# CHAPTER 1

# INTRODUCTION

Mosquitoes are the most important group of insects from the standpoint of human and veterinary medicine. Their ability to vector viral, protozoan, and filarial diseases has made them the target of a variety of biological, chemical, and cultural control strategies. Since the advent of DDT in 1939, organic chemical insecticides, particularly chlorinated hydrocarbons, have been the principle means of control. Their extensive use suppressed mosquito populations, thereby reducing the prevalence of mosquito vectored diseases. However, the yellow fever, filariasis and especially malaria still prevail over many areas of the world. In tropical Africa alone at least 500,000 children die of malaria every year (Axtell, 1979). The development of resistance in mosquito populations to various insecticides, by insecticide use or habitat alteration have created a need for other methods of mosquito control (Merriam and Axtell, 1982).

Biological control agents are considered to be important in an integrated pest management (IPM) approach to mosquito control (Chapman, 1974; Axtell, 1979; Jaronski and Axtell, 1982). However, few agents have been sufficiently studied in field situations of mosquito control strategies. Particularly lacking were studies measuring environmental parameters that might directly affect the success of a biological control agent. Some of the most significant progress in recent years has come from the studies of the fungi, particularly those of the genus Lagenidium.

The fungi Lagenidium giganteum (Couch) has been suggested to be one of the most promising candidates as biolocal control agents for mosquitoes (Roberts, 1974; Umphlett, 1976; Federici, 1981; Jaronski and Axtell, 1982; Merriam and Axtell, 1982). This Oomycetes fungus is a facultative parasite that can be grown saprophytically on a variety of media. In the parasitic phase, motile zoospores are infective either orally or percutaneously in the periclypeal region of mosquitoes. The hyphae are coenocytic, being constricted or not constricted at the septum, branched. When growing on a copepod, Daphne, or mosquito larva, the large segmented hyphae are within the host, but numerous delicate hyphae extend from the host for a distance of one or two millimeters to form a fringe. Hyphae are 6 - 40 µm thick, the segments 50 - 3000 µm long. The hyphae walls contain cellulose giving a purplish reaction with chloroiodide of zinc. The protoplasm has the pale Whitish gleams (Bland et al. 1981). The tip of segment can form a sporangium. The sporangium empties after the zoospore was released, the dimensions of zoosporangia which are 6 - 10 x 50 - 300 µm. (Couch and Romney, 1973). This mass becomes differentiated into a variable number of laterally biciliate zoospores. Zoospores are 8 - 9 x 9 - 10 µm, their movement is rather sluggish (Alexopoulos and Mims, 1979). Sexual reproduction was not observed.

An electron microscopic study of L. giganteum (Domnas et al. 1984) showed that L. giganteum zoospores are morphologically very similar to Peronosporales and Saprolegniales. The zoospore typically showed lipid vacuoles, granular vesicles and vacuoles. The tabular type, mitochondria were present as well as dictyosomes. The particular interest in these preparations was the presence of endoplasmic

reticulum which were also found in tabular structures similar to the tinsel flagellum. Serial sections showed that the mastigonemous were found in the interior, whereas the longitudinal tubes were found in the periphery. These tubes appeared to be hollow, and measurements at this time indicated that they were not microtubular. Many cisternae were presented which contained the fine hairs attached to the mastigonemous.

In screening the organism for its enzymatic capabilities, β-glucosidase activity was demonstrated. The enzyme β-D-glucosidase (β-D-glucoside glucohydrolase, E.C. 3.2.1.21) had been found in bacteria, plants and animals. The β-D-glucosidase for *L. giganteum* showed preferential activity for PNPG indicating the best *in vivo* substrate would be an aromatic glycoside. The *in vivo* source of intracellular aryl-β-glucosidase in fungi was not clear; however, extracellular forms could release glucose from aromatic glucosides for subsequent uptake and catabolism (Brunet and Kent, 1955). The function of *L. giganteum* β-glucosides could be better hypothesized after its localization in the cell was determined. However, if it was located at or in the cell surface, it could then hydrolyse aryl-β-glucosides present in the host insect circulatory system (McInnis, Jr. and Domnas, 1974), releasing glucose for fungal uptake and at the same time releasing toxic aglycones into the insect.

