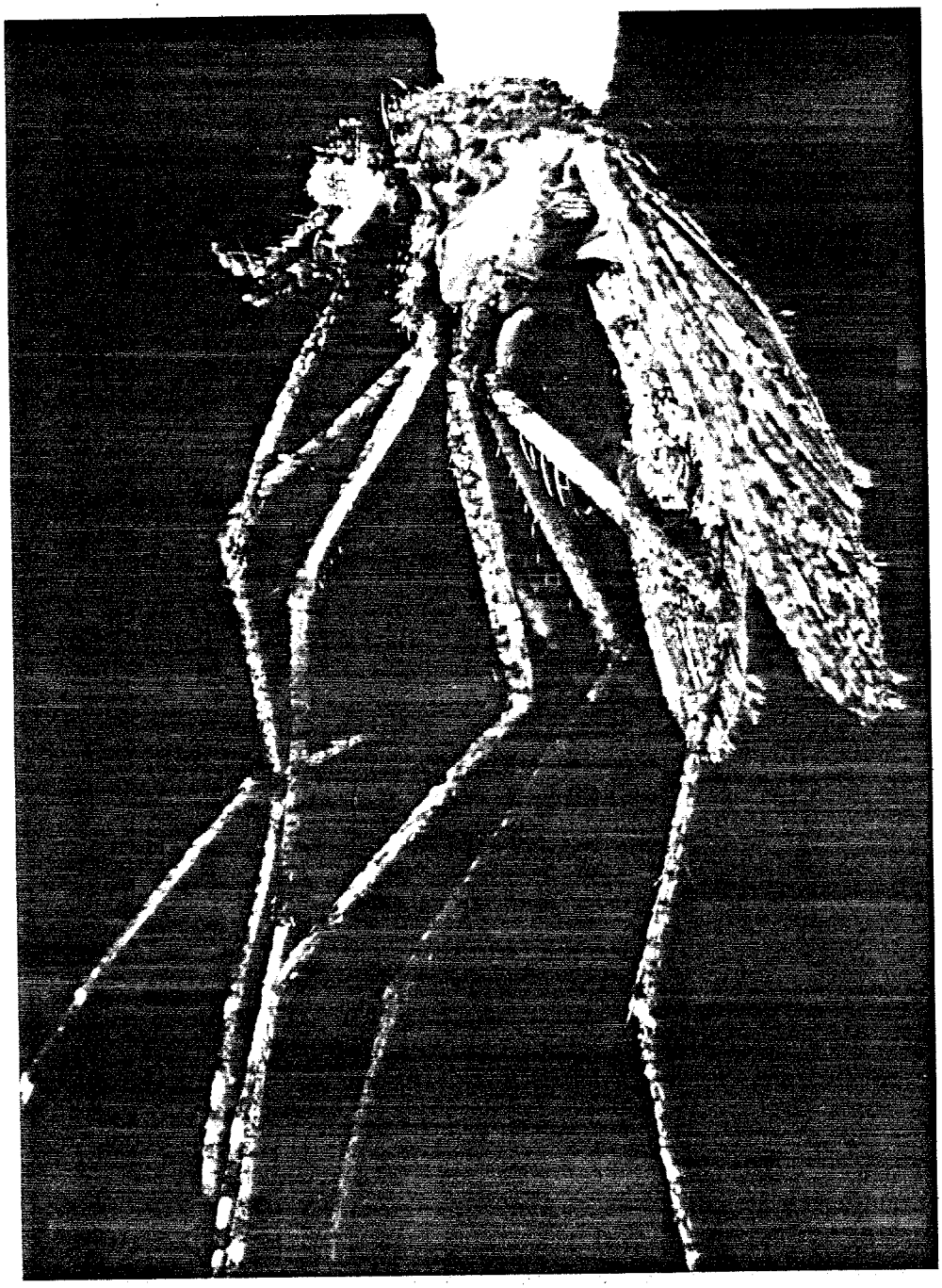

Figure 1. Adult female of Mansonia spp.

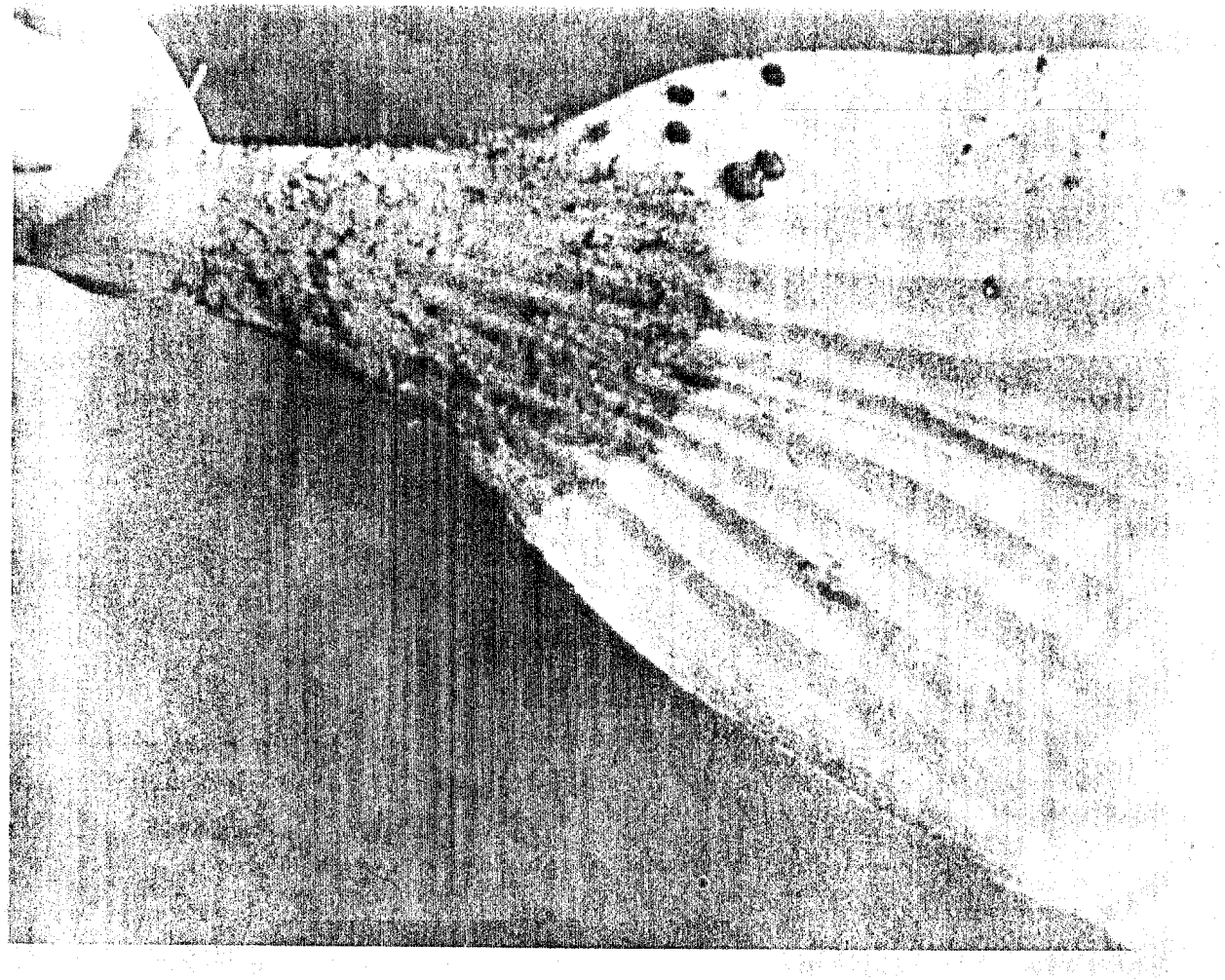

Figure 2. Egg masses of Ma. dyari deposited at base of water lettuce leaflet above the water line.

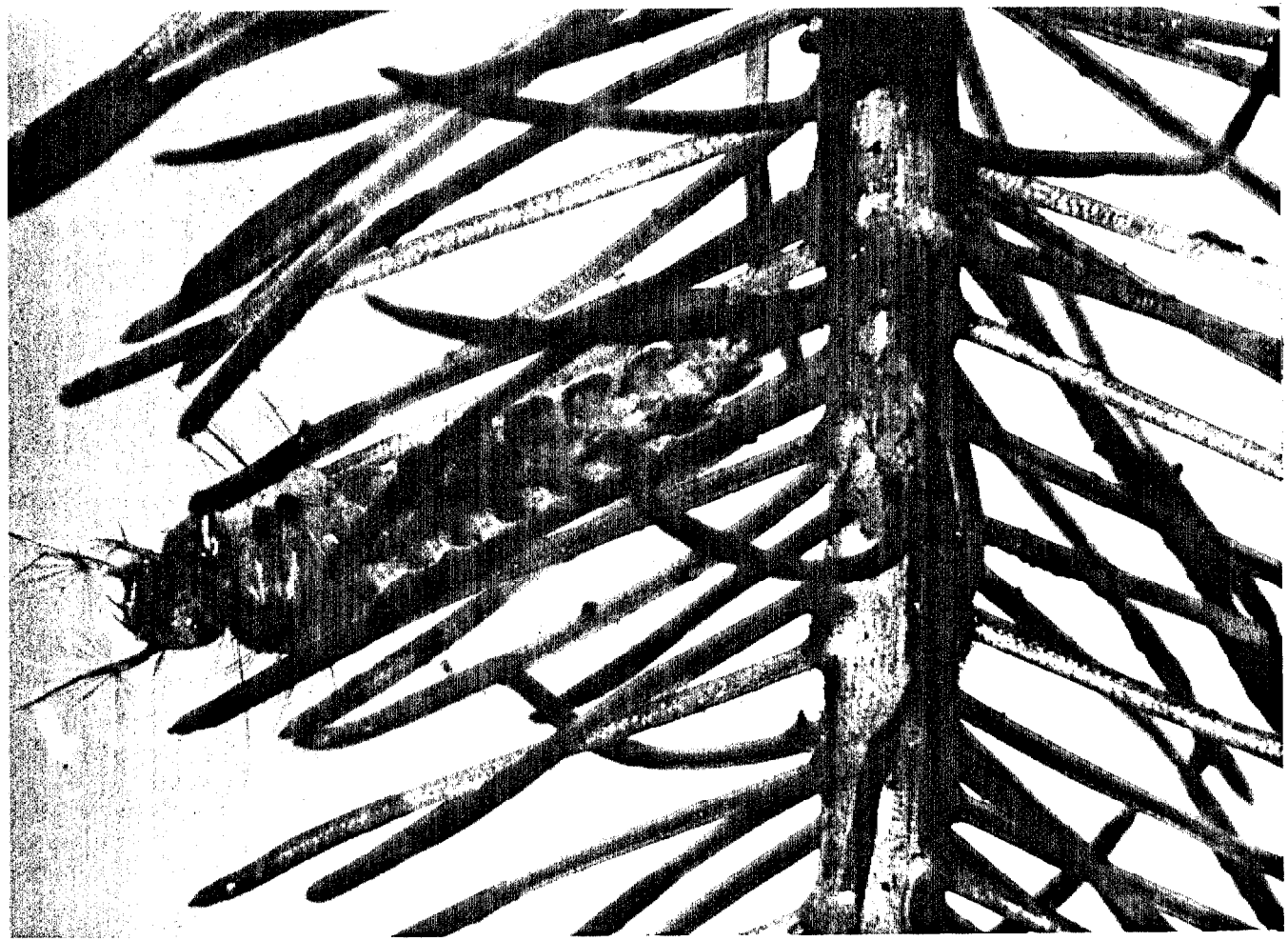

Figure 3. Larva of Mansonia spp. attached to water lettuce root.

Haeger (1979) recognized the necessity for developing specific biological agents for controlling Mansonia mosquitoes, but their feasibility only recently has been investigated (Lord and Fukuda 1990, Lounibos et al. 1992). One of the most promising larval control agents is the fungal pathogen Lagenidium giganteum Couch (Oomycetes: Lagenidiales) (Figure 4). This newly registered microbial agent has the potential to provide sustained biological control of Mansonia mosquitoes (Hornby et al. 1992). Unlike the nonpersistent biorational products formulated from the bacteria, Bacillus thuringiensis Berliner var. israelensis de Barjac serotype H-14, which must be ingested by the mosquito larva, the infective stage of L. giganteum actively seeks out its host (Figure 5), and is-capable of differentiating between the cuticle of mosquitoes and other aquatic organisms (Kerwin et al. 1991). Continuous reduction in larval populations of mosquitoes is possible from a single application of L. giganteum because of its recycling capacity and subsequent amplification of the infective stages.