Life cycle of *L. giganteum*

Life cycle of *L. giganteum* can be divided into:

A. Vegetative phase

The infective form of *L. giganteum* is the biflagellate motile zoospore, and the two distinct portals of entry for mosquito are the mouth and the cuticle. In the majority of the infected larvae, zoospores are concentrated by the mouth parts and penetrate the tissues of the host larvae in the anterior portion of the digestive tract, usually in the region of the pharynx. Myclial growth spreads rapidly from the point of entry throughout the head, then to the haemocoel toward the posterior, infecting the anal segment and anal gills last.

However, in about 2% of the specimens observed, mycelial growth first appeared in the abdomen, anal segment or anal gills. In these larvae the hyphae grew from darkly pigmented spots on the chitinous exoskeleton. Numerous larvae were observed with infections occurring through both portals of entry.

The hyphae which grow in the haemocoele spread rapidly throughout the body, consuming the entire contents except for the chitinous respiratory tract, and then become septate and enter their reproductive phase.

B. Asexual Reproduction (see Diagram 1).

The individual segments of the hyphae become rounded, granular structures known as sporangia and sporangial formation and larval death are usually simultaneous. A fourth stage larva will usually contain about 20,000 sporangia (McCray, Jr., 1985). One,

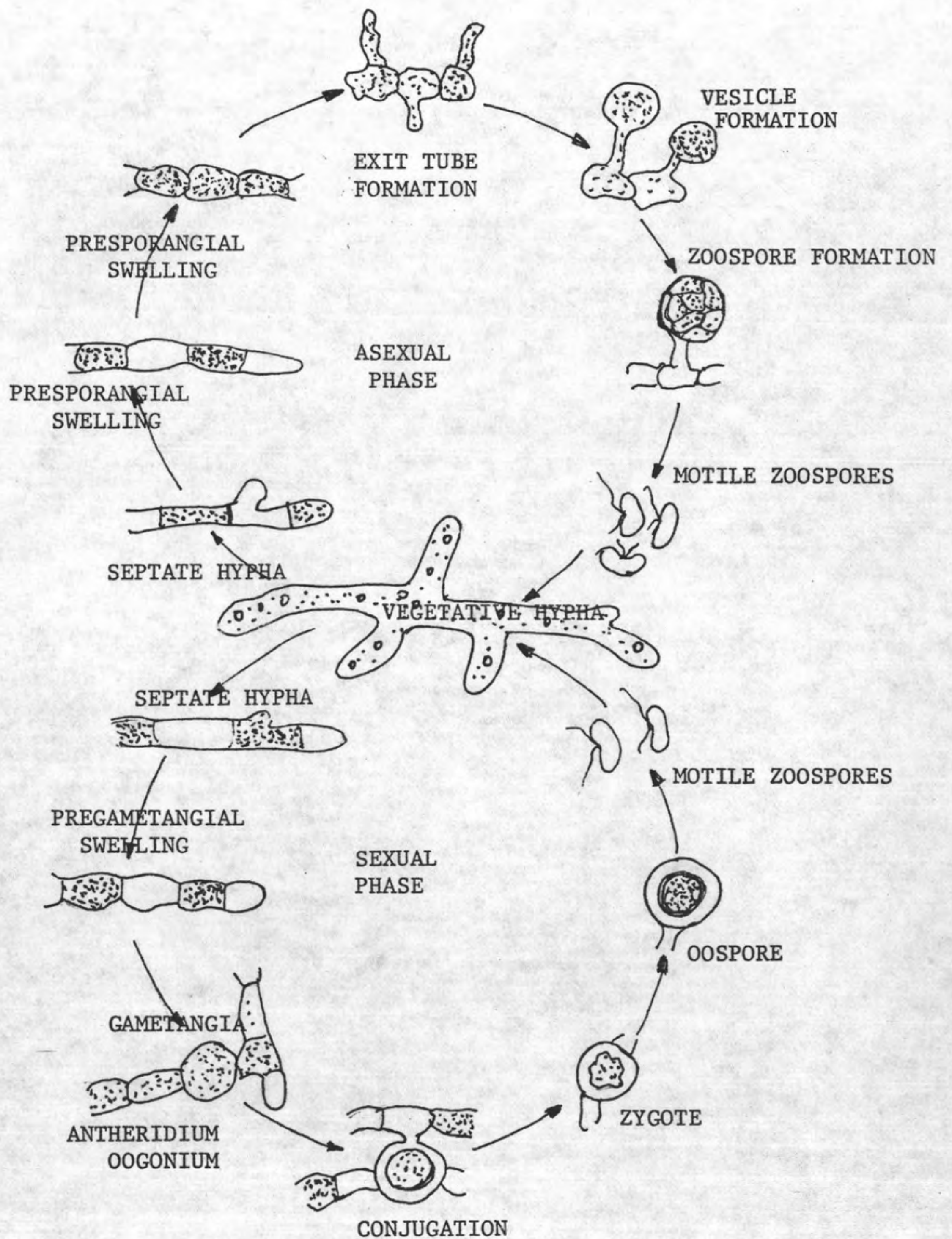


Diagram 1 Life cycle of Lagenidium giganteum

sometimes two, exit tubes are then formed by each sporangium and grow out the body wall of the dead larva. A vesicle is formed at the end of each exit tube, and the entire contents of the sporangium flow into the vesicle. Within the vesicles are formed numerous zoospores (average of 12/vesicle). The wall of the vesicle disintegrates and the motile zoospores are released into the water to repeat the cycle.

C. Sexual reproduction (see Diagram 1)

In the sexual phase, the individual segments of the hyphae become either antheridia or oogonia. The anteridial protoplast moves through a conjugal pore into an adjacent oogonium to form a zygote. This becomes the oospore (resting spore) by formation of a thickened wall around the combined protoplasts. The oospore remains dormant until stimulated to germinate and then releases motile zoospores into the water to repeat the cycles may occur within a single larva and also within a single hypha.

The species of mosquito infected by L. giganteum

L. giganteum was originally found in copepods, Daphnia sp., and the larvae of Culex sp. and Anopheles sp. From preliminary studies, the following is a list of those mosquito species to which experimental infections with L. giganteum have been transmitted in the laboratory at Chapel Hill, North Carolina in summer, 1972 : Aedes aegypti, Ae. atropalpus epactius, Ae. polynesiensis, Ae. triseriatus, Ae. stephensi, Culex pipiens quinquefasciatus, Cx. restuans, Cx. tarsalis, Culiseta incidens.

Umphlett and Huang (1970) isolated their fungus from Culex in 1969 and reported experimental infections of Cx. restuans, An. quadrimaculatus and Psorophora sp. (Umphlett and Huang, 1972). McCray et al. (1973 a) conducted studies in 1969 with an isolate of the original Umphlett strain and reported laboratory infections of Ae. aegypti, Ae. triseriatus, Ae. mediovittatus, Ae. taeniorhynchus, Ae. sollicitans, Cx. quinquefasciatus, Cx. tarsalis, and Cx. nigripalpus, but could not infect several Anopheles species (McCray, Jr., 1985). In laboratory and field studies at the TDL of CDC in Savannah, Georgia, An. albimanus, An. quadrimaculatus, An. stephensi and An. sundaicus were routinely infected. Domnas et al. (1974) reported routine infections of An. quadrimaculatus with L. giganteum in their study on the biochemistry of mosquito infection. Couch and Romney (1973) in their description of L. giganteum, reported the infection of Ae. aegypti, Ae. epactius, Ae. polynesiensis, Ae. triseriatus, An. punctipennis, An. quadrimaculatus, An. stephensi, Cx. tarsalis and Culiseta incidens. Glenn and Chapman (1978) reported a natural epizootic in Cx. territans near Moss Bluff, Louisiana.