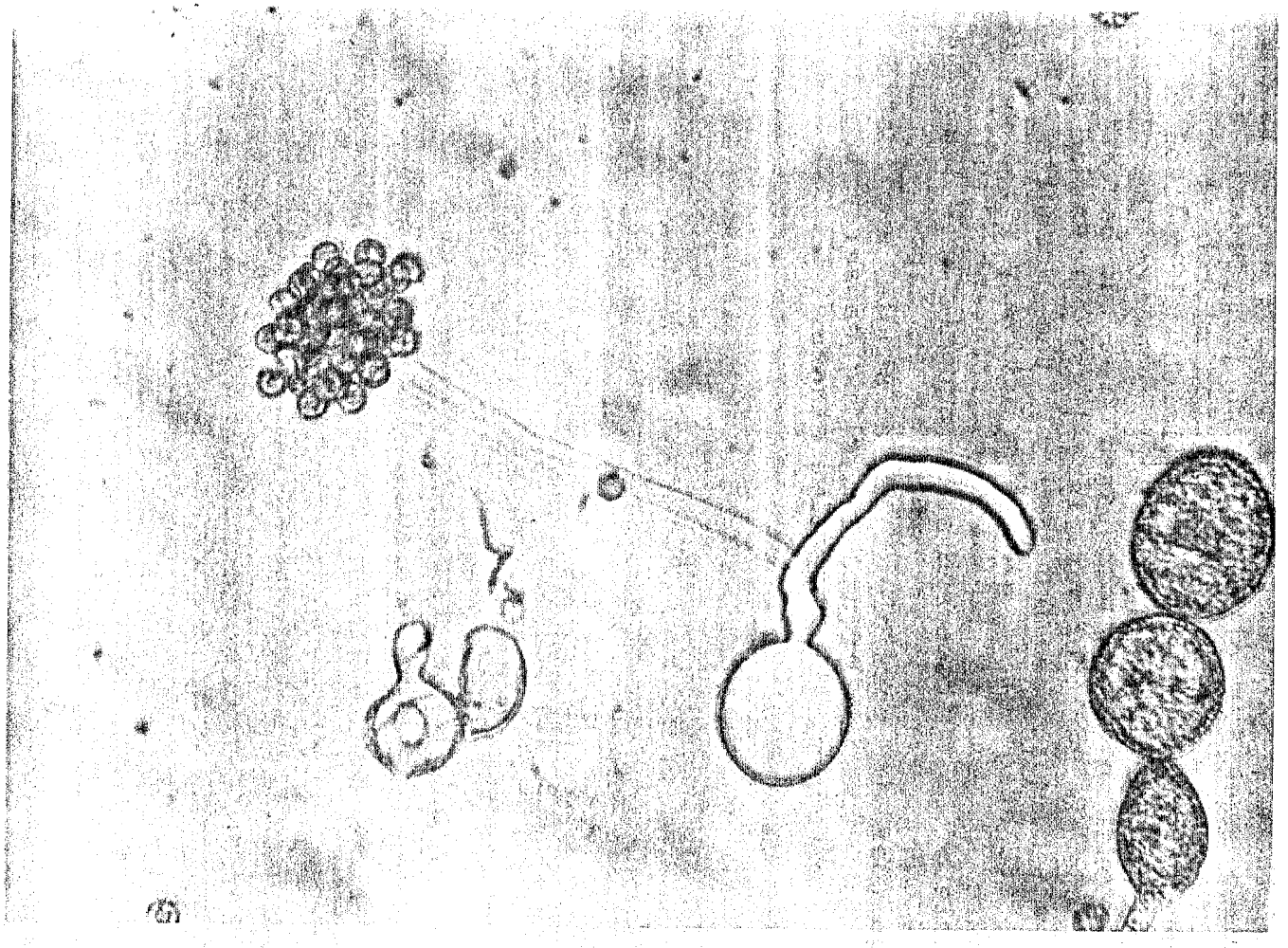

Figure 4. Empty presporangium of L. giganteum with developing zoospores at end of exit tube (after Hornby et al. 1992).

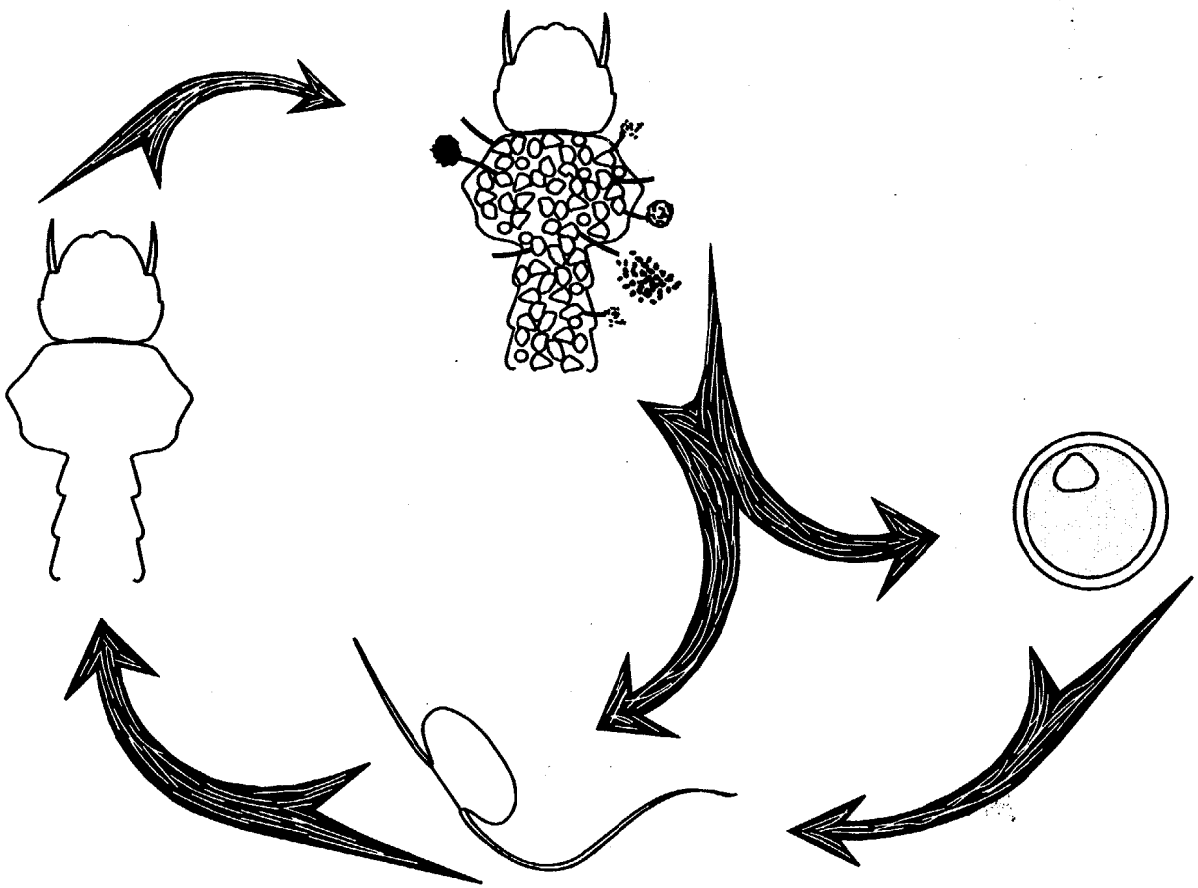

Figure 5. Life cycle of L. giganteum (after Hornby et al. 1992).

MATERIALS AND METHODS

METEOROLOGICAL PARAMETERS

Ambient temperature and relative humidity were measured with a mini-drum hygrothermograph (Oakton Model 08369-70, Cole-Parmer Instrument Company, Chicago, IL) placed inside a small wooden instrument shelter. Temperature and humidity at the project site were measured from April 1993 to May 1994. Rainfall data from January 1993 to May 1994 were provided by Coronet Industries, P.O. Box 760, Plant City, FL.

TEST POOLS

We set up a series of wading pools inside a fenced exclosure (Figure 6). The wading pools (Model GV 200, General Foam Plastics, Norfolk, VA) measured 35 in diameter (88.9 cm) x 8 in deep (20 cm). The exclosure, which was erected to exclude grazing livestock, was located in a pasture adjacent to a waterlettuce infested inactive phosphate pit on the property of Consolidated Minerals, Inc., Plant City, Hillsborough County, Florida (Figures 7 and 8). This particular site was selected because preliminary sampling of the plants indicated the roots supported a heavy infestation of the aquatic stages of M. dyari.

A randomized block design with five blocks was used to test the efficacy of L. giganteum against M. dyari at two treatment levels, 400 ppm and 800 ppm. The experimental design also included an untreated control group. We filled each test pool with 50 liters of water obtained from the phosphate pit with the aid of a self-priming pump (Jabsco Water Puppy, 12 volt Model 6360-1001, Costa Mesa, CA). The water was pumped through a series of sieves (BioQuip, Gardena,

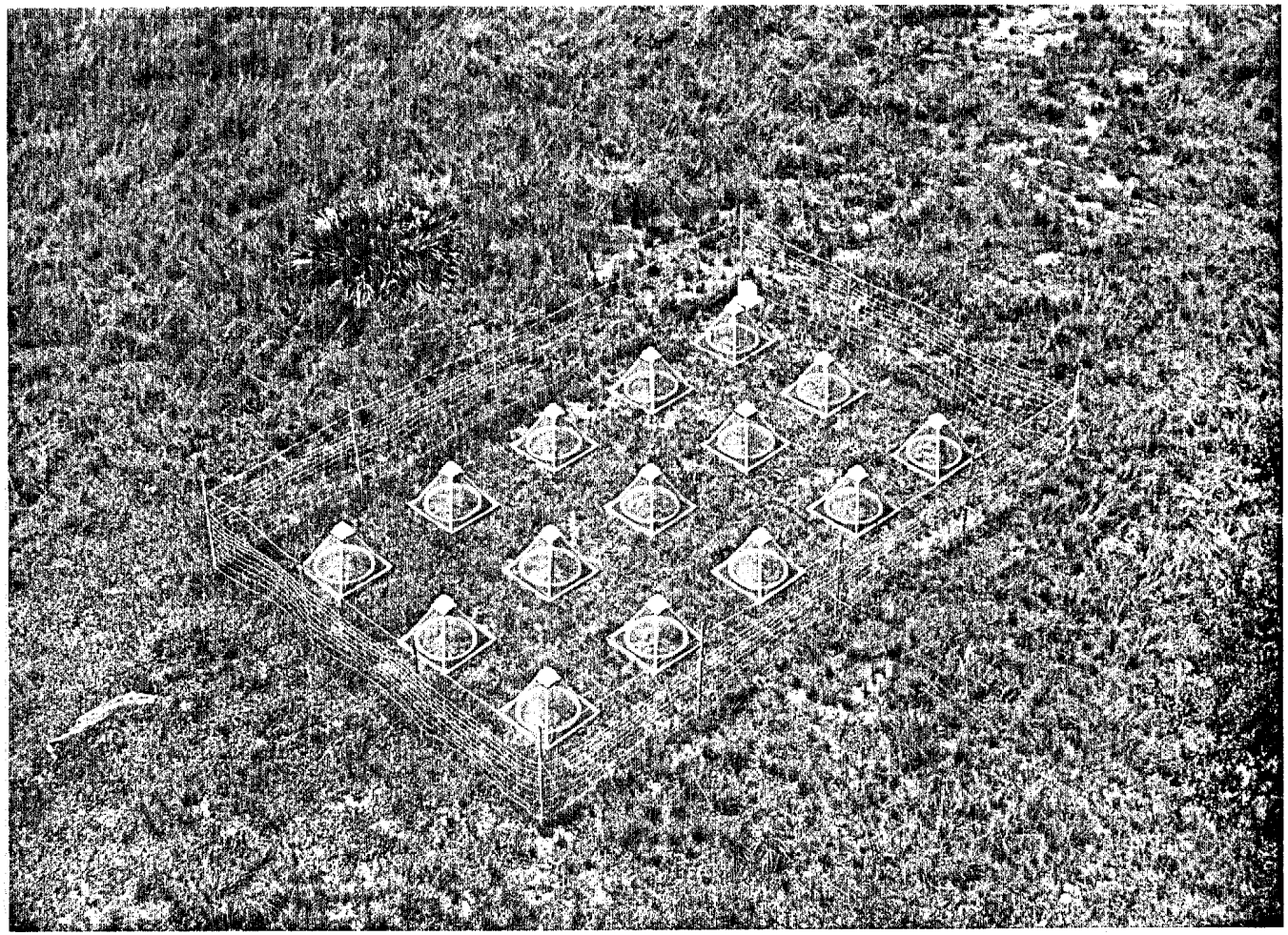

Figure 6. Aerial view of test pools and emergence cages adjacent to inactive phosphate pit.