Jaronski and Axtell (1983) reported experimental field infections in larvae of An. punctipennis, Cx. restuans, Cx. territans, Ae. vexans, Psorophora ferox and Ps. columbiae. Washino (1983) reported infections of An. freeborni in California rice fields. Published reports reported include 2 other anophelines, 7 other Aedes, 4 other Culex, 2 Culiseta spp. and a Psorophora as mosquitoes susceptible to L. giganteum (Umphlett et al., 1972; Couch et al., 1973; McCray et al., 1973; Jaronski 1982). The susceptibility of mosquito larvae by L. giganteum were due to various strains and the

semi-quantitative bioassays used, in which no direct zoospore counts were determined.

Mattingly (1972, 1977) found this organism in eggs of Armigeres dentatus which were sent from Malaya for description. Parasitized eggs lack the outer chorion. The inner chorion was perforated by motile zoospores of the fungus in numerous places. In one instance there were more than 100 perforations in a single egg.

## Ecology Studies of L. giganteum

One of the important attributes that make L. giganteum such a promising biological control agent is its ability to survive seasons of adverse conditions and agricultural crop management practices. Fetter-lasko and Washino (1983) conducted long term in situ studies on seasonality and recycling pattern in California of L. giganteum. Following a single treatment of the fungus it was demonstrated from 1974 through 1980 in aquatic situations associated with flooded rice fields in northern California. In two instances, persistence of the fungus through two winters and a summer without the availability of irrigation water was documented.

The effects of temperature on infection, growth and zoosporogenesis of the fungus were studied by Jaronski and Axtell (1983). North Carolina (NC) and Louisiana (LA) isolates optimally infected Cx. quinquefasciatus at 21 to 29°C. Sporulation by the NC isolate in larvae was optimal from 19 to 32°C; the LA isolate, 18°C. Further more, Jaronski et al. (1984) found that optimum zoospore production on agar plates occurred between 21 and 27°C. and decreased to zero as temperatures approached 15 or 33°C. Larval infection rates by

zoospores had the same pattern with no infection at 12 or $35.5^{\circ}$C. Suboptimal temperatures also caused a delay in the onset of zoosporogenesis. They also indicated that in the mosquito, *L. giganteum* produced zoospore at 15 - $32^{\circ}$C. As the temperature extremes were reached fewer infected cadavers yielded zoospores. Storage of sporangia within larval cadavers at 100 killed the fungus in one week, but McCray (1973) and Umphlett et al., (1972) observed that infected cadavers remained viable for 2 weeks when stored at $15^{\circ}$C. in water. Jaronski and Axtell (1984) studied more about stored *L. giganteum* and found that *L. giganteum* could keep for 6 weeks at $15^{\circ}$C. in SFE agar plates.

In related studies, Merriam and Axtell (1982) demonstrated the ability of both NC and LA isolates to infect larvae of *Ae. taeniorhynchus* increased as salinity of the water decreased. In water containing 1.5 ppt NaCl there was complete inhibition of zoosporogenesis and mosquito infection in each isolate. Zoosporogenesis was ca. 22 times more sensitive to salinity than was mycelial growth.

Further studies were conducted by Jaronski and Axtell (1982) to determine the effects of organic water pollution on the infectivity of the fungus. In unpolluted water, the fungus infected 27 - 100% of *Culex quinquefasciatus* larvae, but no infection occurred in water with low to moderate levels of organic pollution. The pollution prevented the formation of sporogenic vesicles by the fungus and dratically reduced the viability of any zoospores that were produced.

Laboratory studies on infection of L. giganteum in mosquito larvae

Umphlett and Huang (1972) studied infection of L. giganteum in Cx. restuans in the laboratory and indicated that there was a relationship between larval infection and larval age, with infection decreasing as larval age increased. Concurrent studies at the TDL confirmed these observations in relation to 4th instars and pupae. Early 4th instars were routinely infected, late 4th instars were occasionally infected and pupae were never infected. McInnis et al. (1971) suggested that the motile zoospores enzysted on the chitinous exoskeleton and penetrated the cuticle with a germ tube, it was unusual that pupae were not infected. Domnas et al. (1974) in their study of the biochemical change in Cx. quinquefasciatus following infection with L. giganteum discussed this, particularly in relation to the apparent absence of chitinase in L. giganteum. They pointed out that their data did not specifically exclude the possibility that the zoospores had a chitinase system because they did not investigate the physiology and biochemistry of the zoospores.

Laboratory infection studied with Ae. nigromaculis by McCray (1974) noted that this species was so susceptible to the fungus that larvae would frequently die from massive infections in the head and thorax within 24 to 48 hours following exposure and before the hyphae could spread throughout the body. Once these larvae died, mycelial growth stopped and the typical sporangial and vescicular development did not occur. These larvae became at head and thorax, that the determination of infection by microscopic examinations in many instances could not verified. In those specimens in which light infections occurred, the larvae lived long enough for normal mycelial growth,

sporangial formation and subsequent vesicle and zoospore production to occur. These zoospores produced subsequent laboratory infections in both Ae. nigromaculis and Ae. aegypti.

Umphlett and Huang (1972) reported experimental infection of An. quadrimaculatus, McCray et al. (1973 a, b) reported experimental infections of An. albimanus, An. quadrimaculatus, An. stephensi, and An. sundaicus and Domnas et al. (1974) routinely used An. quadrimaculatus in much of their studies on the biochemistry of mosquito infection with this fungus. All noted that infected anopheline larvae died similar to other species, but there was an obvious reduction in the infection rate of a population. McCray (1973) suggested that this was because of the feeding habitats of anopheline larvae, the portal of entry might be restrict to culicular penatration rather than through the double portals of buccal cavity and cuticle. This explanation appeared to be borne out by studies in which anopheline larvae were placed in inoculum less than 1.27 cm. deep. These anophelines routinely grazed the bottom of the container, rather than the surface film, and infections of 90 - 100% were usually obtained.

Ramoska et al., (1982) discussed about the percentage of mortality of An. dirus larvae and stated that Anopheles is a surface feeder while both Cx. quinquefasciatus and Ae. aegypti were depth feeders and are usually found well beneath the water -air interface, the difference in susceptibility between the species might be attributed to the fact that L. giganteum spores are hydrophobic by nature and present very high dosage levels at the bottom of the container where culicines were found.

As further laboratory studies by McCray (1973 a, b) were continued in relation to mode of infection and identifying the

parameters in which this organism could be potentially effective in the control of vector species, efforts were made to obtain infections during the pupal and adult stage or to discover if light infections, initiated during the fourth instar, would result in infected pupae that survived and produced infected adults. Throughout one year of continuous effort which consisted of placing newly formed pupae in active cultures of infective zoospores and providing adults only water containing infective zoospores, no infections were observed in such pupae or adults. Fourth instars which became infected either died prior to pupation or pupated as infected pupae and died.

McCray et al. (1973 b) observed the life cycle of L. giganteum and revealed two modes of action against California mosquitoes. In a permanent body of water, the fungus produces asexual zoospores which were released from the infected larvae to infect other larvae and the cycle is repeated. In intermittently dry and flooded areas, the fungus goes through asexual cycle and produce resting oospores which germinate when rewetted. Field tests in both types of location resulted in dramatically reduced mosquito populations. No infections were found in 1400 other aquatic organisms from the treated sites.

Dean and Domnas (1983) found that L. giganteum produced extracellular protease. The production of the enzyme required a suitable inducer such as protein, and was repressed by glucose. They also reported that the use of specific substrates and inhibitors could demonstrate the presence of collagenase, a trypsin-like protease and a weak elastase from the fungus. Trypsin-like activity was present in the PYG medium after 38 hours of growth, whereas collagenase appeared in 43 hours.

McInnis (1974) demonstrated β- glucosidase activity in screening the organism for its enzymic capabilities. β-glucosidase was interesting in that it was a transferase as well as a hydrolase.

Domnas et al. (1977) indicated that L. giganteum required exogenous sterols before it produced zoospores. Twenty -three different sterols were administered to growing cultures of L. giganteum. These compounds were reisolated after 7 days, along with any metabolites produced from them. The data showed that L. giganteum metabolic route leading to cholesterol was cycloastenol → fucosterol → cholesterol, which was similar to the metabolic sequence observed in many algae, and some transformations that were indicative of obvious intermediate biosynthesis steps were apparent. L. giganteum transformed 24 alkylidane but not 24 -alkyl sterols sterols to cholesterol. The organism reduced cholesta -5, 7 -dienol to cholesterol, metabolized cholestenol to cholesterol, and transformed 3 -ketosteroids to 3 β -hydroxysterols. Coprosterol was changed to cholesterol.