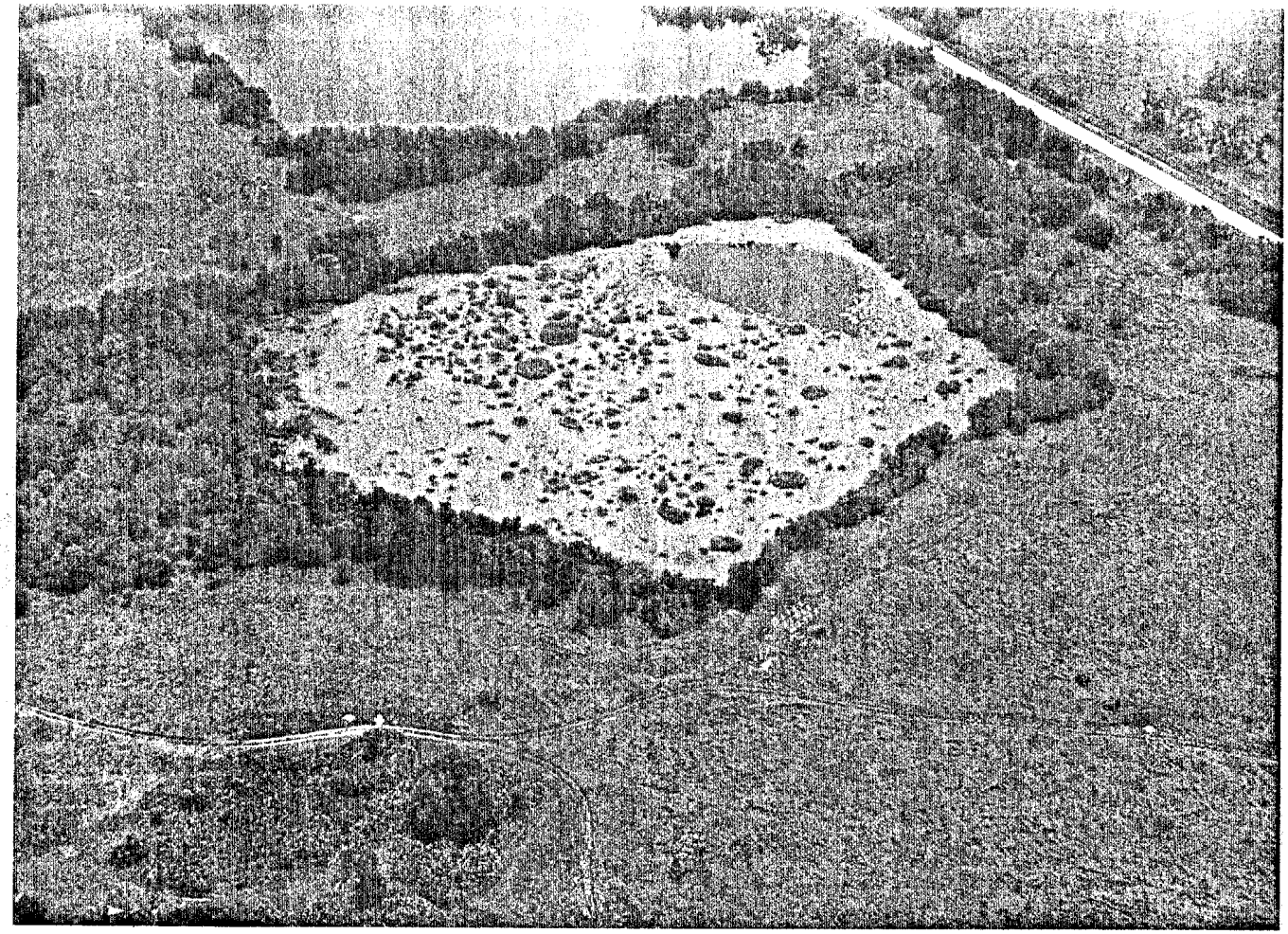

Figure 7. Aerial view of the Coronet Minerals, Inc., inactive phosphate pit in Coronet Junction, Hillsborough County, Florida.

Figure 8. Close-up of waterlettuce mat on surface of inactive phosphate pit, Coronet Junction, Florida.

CA) with Nos. 20, 40 and 100 wire mesh to exclude extraneous mosquito larvae and potential predators such as mosquitofish, Gambusia spp., (Lounibos et al. 1992), dragonfly and damselfly naiads, dytiscid beetle larvae and nymphs of predaceous aquatic Hemiptera (Morris et al. 1986). We then placed 30 small to medium sized waterlettuce plants in each wading pool after removing additional predators and mosquito larvae associated with the plants' roots. This was accomplished with the aid of an ultrasonic cleaner (Mettler Cavitator Model No. 10365, Anaheim, CA) powered by a portable gas generator. The root systems of 5 to 10 plants at a time were exposed to ultrasonic treatment for approximately five minutes. Previous studies (J. P. Cuda, unpublished data) showed this extraction technique to be effective without adversely affecting normal growth and development of water lettuce plants.

Pyramidal emergence screen cages (Slaff et al. 1984) were placed over the pools to collect adult M. dyari mosquitoes emerging from the test pools and also to prevent unwanted mosquitoes (e.g., container breeding Aedes and Culex spp.) from ovipositing in the pools. The emergence cages were supported by a duplex wooden framework consisting of an inner base of 2 in (5.1 cm) x 2 in (5.1 cm) x 37 in (94.0 cm) attached to an outer base of 2 in (5.1 cm) x 4 in (10.2 cm) x 40 in (101.6 cm). The framework not only supported the pyramidal emergence cages but also served as 'a barrier to prevent small mammals from gaining access to the test pools.

The tops of the emergence cages were closed with a section of 10 in (25.4 cm) x 10 in (25.4 cm) plexiglass. Each piece of plexiglass was fitted with the top and threaded base of a BioQuip Mosquito Breeder (BioQuip Products, Gardena, CA). The top 3 cm of the threaded base was sawed off and secured to the plexiglass with hot glue. This arrangement facilitated the passage of the adult M. dyari mosquitoes through a narrow opening created by the plastic baffle and into a collection chamber. A cement lawn sprinkler head donut with an inside diameter of 6 in (15.2 cm) was placed over each plexiglass top to keep it secured to the emergence cage during inclement weather.

Between 27 August and 28 December 1993, we added 25 first and second instar M. dyari larvae to each pool twice a week in order to provide a continuous supply of hosts for the fungus to infect and also to facilitate recycling. Because of the magnitude of the number and size requirements of larvae needed (750 small larvae per week), we had to develop a quick yet efficient technique for collecting larvae of the appropriate size on a routine basis. This was accomplished by the

following procedure. Ten waterlettuce plants were randomly collected in the vicinity of the shoreline of the phosphate pit. Each plant was gently lifted from the surface of the water and placed in a 5 gal (18.9 liter) bucket containing 1-2 gals (3.8 - 7.6 liters) of filtered pond water. The mosquito-laden roots were then vigorously shaken in the water for a few seconds to dislodge the larvae and then the plant was held above the bucket with the root system exposed to facilitate detachment of the larvae as the water drained away from the roots. This procedure was repeated two or three times, depending on the size of individual plants. After the initial extraction process was completed, the contents of the bucket were poured through a series of sieves with nos. 6, 20 and 40 wire mesh. The nos. 6 and 20 wire mesh effectively separated extraneous plant material and large mosquito larvae (third and fourth instars), respectively, from the smaller larvae (first and second instars). The latter constituted the principle contents of the no. 40 mesh sieve. The 40 mesh sieve was then immediately rinsed in a another bucket containing filtered pond water. Aliquots of this final rinsate were then transferred to a 12 in (30.5 cm) x 8 in (20.3 cm) x 2 in deep (5.1 cm) white porcelain pan for final sorting and counting.

The supply of larvae was also supplemented by examining waterlettuce plants in the phosphate pit for egg masses and transporting the egg-laden leaves to the laboratory for subsequent development. In the laboratory, we placed excised leaf sections (ca. 1 cm^2) with the eggs firmly attached into three 12 in (30.5 cm) x 9.5 in (24.1 cm) x 4 in (10.2 cm) acrylic trays until larval eclosion. The trays were half filled with filtered water from the phosphate pit and contained juvenile water lettuce plants germinated from seeds. The waterlettuce seedlings provided sufficient root material for the larvae to attach and obtain oxygen during the development. The larvae were also provided with cultured Paramecium (Carolina Biological Supply Co., Burlington, NC) as a food source (Haeger 1960) until they were mature enough (second instars) to transfer to the wading pools. The trays were held in an environmental chamber (Biotronette Mark III, Model 846, Lab-Line Instruments, Inc., Melrose Park, IL) with four 40-watt, rapid-start 48" (121.9 cm) full spectrum "grow lamp" type fluorescent tubes. The L:D photoperiod was 14:10 and the thermostat was set at 15.6 C^0. This thermostat setting produced an internal daily temperature range between 21.1 and 28.9 C^0.

WATER QUALITY

Unlike other microbial control agents which have a stable shelf life (e.g., Bacillus thuringiensis var. israelensis , or simply Bti), the only formulations of L. giganteum available at the present time maintain their viability for less than 10 days and must be used in fairly clean water (Jaronski and Axtell 1982) with low salinity (Merriam and Axtell 1982). Because low measurements of certain water quality parameters such as TDS, $CaCO_3$, COD and PO_4 have been correlated with fungal efficacy (Kramer 1990), we flushed the test pools with filtered water from the phosphate pit twice a week to minimize stagnation and monitored water quality once a week during the study period (August through December 1993).

We obtained a water sample (250 ml) from eight or nine randomly selected test pools (2 or 3 samples from each treatment group) per week along with three samples from the phosphate pit for comparison. We measured acidity alkalinity, NaCl, chemical oxygen demand (COD), color, conductivity, CO_2, dissolved oxygen (DO), hardness, NO_3-nitrogen, pH, PO_4, total dissolved solids (TDS), turbidity, and water temperature. All water quality parameters except for COD, conductivity, DO, pH, TDS and water temperature were measured with a portable water quality test kit (Hach Model DR-EL 19213B, Ames, IA). The micro-COD test method (Bioscience, Inc., Bethlehem, PA) was used to measure COD. This procedure required the use of a spectrophometer (Bausch and Lomb Spectronic 20, Milton Roy, Rochester, NY) set at a wavelength 440 nm. Conductivity and TDS were measured with a conductivity meter (Digital Conductivity Meter Model 09-326-2, Fisher Scientific, Orlando, FL), DO with a portable dissolved oxygen meter (Hach Model 16046, Ames, IA) and pH with a portable meter (Accumet Model 1001, Fisher Scientific, Orlando, FL).

Maximum and minimum water temperatures were recorded continuously in one of the pools with a maximum-minimum thermometer (Taylor Model 5458, Fletcher, NC) from August 1993 to May 1994 (Figure 9).

FUNGAL CULTURE

The strain of L. giganteum we used in this study originated from a culture provided by J. Kerwin, University of Washington, Seattle, and formerly of the University of California. Because it originated in California, this particular isolate is referred to as the California (CA) isolate (ATCC, 12301 Parklawn Drive, Rockville, MD, 20852,

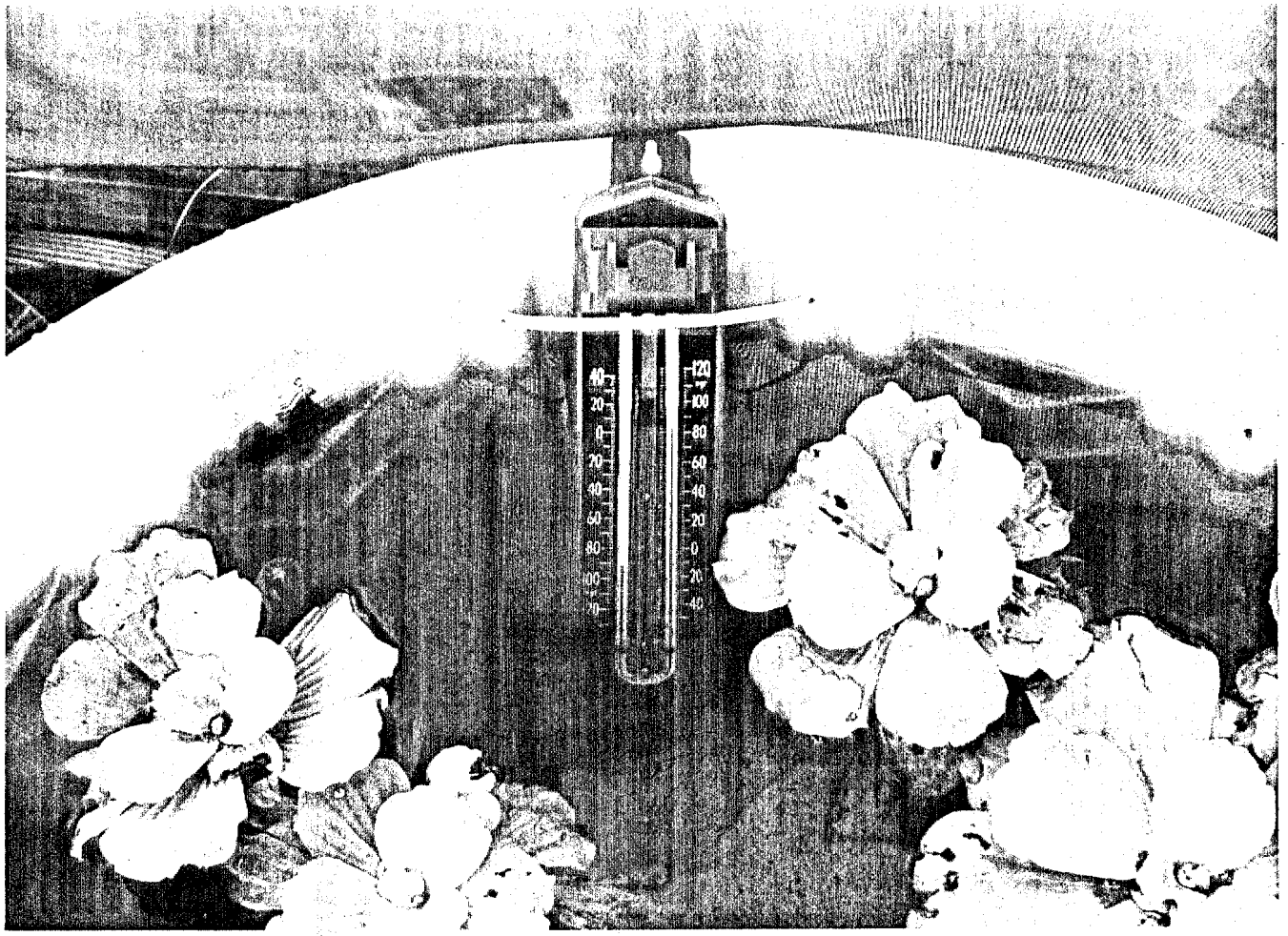

Figure 9. Maximum-minimum thermometer shown in recording position in one of the test pools.