Warner et al. (1981) analyzed alkanes associated with the mycelia of L. giganteum, careful studies showed that no alkane biosynthesis or transformation was detected, indicating little or no alkane metabolism. The importance of using clean techniques, high -purity solvents and proper media controls was emphasized.

Lord and Roberts (1985) found that the zoospores of L. giganteum rapidly lose motility when dispersed in deionized water. Several organic solutes were tested for their effect in prolonging zoospore activity. Peptone at 0.2 and 0.05 g/l was more effective than methionine and glucose, individually or in combination. The use

of 0.2 g/l of peptone as a medium for bioassays of L. giganteum against 3-day-old Aedes aegypti reduced the mean $LD_{50}$ to 12.9 zoospores/ml as compared to 133 with field water and 124 with deionized water. The use of peptone also dramatically improved the reproducibility of the assays and the goodness of fit of the resultant probit regression lines. The mean $X^2$ values were 7.4 for 0.2 g/l of peptone, 26.8 for field water, and 47.8 for deionized water. It is suggested that the erratic results obtained from use of deionized water were due to variation in the osmatic stress to which the zoospores were exposed, depending on the amount of debris that was introduced into the assays along with the mosquito larvae.

Domnas et al. (1982) indicated that considerable variation in many experiments was encountered, with identical mycelial preparations yielding zoospore numbers ranging from zero to 40,000/ml. One reason for the variation might be that the zoospores gather in the upper surface layer of the containing vessel.

Elliot et al. (1978), Haskins et al. (1977) and Hendrix (1980) noted that L. giganteum were unable to synthesize sterols and required exogenous sterols for sexual and asexual reproduction. Kerwin and Washino (1983) suggested that L. giganteum produced oospores in vitro when supplied with exogenous sterols. Following prolonged maintenance on sterol -free medium, the fungus retained its ability for zoosporogenesis, but oosporogenesis did not occur.

Boswell et al. (1977), studied the methods of inducing zoosporogenesis of L. giganteum. Ether extracts of the seeds of hemp, flax, cotton or soybean, or of freeze -dried mosquito larvae

stimulated zoosporogenesis when the fungus was incubated on PYG agar provided hyphal segments (zoosporangia) were grown in contact with the extracts and were submerged in distilled water. The sterols cholesterol, campesterol, beta -sitosterol and stigmasterol all induced zoosporogenesis in L. giganteum. Zoospores induced by these substances were viable and produced infections in Ae. aegypti larvae. Results showed that L. giganteum had an absolute requirement for sterols for zoosporogenesis.

Prior to anticipated field studies of Merriam and Axtell (1982) efforts were made to determine some environmental conditions, physical and chemical, that might limit or severely modify this parasite's effectiveness. In a series of temperature studies, it was found that at temperatures near 38°C and below 16°C, the fungus was non- infective. While temperatures around 38°C interfered with infection by the zoospores and also killed them, temperatures around 16°C merely interfered with infection. If the water became warmer during the day of application, some infection did result.

Domnas et al. (1982) noted that initial results showed very few to no zoospores produced above 30°C and below 18°C. Temperatures of 20 - 25°C were optimal, with an average of 39,000 zoospores/ml at 25°C in Z medium. Fetter -Lasko and Washino (1978) tentatively concluded (but presented no data) that persistently high water temperatures limited mosquito infection by L. giganteum.

Merriam and Axtell (1982) noted that some member of the genus Lagenidium were known pathogens of marine organisms and also because in the laboratory L. giganteum readily infected the salt

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marsh mosquito species (when tested in fresh water), there was a dual interest in the effects of salinity on infection and survival. In laboratory tests with saline solutions made from evaporated sea salts and _Ae_. _taeniorhynchus_ and _Ae_. _sollicitans_, it was found that salinity levels above 0.7% severely impaired the infectivity of suspensions of zoospores. When larvae of the salt marsh species that had been infected in fresh water were placed in similar saline solutions to complete their development, normal exit tubes and vesicles failed to develop and produce active, infective zoospores. Whether this was the result of the total dissolved salts present in sea water (about 3.5% by weight) or by chloride ions only was not known. Limited data indicated that unusually high concentrations of the chloride ions, 250 mg/liter or higher, had a deleterious effect upon the fungus, but this level was seldom encountered in the fresh water environment notable exceptions being the seepage ditches around rice fields in the central valley of California or other areas of intensive irrigation practices which result in a buildup of salts.

To further define the environmental limits in the laboratory, several studies were conducted with various pH levels. Because McInnis (1971) found that a pH of 6.0 and a low phosphate concentration, less than 5.0 mM/100 ml, produced maximum production, most studies of TDL used pH 6.0 as the norm at a range from 3 to 9. These test indicated that the fungus was able to survive pH levels of 3, the lowest level tested, but that the mosquito larvae were not. Actually, infections became erratic at pH 4, but it was believed that this was due to erratic larval survival. The effectiveness of the fungus was severely hampered and infections were also erratic at pH 9. Additional pH tests

above 9 resulted in no infections. Optimum larval infections occurred at all instars between pH 6 to pH 7.

Peabody (1974) investigated the effects of pH and certain buffering compounds that might affect infection in larval hosts. She found that different buffers at the same pH produced varying levels of infections and that variation in buffer concentration at the same pH was found to influence the infection process. For example, when using tris maleate or tris HCl at pH ranges from 7 to 9, infection rates were 95 - 100%, whereas no infection occurred in any of the buffers at pH 4 or 5, and 38% infections was obtained at pH 10 when using a carbohydrate buffer. In comparing several buffers at the same pH, she used 0.005M citrate, 0.005M phosphate, and 0.005M tris maleate at a pH of 6.0 and obtained infection rates of 2.0, 9.8 and 100.0% respectively and similar effects with these and 0.005 tris HCl and 0.005 tris with 0.2M HCl buffers of a pH of 8.0 -8.5. Since _L. giganteum_ sporulates in water ranging from pH of 5.5 - 9.0 and the spores are infective in the same range, it appears that the pH of most natural breeding habitats of mosquitoes would not constitute a barrier to infection.

Axtell et al. (1982) observed the sporulating fungus cultures in polluted water and revealed that organic pollution interfered with zoosporogenesis and with viability of any zoospores that had been produced. They indicated that _L. giganteum_ had little of no potential as a biological control agent in habitats having even slight organic pollution. In unpolluted water, however, the fungus caused epizootics and recycle itself as long as water temperatures were favourable for its growth.

Merriam and Axtell (1982) also examined the interaction of certain mosquito larvicides, and herbicides used for weed control, with the growth and zoosporogenesis of L. giganteum. Based on the inhibition of mycelial growth on peptone-yeast-glucose agar, and of zoosporogenesis on hemp-seed agar, the least toxic compounds were premethrin and the insect growth regulators, diflurbenzuron and methoprene. Most of the organophophates and carbamates tested, and the herbicides alachlor and atrazine, had moderate toxicity (100 ppm > $IC_{50}$ > 75 ppm). Fenthion, DDT, chloropyrifos, lindane, toxaphene and captan (a fungicide) were the most toxic ($IC_{50}$ < 5.0 ppm).

McCray (1973) and Womeldorf et al. (1972) found that L. giganteum persisted at least 5 yr. despite cyclical drying of the habitats in California. In Louisiana, Glenn and Chapman (1978) indicated that the fungus persisted for 3 years among Cx. territans, however, overwintering studies in North Carolina Jaronski (1982) revealed that L. giganteum did not survive a winter during which water temperatures fell to 4°C. and surface ice formed on the experimental lagoons.

Infection studies on Non-target organisms

McCray et al. (1973), Brown et al. (1977) and Washino (1977) reported the cumulative evidence of susceptibility of non-mosquito hosts infered that L. giganteum is restricted to the Culicidae and Chaoborus astictopus.