USA, Accession No. 52675). In this study, L. giganteum was cultured as a 10 liter batch fermentation in a LSL Biolafitte 20 liter steam-sterilizable, in-place fermentor (Model BL 20.2). The growth media consisted of yeast extract (1.25 gm/1, Difco Laboratories, Detroit MI), dextrose (0.85 gm/1, J. T. Baker Chemical Co., Phillipsburg, NJ), $CaCl_2 \cdot H_2O$ (0.59 gm/l Fisher Scientific, Fairlawn, NJ), $MgCl_2 \cdot 6H_2O$ (0.41 gm/l, Fisher Scientific, Fairlawn, NJ), Tris-HCl buffer (10 ml/l, 1 M solution, pH 8.0, Fisher Scientific, Fairlawn, NJ), chicken egg yolk (3 per 10 liters). Fermentation was performed at 33° C, pH 6.8, 10 liter air per min, with 100 rpm agitation. The media was inoculated with L. giganteum zoospore and the culture was harvested 48 hours later as presporangia for use in this research project. Lagenidium giganteum has been continuously cultured in the laboratory at the Lee County Mosquito Control District for approximately nine years prior to our field testing against M. dyari.

LABORATORY ASSAYS

Eggs and larvae of Ma. dyari were collected from water lettuce plants and shipped to Lee County Mosquito Control District for bioassays. Eggs were hatched in reverse osmosis (R/O) water with field-collected giant duckweed, Spirodela polyrhiza (L.) Schleiden provided as an attachment substrate. On the second day following eclosion, we transferred individual giant duckweed plants with larvae attached to 50 ml pyrex beakers containing 30 ml of R/O water. Ten larvae were placed in each beaker for the assay. Lagenidium giganteum was added to the beakers in volumes ranging from 1 to 4 ml to achieve the appropriate assay concentration. Three replicates were performed and mortality was evaluated at 24 hrs posttreatment. The effects of host age on the infectivity of L. giganteum to Ma. dyari were also tested by subjecting field-collected third and fourth instar larvae to similar assays.

Because several key factors pertaining to the strain of the fungus and its propagation will ultimately affect field performance (Kerwin and Washino 1987), a final laboratory assay was conducted on 6 Sep 1993 with field-collected second instar larvae. The larvae were collected on 5 Sep 1993 when the test pools were first inoculated with the fungus. This final assay was performed in 500 ml beakers using water from the phosphate pit to ensure the strain of L. giganteum in culture was still highly infective to Ma. dyari.

FUNGUS APPLICATION

Lagenidium giganteum was applied to the appropriate test pools in a liquid suspension of presporangia (Figure 10). A total of 20 ml and 40 ml of the suspension was added to each pool designated as 400 ppm and 800 ppm treatment levels, respectively, on 5 September and again on 5 November 1993. The liquid suspension was applied to each pool (5 pools/treatment level) with the aid of a 10 ml disposable pipette fitted with a rubber pipette bulb.

SAMPLING PROCEDURES

Following the first inoculation of *L. giganteum* into the test pools, we determined the efficacy of the fungus indirectly by counting and recording the number of adults collected in the emergence traps. Adult samples were collected weekly during the study period.

Larval mortality attributed to *L. giganteum* was also determined by using sentinel larvae. This technique facilitated direct mortality counts which would have been difficult if not impossible because of the habit of *Mansonia* larvae and pupae to remain firmly attached to the roots of waterlettuce via the air tubes. From 5 November to 21 December, five second instar larvae were confined in a floating plastic container placed in each test pool similar to that described by Undeen and Becnel (1994) (Figures 11 and 12). The only difference was the size of the corks used for flotation and method of affixing the corks to the container. Four equally-spaced holes large enough to accommodate # 2 laboratory corks (American Scientific Products, McGraw Park, IL) were made along the rim of the container. The corks were inserted into the holes from the outside. In this way, the corks not only buoyed the container but the protruding corks exposed on the inside also served as a discernible point of attachment for the normally cryptic larvae. A standard wooden golf tee was inserted into the drain hole of each container when larvae were added to prevent their escape and removed between sampling dates to facilitate dispersal of the recycled zoospores into the container. Sentinel larvae constituted 20 % of the total larval sample added and were collected twice a week when new larvae were added to each test pool (see above). Sentinel larvae (alive and dead) were collected, placed in 2 dram glass vials and returned to the laboratory where they were counted and observed microscopically to confirm fungal infection. Before microscopic examination, larvae were mounted individually on glass slides with Macroinvertebrate Mounting Medium (Polysciences, Inc., Warrington, PA).

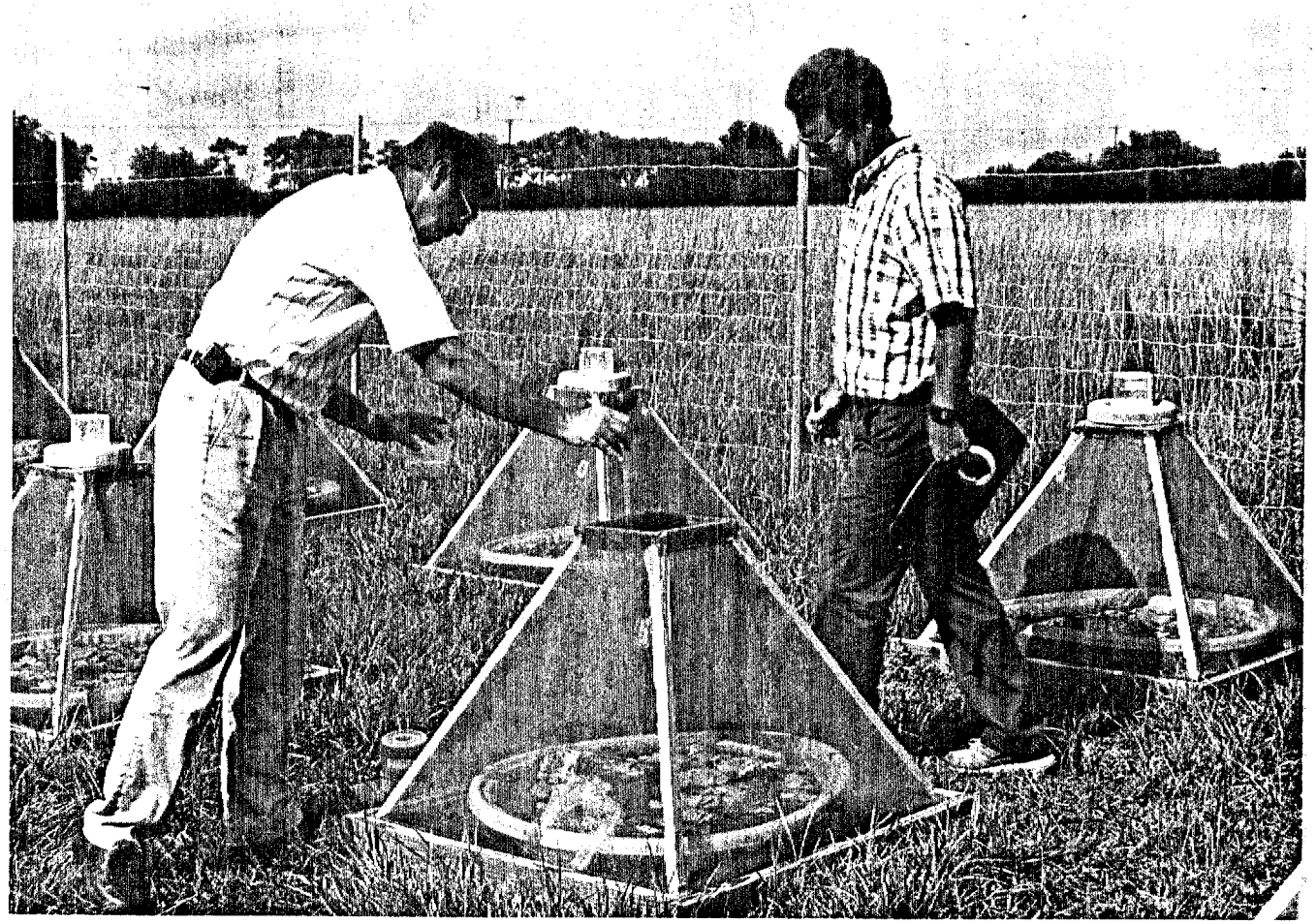

Figure 10. Inoculation of test pools with L. giganteum after removal of plexiglass top of emergence cage. Livestock exclosure in background.

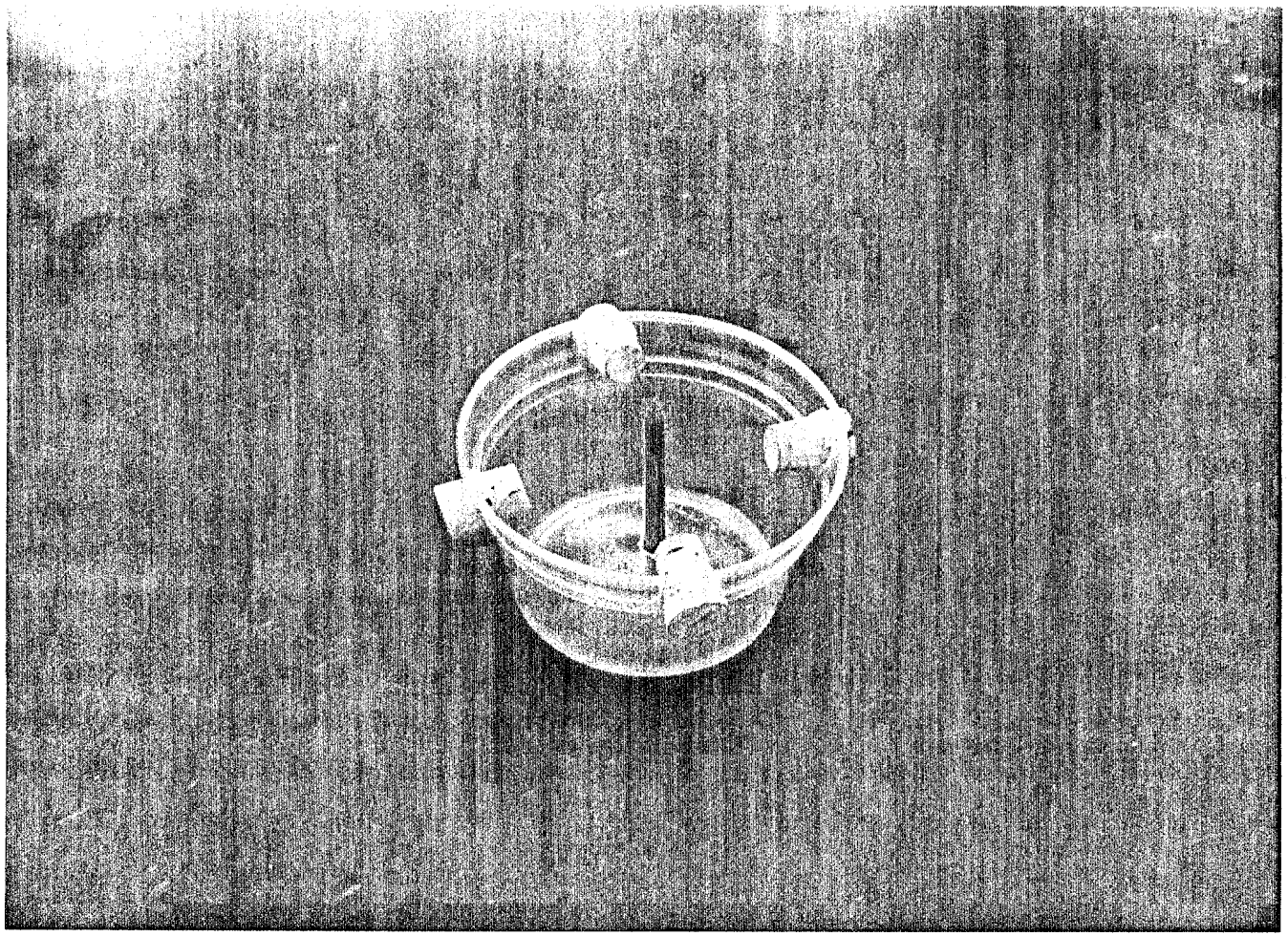

Figure 11. Sentinel larvae sampling device (modified from Undeen and Becnel 1994).