Although all available literature on the Lagenidiales and the genus Lagenidium reveals these water molds to be very host specific, the possible effect if this species of Lagenidium on

nontarget aquatic organisms which may be essential food chain species in the environment was considered early in the study. Cyclops, Daphnia, Scapholeberis, several additional unidentified species of copepods and cladocerans, polychaetes, dytiscids and chrionomids were collected from naturally occurring sites and tested at TDL by placing approximatedly equal numbers in treated and control containers. The tests on these organisms were repeated 3 or more times over a 1 -year period. The species were tested separately in 250 ml of water to which was added about one million zoospores daily (Monday - Friday) for 2 weeks. Infectivity and pathogenicity of the zoospores were validated in every test by the introduction and subsequent infection and mortality of Culex quinquefasciatus or Ae. aegypti larvae. Population estimates and actual counts of the nontarget organisms revealed no greater survival among the control than in the treated organisms. Microscopic examinations of young and adult organisms revealed no fungal infections. Adults were observed daily with eggs; abundant young specimens, from newly hatched through all stages, were routinely observed. Microscopic examination of centrifuged detritus showed fungal infection only in mosquito larvae.

Laboratory Studies on Invertebrate Cell Line

Invertebrate cell line produced from mosquitoes, were from Aedes albopictus (C6/36) and Ae. pseudoscutellaris (LSTM-AP-61). Recently, mosquito cell lines were successfully used for the isolation of dengue virus strains from acute human sera and for detection of viral growth (Race et al., 1979). There was no report on using

mosquito cell lines for pathogenic fungi. The reason for using mosquito cell line in this study was that *L. giganteum* showed a high percentage of infection of mosquito larvae and it can grow on artificial media. The mosquito cell line was used for studying fungal growth and infection. The mosquito cell line which was used in this study was C6/36 which was produced from *Ae. albopictus* larvae discovered by Singh and Paul (1969). The results of their study showed that *Ae. albopictus* cell line was more sensitive for isolating dengue viruses from human serum than other cell lines. Chapell et al. (1971) also indicated that this line was better for isolation of dengue virus from mosquitoes than infant mice during the 1969 epidemic in Puerto Rico.

*L. giganteum* are promising biological control agents, are facultative parasite in nature, specificity for mosquito larvae. The idea for culturing the fungus on invertebrate cell lines was to study the pathogenicity and infectivity of the fungus. The recent successful development of an *in vitro* culture technique for oospores will make possible a carefully designed, large-scale field trial to assess the efficacy of the fungus as a practical biological control agent for mosquitoes.

## Hypothesis

The fact that *Lagenidium* sp. is a biological control agent of mosquito larvae in other countries, it might be possible to use it as a means of biologically controlling the quantity of mosquito larvae in Thailand.

Objectives

1. Study the life cycle of Lagenidium giganteum, an imported strain;

2. Study the possibility of using Lagenidium sp. for mosquito larval control in the laboratory.

3. Feasibility study in tissue culture.

Scope : Experiments in laboratory

Procedure of the studies:

1. Study of Lagenidium giganteum, an imported strain. Process of maintenance, propagation in liquid medium and study life cycle.

2. Study life cycle of three important mosquito vectors of disease;

2.1 Aedes aegypti

2.2 Anopheles dirus

2.3 Culex quinquefasciatus

3. Experimental design test for susceptible mosquitoes

3.1 Specific virulence of Lagenidium sp. to host

3.2 Parameters induce specificity for infection

3.2.1 type of medium

3.2.2 inoculum size

3.2.3 acidity (pH) of water

3.2.4 temperature of water

3.2.5 different kinds of water (natural breeding)

3.2.6 different BOD and COD

4. Study of light microscope study for target organs of host by histopathology.

5. Study of *Lagenidium* of propagation in invertebrate cell culture.

## Usefulness

1. To find out from the preliminary data about steps and suitable conditions in using *Lagenidium* sp. for controlling populations of mosquito larvae in Thailand.

2. *Lagenidium* spp. appear to be useful for mosquito control because they are largely restricted to the family Culicidae and they maintain themselves in the mosquito environment year after year. Natural infections indicate that significant control can be achieved with these fungi.