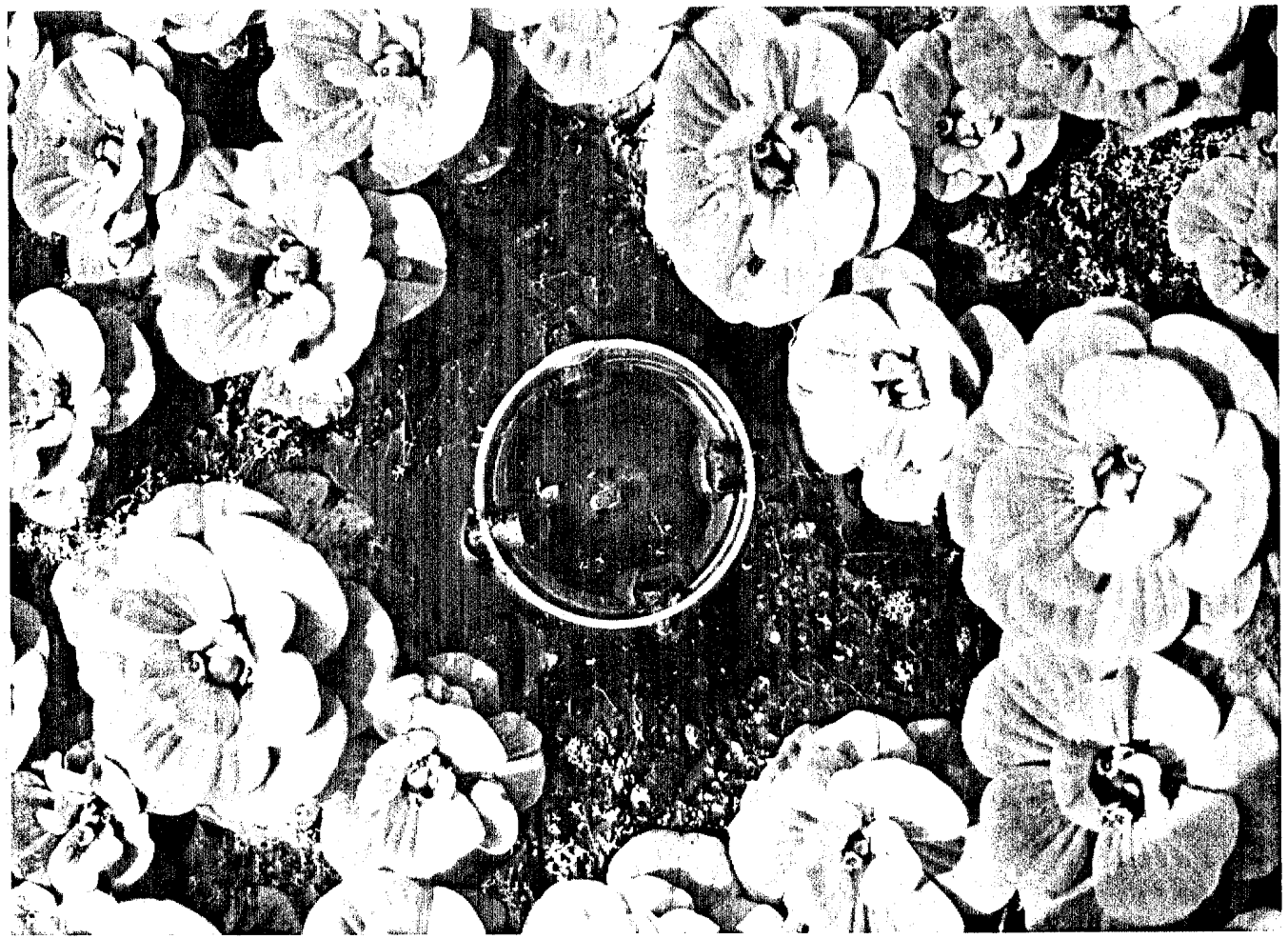

Figure 12. Larval sampling device surrounded by water lettuce in one of the test pools.

STATISTICAL ANALYSES

Data were subjected to analysis of variance using the GLM procedure for water chemistry data (unbalanced) and the ANOVA procedure for the mortality data obtained from sentinel larvae (SAS Institute, 1988). Treatment mortality data for sentinel larvae were adjusted for control mortality by Abbott's formula (Abbott 1925). The arc sine percent$^{\frac{1}{2}}$ formula was used to transform the percent mortality data before analysis. Means were separated by Tukey's studentized range test. The TEST procedure for paired comparisons was used to analyze the water lettuce population data. Dosage-mortality data from the laboratory bioassays were analyzed by probit analysis (POLO-PC, LeOra Software, Berkeley, CA) to determine optimal concentration (in ppm) for infection by the fungus.

RESULTS AND DISCUSSION

ABIOTIC FACTORS

Weather conditions were monitored continuously at the study site prior to and during the actual testing phase. The mean monthly temperatures at the project site for September and November 1993 when the test pools were inoculated with L. giganteum were 25.9° C (range 22-28° C) and 17.2° C (range 7-24° C), respectively (Figure 13). The mean relative humidity during this same time period exhibited very little variability, ranging from only 77.8% to 78.4% (Figure 13).

A substantial amount of rainfall occurred in the vicinity of the project site during 1993 which may have affected certain water quality parameters in the test pools (see below and Hornby et al. 1992). Approximately, 60% of the total rainfall recorded for 1993 (163.68 cm) occurred between the months of May and October (Figure 14) which coincided with the September inoculation of the test pools with the fungus. The total rainfall for the months of June, July, August and September was 20.27, 25.68, 28.70 and 23.04 cm, respectively.

According to Jaronski and Axtell (1983b), the optimal range of water temperature for infection of mosquito larvae by L. giganteum is 21-29° C. Furthermore, McCray (1985) reported that L. giganteum is non-infective at water temperatures above 37° C and below 16° C. Water temperatures around 38° C are lethal to the zoospore but temperatures less than 16° C only delay infection if water temperatures increase during the day of application or presumably during the recycling phase. Water temperatures in the test pools clearly exceeded the upper limit for fungal viability before the pools were inoculated in September (Figure 15). Maximum water

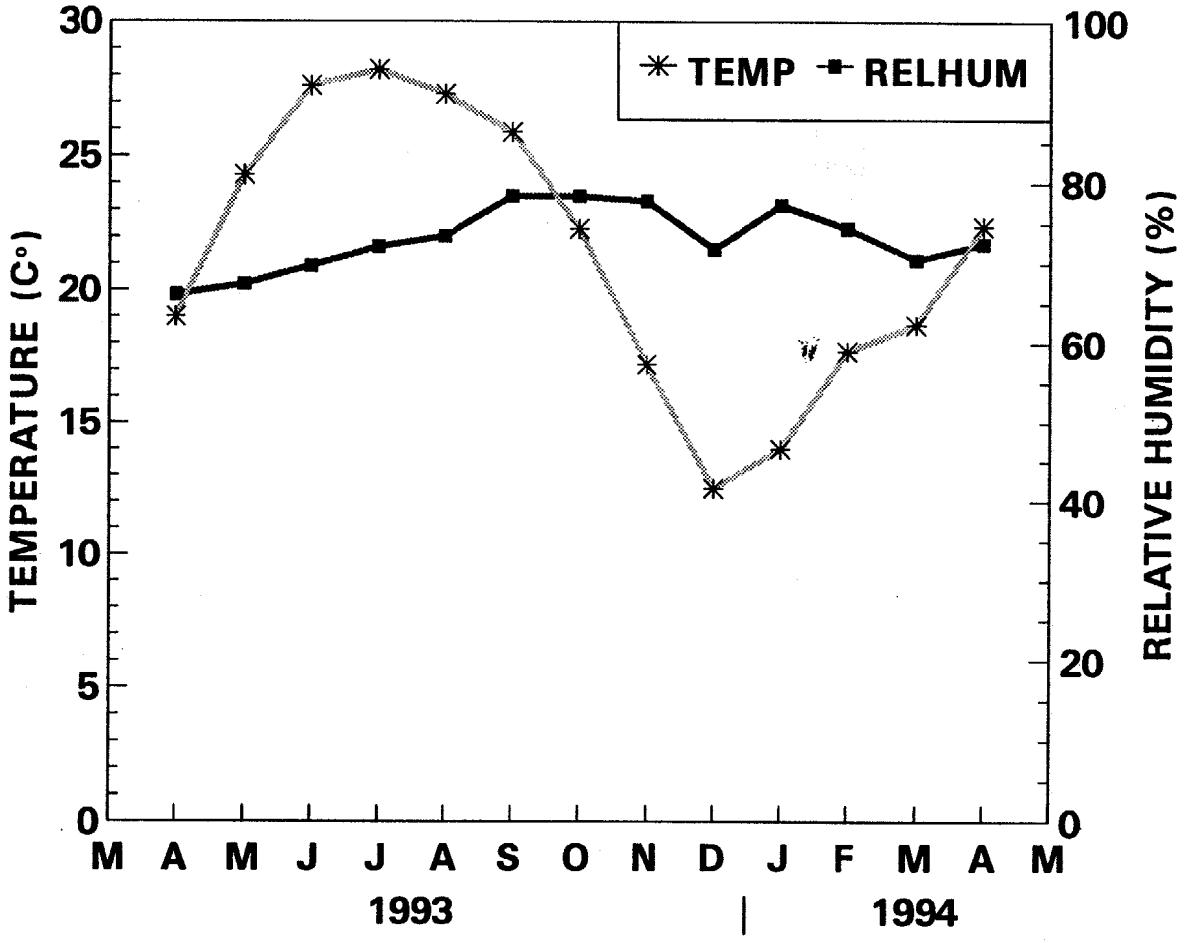

Figure 13. Monthly means of temperature and relative humidity recorded at the project site during 1993-1994, Coronet Junction, Florida.

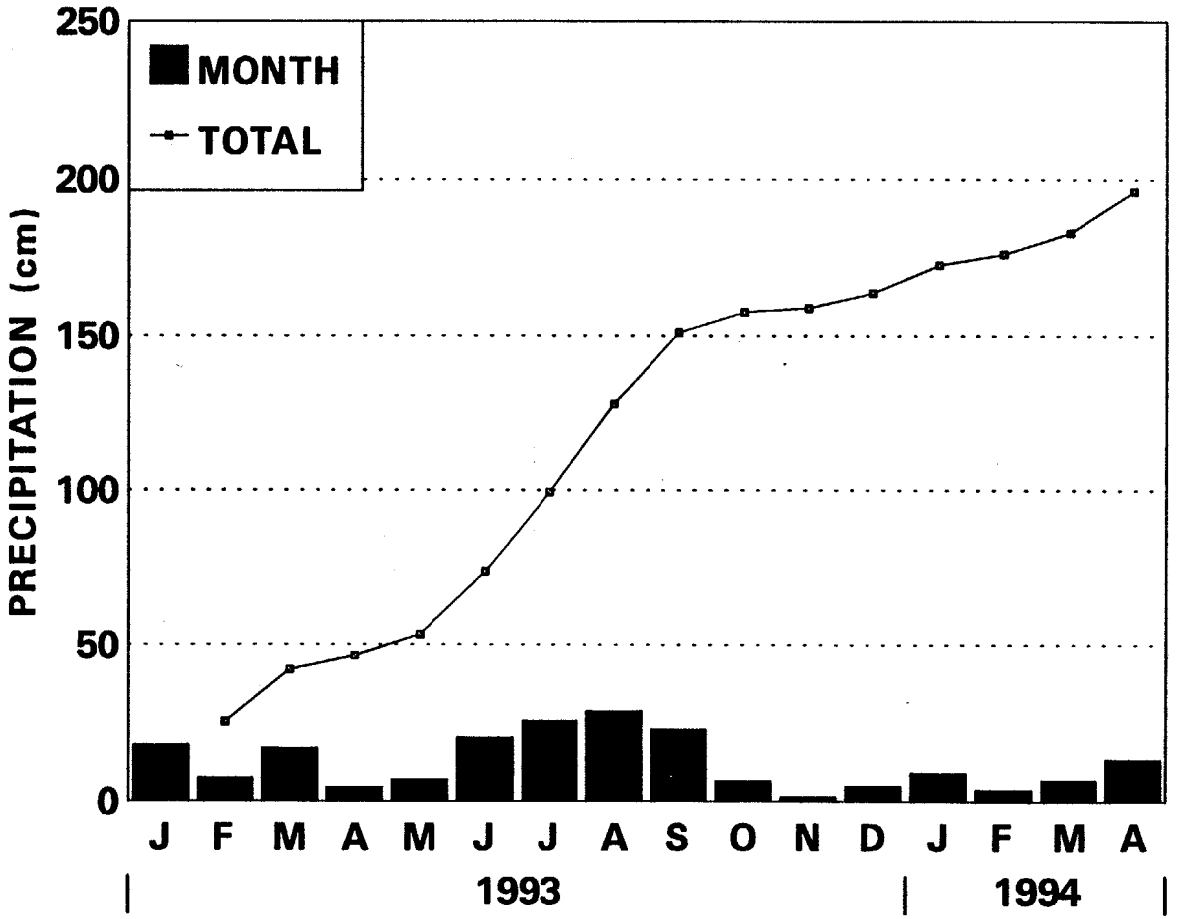

Figure 14. Monthly total and cumulative precipitation recorded by Consolidated Industries, Inc., during 1993-1994, Coronet Junction, Florida.

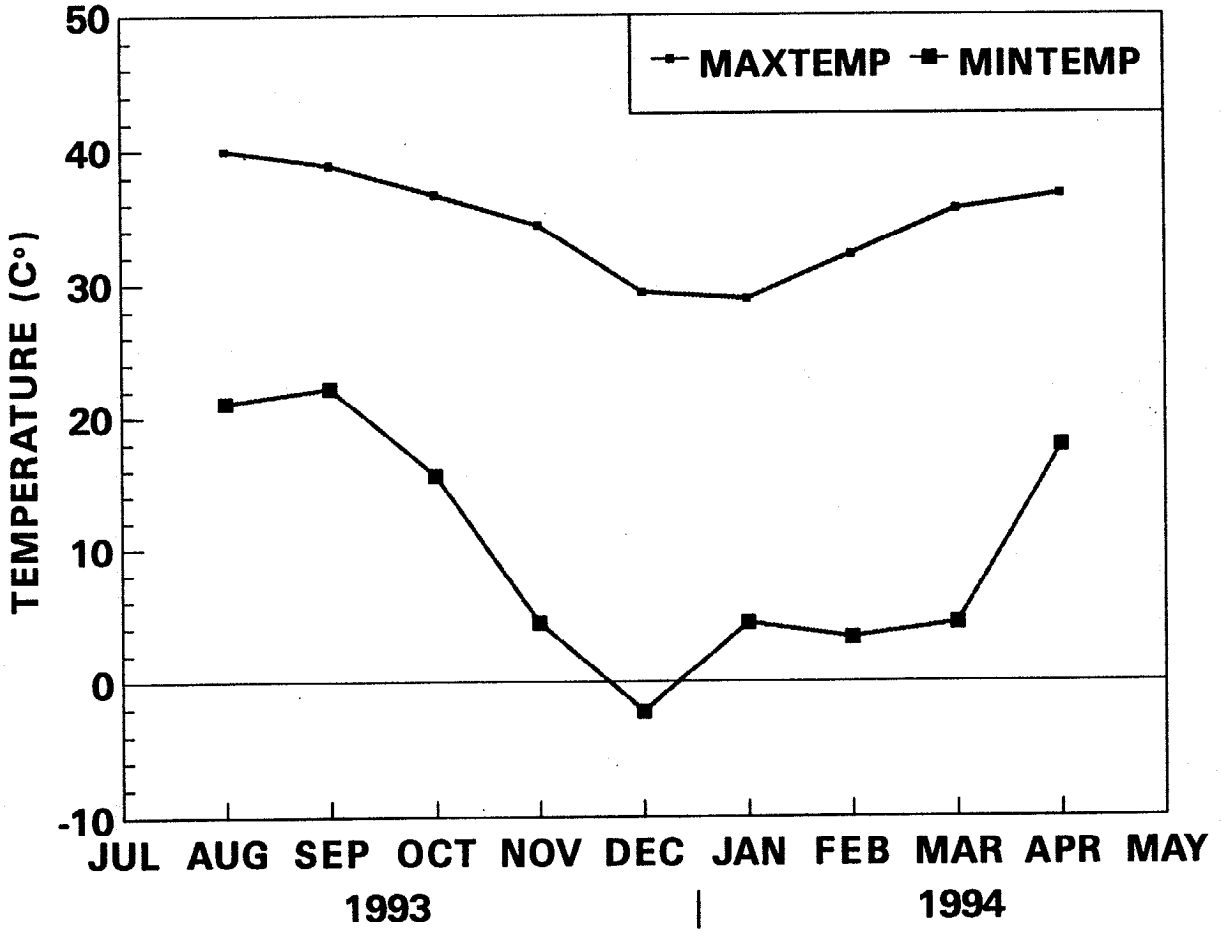

Figure 15. Monthly maximum and minimum water temperature recorded in one test pool at the project site during 1993-1994, Coronet Junction, Florida.

temperature ranged from as high as 40° C in mid-August 1993 to 28.9° C during the last week of January 1994. Similarly, the minimum water temperature extremes occurred in September 1993 (22.2° C) and December (-2.2° C) (Figure 15). Previous studies with Culex quinquefasciatus Say showed that a constant water temperature of 34° C or a variable water temperature regime between 30 and 35°C did not inhibit fungal infection (Patel et al. 1991). Water temperatures in our test pools did not exceed 35.6° C between November 1993 and March 1994 which coincided with the second inoculation and recycling phase of the fungus.

The ability of L. giganteum to produce zoospores and infect mosquito larvae is also affected by the chemistry of the water in which the larvae are developing (Lord and Roberts 1985a, Kramer 1990, Woodring and Kaya 1992). The data on water chemistry encountered in the water samples collected during the course of this study are summarized in Table 1. No significant differences in the levels of chloride, COD, color, nitrate or turbidity were observed in water samples collected from the phosphate pit and test pools. However, statistically significant differences in acidity, alkalinity, conductivity, CO_2, DO, hardness, pH, phosphate and TDS occurred between the phosphate pit and test pool samples. No differences in these same water chemistry parameters were apparent among the test pool samples. It is noteworthy the values for conductivity, COD, hardness, nitrate, pH, phosphate TDS for both the phosphate pit as well as the test pool samples were below levels determined to be inhibitory to the fungus (Jaronski and Axtell 1982, Kramer 1990). Moreover, the lower values for some of the water chemistry parameters observed in the test pools (e.g., alkalinity, conductivity, CO_2, hardness, phosphate and TDS) may have resulted from dilution of the test pool water by the frequent and sometimes heavy rainfall events between sampling dates.

BIOTIC FACTORS

Clearly, if the population of waterlettuce plants declined appreciably in the test pools during the course of this study, then the experimental population of Ma. dyari mosquitoes developing in each test pool also would have been compromised due to the loss of suitable habitat, i.e., plant roots for attachment of larvae and pupae. When we assessed the plant population in each test pool in April 1994 at the end of the study (Table 2), we discovered the water lettuce population increased significantly from the original 30 plants placed in the test pools in August 1993 (paired t-test, $t=4.38$, $df=14$, $p < 0.001$). Because the plant populations were

Table 1. Water quality parameters of experimental pools and inactive phosphate pit, Coronet Junction, Hillsborough County, Florida.

	Treatments ($\bar{x}$ ±SD)[1]							
Parameter (Unit)	N	Pit	N	Control	N	400 ppm	N	800 ppm
ACIDITY (mg/l)	47	0.26(1.05) a	48	1.46(2.24) b	46	1.48(2.17) b	44	1.06(2.03) ab
ALKAL INITY (mg/l)	41	31.71(5.20) a	44	20.11(5.95) bc	40	18.50(4.56) c	38	20.79(6.93) b
CHLORIDE (mg/l)	43	8.66(2.90) a	45	8.24(3.94) b	42	6.98(3.61) a	41	8.60(5.06) a
COD (mg/l)	42	66.30(22.28)a	47	75.31(21.72)a	45	75.08(19.86) a	43	74.30(19.40)a
COLOR (units)	47	64.04(19.38)ab	48	62.40(21.14)b	46	67.07(25.96) ab	44	73.98(26.73)a
CONDUCTIVITY (µmhos/cm)	43	116.69(23.81)a	42	79.69(26.29)bc	41	70.71(23.61) c	41	85.00(38.10)b
CO_2 (mg/l)	47	17.02(6.17) a	48	6.83(2.221)b	46	6.78(2.17) b	44	6.86(1.90) b
DO (mg/l)	43	4.16(3.90) a	48	6.92(1.02) b	46	6.69(1.07) b	43	6.38(1.32) b
HARDNESS (mg/l)	42	34.40(7.09) a	43	20.88(8.18) bc	40	19.88(6.65) c	38	23.29(10.35)b
NO_3 (mg/l)	41	0.07(0.07) a	44	0.09(0.08) a	41	0.11(0.07) a	38	0.08(0.07) a
pH	44	6.40(0.17) a	48	6.60(0.35) b	46	6.45(0.49) ab	44	6.51(0.36) ab
PO_4 (mg/l)	40	2.71(0.24) a	42	1.69(0.70) c	39	1.93(0.68) bc	37	2.03(0.68) b
TDS (gm/l)	43	77.27(15.33)a	42	53.32(17.95)bc	41	47.20(15.66)c	41	56.48(25.48)b
TURBIDITY (NTU)	44	15.79(7.13) a	47	13.89(7.68) a	46	14.10(9.47) a	44	16.00(9.50) a

[1]Means within a row followed by the same letter are not significantly different (p=0.05, Waller-Duncan Test, [SAS Institute 1988]).

Table 2. Productivity of water lettuce, Pistia stratiotes L., in test pools between August 1993 and April 1994, Coronet Junction, Hillsborough County, Florida.[1]

Variable	N	$\bar{x}$ (±SD)	Min	Max
Pool	0	-	-	-
Treatment	0	-	-	-
Pretest	15	30.0(0)	30.0	30.0
Post test	15	53.9(19.7)	21.0	85.0
Difference[2]	15	23.9(19.7)	-9.0	55.0

[1]A total of 30 plants was placed in each test pool approximately one month prior to field tests with Lagenidium giganteum Couch.

[2]Paired-comparisons t test (t=4.38, p=0.0009, [SAS Institute 19881).

actually increasing during the field testing of the fungus and since predators were effectively excluded by filtration and ultrasound techniques, then it would be reasonable to assume that any measurable decline in emergence of Ma. dyari in the 400 and 800 ppm treatment pools should be attributable only to the effects of L. giganteum when compared to the untreated control pools.

BIOASSAYS

A total of 95 egg masses and approximately 4,000 larvae (all instars) of Ma. dyari collected between January and November 1993 were used in laboratory strain selection and dosage-response studies. Comparisons of lethal concentrations of L. giganteum in three replicates showed mortality of two-day-old neonate larvae in R/O water was the same and the slopes were parallel at the 95 % confidence level (g= .144, .145; same, parallel; respectively). The LC_{50} of the combined replicates was 107.7 ppm (91.8 - 125.4 ppm, 95 % confidence interval).

Following the inoculation of the test pools on 5 September, a final assay was performed on 6 September at the Lee County Mosquito Control District with second instar larvae in field water collected-from the phosphate pit to confirm infectivity of the laboratory selected isolate of fungus against M. dyari. The results of this bioassay are shown in Figure 16. Second instar larvae were highly susceptible to the Lee County strain of L. giganteum (1g-pc3 f4 8-28). The average % mortality was 99.6% across the full range of treatment concentrations. Furthermore, a series of laboratory bioassays performed with third and fourth instars indicated that mature larvae of M. dyari were either not infected or were susceptible to infection by the fungus only at very high application rates (>200 ppm). This finding is consistent with that of other researchers (Umphlett and Huang 1972, McCray et al. 1973, Guzman and Axtell 1987, Kerwin and Washino 1987, Lord and Roberts 1987, Woodring and Kaya 1992) who observed that later instars of normally susceptible mosquito species are more or less immune to infection by the fungus, possibly due to changes in chemical cues required for host recognition or an increase in cuticle thickness that occurs during larval development (Woodring and Kaya 1992).

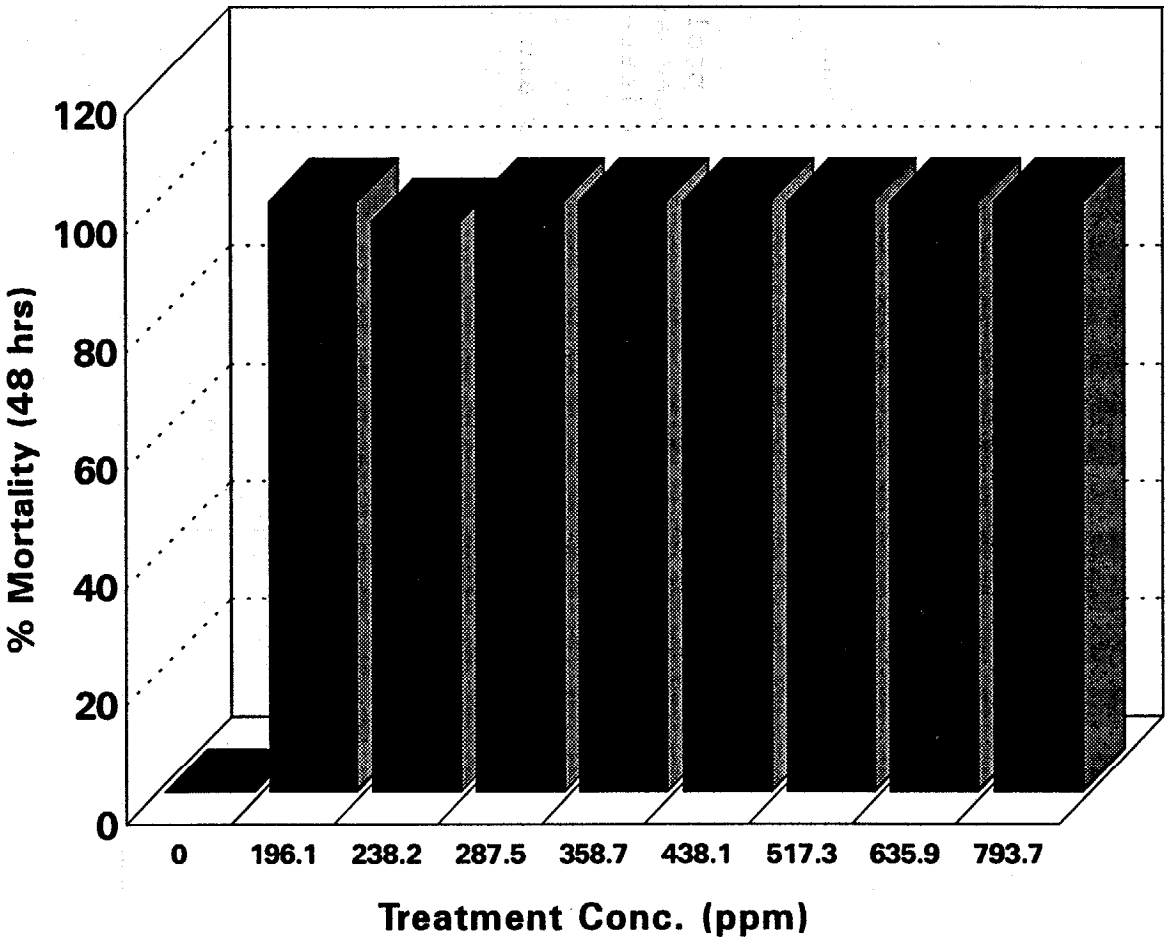

Figure 16. Laboratory bioassay of L. giganteum against field collected second instar larvae of Ma. dyari. Assays conducted in water from inactive phosphate pit.

PHENOLOGY OF *MANSONIA DYARI*

Slaff and Haefner (1985) observed the following seasonal distribution pattern of *Ma. dyari* in the central Florida phosphate mining region of Polk County: Peak populations of mosquitoes occur in late summer and early fall with adult emergence declining by December. *Ma. dyari* is not abundant in the springtime which reflects the inability of this neotropical species to undergo obligatory diapause in the larval stage. Cessation of development in the larval stage is apparently due to low water temperatures. An increase in metabolic activity and subsequent development in the spring is associated with a concomitant increase in water temperature.

The emergence pattern of *Ma. dyari* observed in this study is shown in Figure 17. Adult emergence occurred from September to December 1993 and again in March 1994 with peak emergence occurring in October. Our data are similar to that of other researchers (Bidlingmayer 1968, Slaff and Haefner 1985) except we used the frequency of emergence events rather than absolute numbers of adults to illustrate the temporal distribution of *Ma. dyari*. The actual number of adults that emerged successfully from the test pools was less than expected (approximately 100 mosquitoes) given the number of second/third instar larvae added to each pool (n = 1000) between August and December 1993. Perhaps the low rate of emergence (< 1.0 %) may have resulted from the extremely high water temperatures observed in the test pools from August to November which could have adversely affected larval survival. Another factor may simply have been an artifact of the experimental design. According to Provost (1949), larval density and survival of *Ma. dyari* is substantially greater on waterlettuce when the aerial portion of the plant exceeds 6 in. (15.2 cm) in height. Our small test pools would not have been able to accommodate plants of this size because they have a root system which extends 2 to 3 feet (61 to 91 cm) below the surface of the water.

EFFICACY of *LAGENIDIUM GIGANTEUM*

We assessed the effect of *L. giganteum* on the survival of *Ma. dyari* indirectly by monitoring the frequency of adult emergence following treatment of the test pools with the fungus (Figure 18). The adjusted mortality rates (Abbott 1925) observed in the 400 ppm and 800 ppm test pools were 81.8 % and 72.7 %, respectively. The combined rate of adult emergence was reduced by more than 77 % for both treatment levels when compared to the untreated controls.

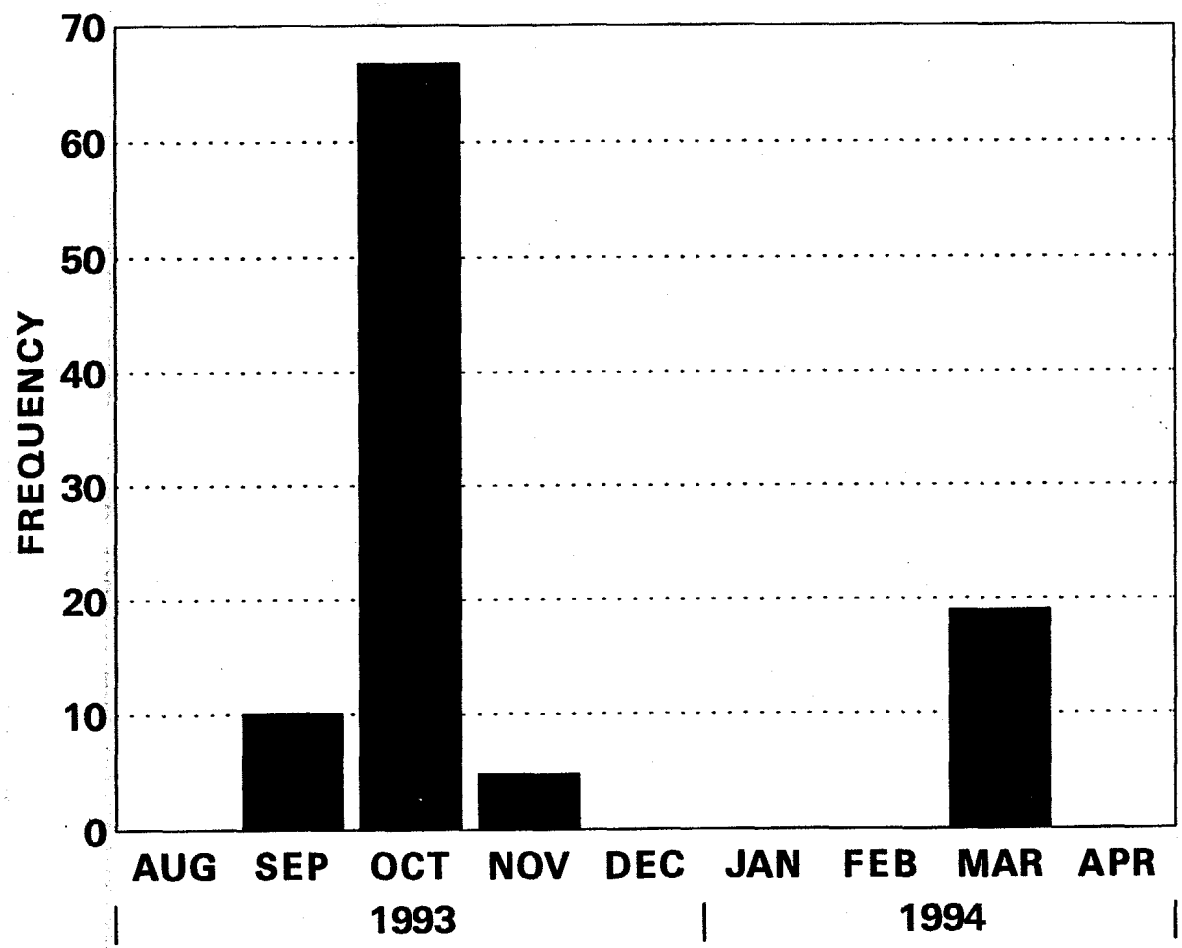

Figure 17. Seasonal emergence pattern of adult Ma. dyari produced in cages test pools during 1993-1994, Coronet Junction, Florida.

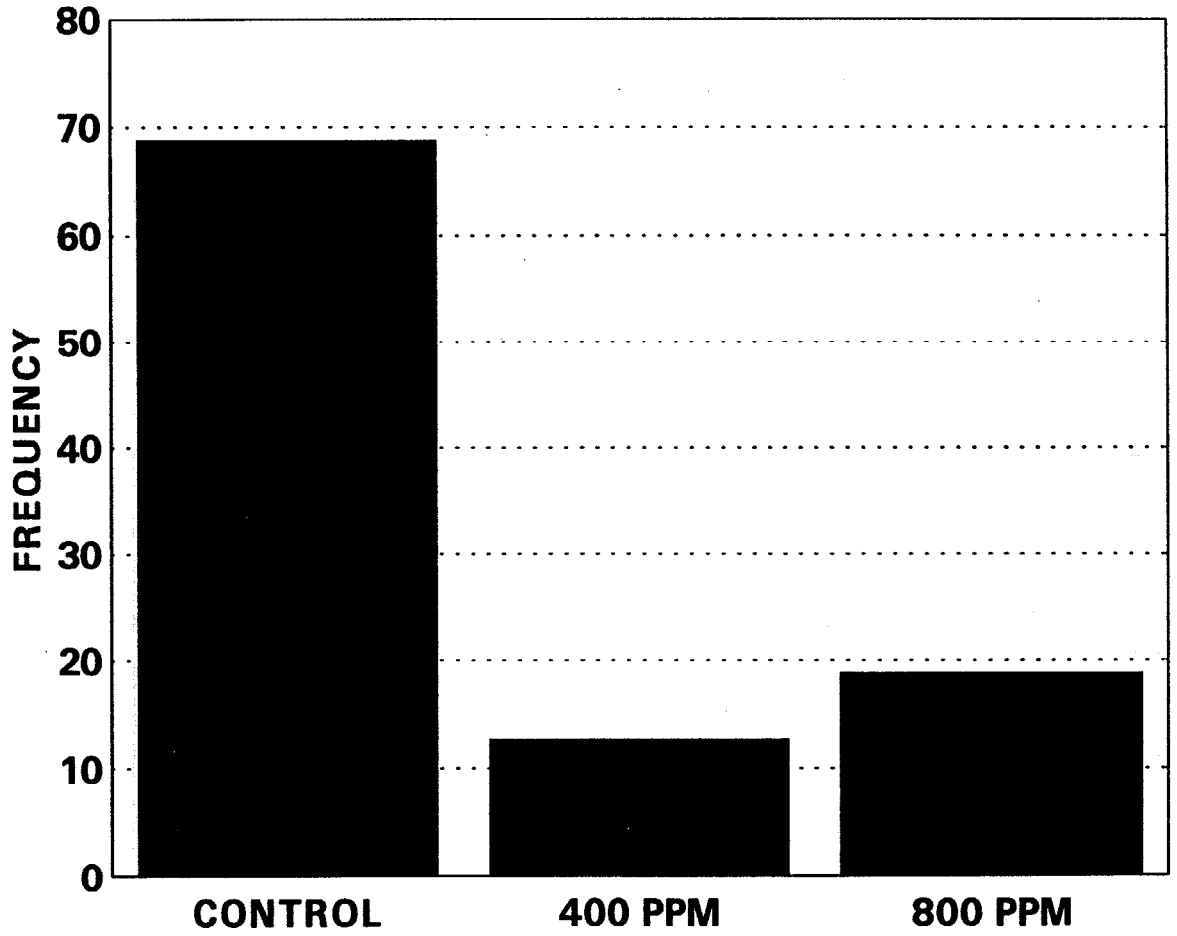

Figure 18. Frequency of emergence of adult Ma. dyari produced in test pools treated with L. giganteum compared to untreated test pools.

The data on the sentinel larvae provided a more direct assessment of the fungus and confirmed the results obtained by adult emergence. Percent mortality of sentinel larvae in the treated pools was significantly higher (ANOVA; df= 2, p = 0.05) than in the untreated controls (Figure 19). Mean adjusted mortality of sentinel larvae in the 400 ppm and 800 ppm treated pools over a six week period was 59.4 and 87.8 8, respectively. Larval mortality averaged 73.6 % for both treatments. No significant differences were detected in the level of larval mortality obtained with either the 400 or 800 ppm treatment rates (Table 3).

We observed evidence of recycling of the fungus seven days after inoculation of the pools and approximately every 14 days thereafter (Figure 19) until we stopped adding first and second instar larvae to the pools in December 1993. Continuous mortality in the treatment pools was apparent for a period of 46 days posttreatment and resulted from the fungus being recycled through dead larvae and reinfecting new hosts.

Table 3. Percentage mortality comparisons of sentinel larvae of Mansonia dyari Belken, Heinemann and Page exposed to Lagenidium giganteum Couch in test pools, Coronet Junction, Hillsborough County, Florida, November-December 1993.

Treatment Comparisons[1]	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
800 ppm-400 ppm	-0.39	16.65	33.68	
800 ppm-Control	22.13	39.16,	56.20	*
400 ppm-800 ppm	-33.68	-16.65	0.39	
400 ppm-Control	5.48	22.52	39.55	*
Control-800 ppm	-56.20	-39.16	-22.13	*
Control-400 ppm	-39.55	-22.52	-5.48	*

[1]Significant comparisons are indicated by (*), one-way ANOVA, Tukey's range test, p=0.05, df=15, (SAS Institute 1988). Mortality data transformed (arcsin sqrt [%/100]) before analysis.

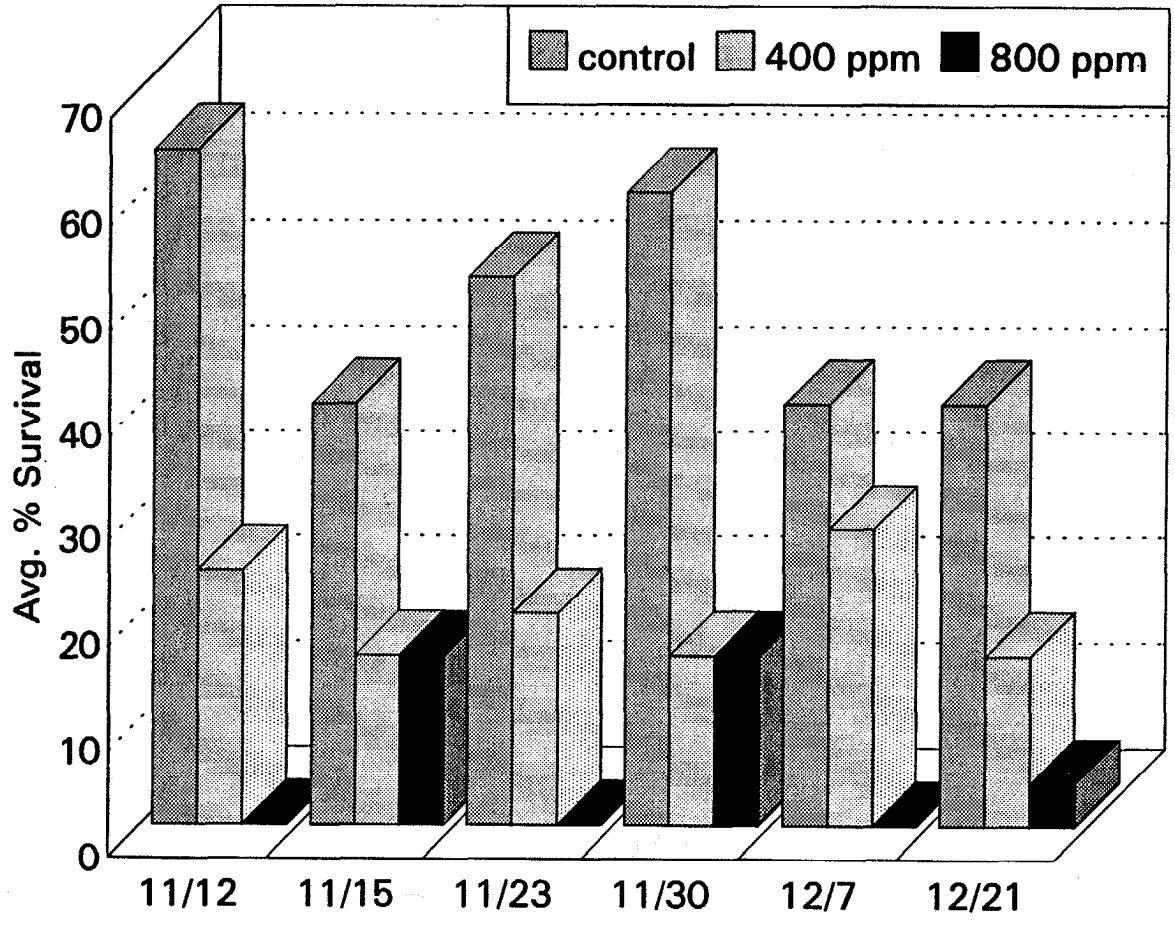

Figure 19. Survival of sentinel larvae of Ma. dyari in test pools treated with L. giganteum compared to untreated test pools.

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this project was to assess the potential of the microbial pathogen L. giganteum as a biological control agent of Mansonia mosquitoes in the phosphate mining region of central Florida. Because Mansonia mosquitoes are significant pests in Florida, and especially in phosphate mining areas (Morris et al. 1986), this study provided us with the first opportunity to evaluate the impact of the fungus on immature and adult populations of Ma. dyari under semi-natural conditions. Mansonia dyari was selected as the target species for this investigation because the immature stages occur preferentially on waterlettuce, a free-floating macrophyte commonly found in inactive phosphate pits. According to Morris et al. (1986), an acre of waterlettuce can yield as many as 30 million adults of Ma. dyari. Clearly, Ma. dyari is of considerable importance to the phosphate industry because of its aggressive biting behavior, its ability to transmit St. Louis and Venezuelan encephalitis viruses (Gorgas Memorial Laboratory 1977) and its capacity to migrate many miles from its larval habitat to plague nearby commercial and residential developments.

Of particular interest was the extent of adult and larval population reduction of Ma. dyari caused by inoculative releases of L. giganteum during an entire breeding season. Our research showed that emergence of adult Ma. dyari between August 1993 and March 1994 was reduced by more than 77% compared to untreated controls. Furthermore, percent mortality of sentinel larvae in the treated pools was significantly higher than in the untreated pools. Evidence of recycling of L. giganteum was also apparent via the sentinel larvae. These research findings are consistent with that of other researchers (Guzman and Axtell 1987, Hornby et al. 1992, and Mulla et al. 1992) who observed reduction in host mosquito

populations following inoculative treatments with this entomopathogenic fungus.

Water quality appears to be an important factor for transmission of L. giganteum. Fresh water with low levels of organic pollution is essential for optimal infection (Lord and Roberts 1985, Guzman and Axtell 1987, Kramer 1990, Woodring and Kaya 1992). Our study showed that the phosphate pit environment had levels of total dissolved solids, hardness, conductivity, chemical oxygen demand, nitrate and phosphate low enough to support high infection rates of larval Ma. dyari. It is unclear from this wading pool study how water depth may affect applications of L. giganteum in waterlettuce-infested unreclaimed phosphate fits, where the depth of the pits may exceed 40 feet (F. Sweat, Consolidated Minerals Inc., pers. comm.) . However, the tendency of the free-swimming infective stage of the fungus, or zoospore, to migrate upwards in the water column (Domnas et al. 1982) where it actively seeks out its host should produce high infection rates among larvae of Ma. dyari. Infected larvae apparently remain firmly attached to the roots of water lettuce even after they succumb to the nutrition-depleting effects of the fungus (J. A. Hornby, unpublished data). In this scenario, newly differentiated zoospores can exit the root-attached cadavers and search for new hosts in the root zone before energy reserves are depleted. Infection of new hosts should continue indefinitely because mild winters and an abundance of water-lettuce permit almost continuous breeding of Ma. dyari (Lounibos et a1.1992). Further research will be required to fully elucidate the epizootiology of L. giganteum in this system.

In addition to Ma. dyari, the phosphate mining region of central Florida produces enormous populations of the closely-related Ma. titillans (Walker) and Coquillettidia perturbans (Walker)(Morris et al. 1986). The biologies of these species are similar to Ma. dyari in that the larvae develop on the root systems of the massive stands of emergent and floating vegetation growing in the mined areas (Morris et al. 1986). Waterhyacinth and floating cattail mats, Typha spp., produce large numbers of Ma. titillans whereas larvae of Cq. pertubans are found only on rooted cattails (Slaff and Haefner 1985). It would be worthwhile to know whether L. giganteum would be a more practical alternative to chemical herbicides currently being used to control the aquatic vegetation which supports larval development of these species.

REFERENCES

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Bidlingmayer, W.L. 1968. Larval development of Mansonia mosquitoes in central Florida. Mosq. News 28: 51-57.

Cofrancesco, A.F. 1991. A history and overview of biocontrol technology, in Proc. 25th Annual Mtg., Aquatic Plant Control Program. Misc. Paper A-91-3. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 117-123.

Darsie, R.F. and R.A. Ward. 1981. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico, Mosq. Syst. Supplement 1. Fresno: Amer. Mosq. Control Assoc.

Department of Natural Resources. 1990. Florida's aquatic plant management program. (unpublished report).

Domnas, A.J., S.M. Fagan and S. Jaronski. 1982, Factors influencing zoospore production in liquid cultures of Lagenidium giganteum (Oomycetes: Lagenidiales). Mycologia 74: 820-825.

Gorgas Memorial Laboratory. 1977(1978). Annual report. Government Printing Office, Washington, D.C.

Grodowitz, M.J. and A.F. Cofrancesco. 1990. Effects of herbicides on Neochetina eichhorniae, a biocontrol agent of waterhyacinth, in Proc. 24th Annual Meeting, Aquatic Plant Control Program. Misc. Paper A-90-3. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 65-74.

Guzman, D.R. and R.C. Axtell. 1987b. Population dynamics of Culex quinquefasciatus and the fungal pathogen Lagenidium giganteum (Oomycetes: Lagenidiales) in stagnant water pools. J. Amer. Mosq. Control Assoc. 3: 442-449.

Haag, K.H. and G.R. Buckingham. 1991. Effects of herbicides and microbial insecticides on the insects of aquatic plants. J. Aquat. Plant Manage. 29: 55-57.

Haeger, J.S. 1960. A technique for rearing Mansonia (Mansonia) titillans (Walker) in the laboratory. Preliminary studies on plant preference for egg laying and larval attachment to plant and artificial material, in Report of the 31st Ann. Mtg. Florida Anti-Mosq. Assoc., April 24-27, Tampa, Florida. pp. 19-21.

_____. 1979. The ecology and control of mosquitoes associated with aquatic habitats: conflicting environmental goals, in Proc. 50th Mtg Florida Anti-Mosq. Assoc., April 24-27, Tampa. pp. 77-78.

Hornby, J.A., B. Cotterill and T.W. Miller, Jr. 1992. Larval mosquito control by Lagenidium giganteum. J. Florida Mosq. Control Assoc. 63: 82-86.

Jaronski, S.T. and R.C. Axtell. 1982. Effects of organic water pollution on the fungus Lagenidium giganteum (Oomcytes: Lagenidiales) for larvae of Culex quinquefasciatus (Diptera: Culicidae): field and laboratory evaluation. J. Med. Entomol. 19: 255-262.

_____. 1983b. Effects of temperature on infection, growth and zoosporogenesis of Lagenidium giganteum, a fungal pathogen of mosquito larvae. Mosq. News 43: 42-45.

Kerwin, J.L. and R.K. Washino. 1987. Ground and aerial application of the asexual stage of Lagenidium giganteum for control of mosquitoes with rice culture in the central valley of California. J. Amer. Mosq. Control Assoc. 3: 59-64.

Kerwin, J.L., D.F. Grant and M.L. Berbee. 1991. Specific induction of encystment of Lagenidium giganteum zoospore by concanavalin A and derivatives of chitin and chitosan. Protoplasma 161: 43-51.

King, W.V., G.H. Bradley, C.N. Smith and W.C. McDuffie. 1960. A handbook of the mosquitoes of the southeastern United States. U.S. Dep. Agri. Handbook No. 173.

Kramer, V.I. 1990. Laboratory evaluation of Lagenidium giganteum (Oomycetes: Lagenidiales) in water from Contra Costa County, California, mosquito sources. J. Amer. Mosq. Control Assoc. 6: 79-83.

LeOra Software. 1987. POLO-PC a user's guide to Probit or LOgit analysis, 1119 Shattuck Ave., Berkeley, CA, 94707.

Lord, J.C. and D.W. Roberts. 1985a. Effects of salinity, pH, organic solutes, anaerobic conditions, and presence of other microbes on the production and survival of Lagenidium giganteum (Oomycetes: Lagenidiales) zoospore. J. Invert. Pathol. 45: 331-338.

_____. 1987. Host age as a determinant of infection rates with the mosquito pathogen Lagenidium giganteum (Oomycetes: Lagenidiales). J. Invert. Pathol. 50: 70-71.

Lord, J.C. and T. Fukuda. 1990. Relative potency of Bacillus thuringiensis var. israelensis and Bacillus sphaericus 2362 for Mansonia titillans and Mansonia dyari. J. Amer. Mosq. Control Assoc. 6: 325-326.

Lounibos, L.P. and G.F. O'Meara. 1982. Mosquitoes and aquatic plants. Aquaphyte 2: 1-2.

Lounibos, L.P. and J.R. Linley. 1987. A quantitative analysis of underwater oviposition by the mosquito Mansonia titillans. Physiol. Entomol. 12: 435-443.

Lounibos. L.P. and L.B. Dewald. 1989. Oviposition site selection by Mansonia mosquitoes on water lettuce. Ecol. Entomol. 14: 413-422.

Lounibos, L.P. and R.L. Escher. 1985. Mosquitoes associated with water lettuce (Pistia stratiotes) in southeastern Florida. Florida Entomol. 68: 169-178.

Lounibos, L.P., N. Nishimura and L.B. Dewald. 1992. Predation of Mansonia (Diptera: Culicidae) by native mosquito fish in southern Florida. J. Med. Entomol. 29: 237-241.

Lounibos, L.P., V.L. Larson and C.D. Morris. 1990. Parity, fecundity and body size of Mansonia dyari in Florida. J. Amer. Mosq. Control Assoc. 6: 121-126.

McCray, E.M., Jr. 1985. Lagenidium giganteum (Fungi), in Biological Control of Mosquitoes, Amer. Mosq. Control. Assoc. Bull. No. 6. H. Chapman, ed. pp. 87-98.

McCray, E.M., Jr., D.J. Womeldorf, R.C. Husbands and D.A. Eliason. 1973. Laboratory observations and field tests with Lagenidium against California mosquitoes. Proc. Calif. Mosq. Control Assoc. 41: 123-128.

McDonald, J.L. 1973. Preliminary results on experimental detection of Mansonia uniformis (Theob.) mosquito immatures. Mosq. News 30: 614-619.

Merriam, T.L. and R.C. Axtell. 1982. Salinity tolerance of two isolates of Lagenidium giganteum (Oomycetes: Lagenidiales), a fungal pathogen of mosquito larvae. J. Med. Entomol. 19: 388-393.

Morris, C.D., M. Slaff, R. Parsons, J.D. Haefner, J.L. Callihan, D. Bailey, D. Kline, J. Nemjo and K. McClain. 1986. Investigations of methodologies for management of mosquito populations in phosphate mining areas. Florida Institute of Phosphate Research. 03-015-043.

Mulla, M.S., J.D. Chaney and J.W. Kerwin. 1992. Field evaluation of Lagenidium giganteum: initial activity, persistence, and recycling capabilities, in Mosquito Control Research, Annual Report 1992. J. Coats and L. Chase, eds. pp. 18-20.

Nayar, J.K. 1990. Mosquito-borne dog heartworm disease. Florida Mosq. Control Factsheet. Vero Beach: Florida Medical Entomology Laboratory, IFAS-University of Florida, Florida Department of Health and Rehabilitative Services.

Patel, K.J., L.M. Rueda, R.C. Axtell and R.E. Stinner. 1991. Temperature-dependent development of the fungal pathogen Lagenidium giganteum (Oomycetes: Lagenidiales) in larvae of Culex quinquefasciatus (Diptera: Culicidae). J. Med. Entomol. 28: 95-100.

Provost, M.W. 1950. Mansonia studies in Leesburg in 1949. Florida State Board of Health. Mimeo. Circ. (unpublished)

_____ 1976. Mansonia mosquitoes: generations per year in Florida, in Proc. 47th Meeting of the Florida Anti-Mosquito Association, 2-5 May, Longboat Key. pp. 24-27.

SAS Institute. 1988. SAS/STAT User's Guide. SAS Institute, Cary, NC.

Slaff, M. and J.D. Haefner. 1985. Seasonal and spatial distribution of Mansonia dyari, Mansonia titillans, and Coquillettidia perturbans (Diptera: Culicidae) in the central Florida, USA, phosphate region. J. Med. Entomol. 22: 624-629.

Slaff, M., J.D. Haefner, R.E. Parsons and F. Wilson. 1984. A modified emergence trap for collecting mosquitoes. Mosq. News 44: 197-199.

Southwest Florida Water Management District. 1988. Ground-water resource availability inventory: Hillsborough County, Florida. (unpublished report).

Umphlett, C.J. and C.S. Huang. 1972. Experimental infection of mosquito larvae by a species of the aquatic fungus Lagenidium. J. Invert. Pathol. 20: 326-331.

Undeen, A.H and J.J. Becnel. 1994. A device for monitoring populations of larval mosquitoes in container habitats. J. Amer. Mosq. Control Assoc. 10: 101-103.

Van den Assem, J. 1958. Some experimental evidence for the survival value of the root piercing habits of Mansonia larvae to predators. Entomol. Exp. Appl. 1: 125-129.

Wesenberg-Lund, C. 1918. Anatomical description of the larva of Mansonia richiardii found in Danish freshwaters. Vidensk. Medd. Dan. Naturhist. Foren. 69: 277-328.

Woodring, J.L. and H.K. Kaya. 1992. Infectivity of Lagenidium giganteum to Culex tarsalis (Diptera: Culicidae) in rice field waters: laboratory evaluation. Environ. Entomol. 21: 183-190.