EFFICACY OF ENCAPSULATED *LAGENIDIUM GIGANTEUM* (OOMYCETES: LAGENIDIALES) AGAINST *CULEX QUINQUEFASCIATUS* AND *AEDES AEGYPTI* LARVAE IN ARTIFICIAL CONTAINERS

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ABSTRACT. Presporangial mycelia of *Lagenidium giganteum* cultured on sunflower seed extract were encapsulated in calcium alginate and added once (July 18) to outdoor (Raleigh, NC) caged tires, wood and concrete containers populated with first instars of *Culex quinquefasciatus* or *Aedes aegypti*. First instars were added twice weekly (for 10 wk) to simulate natural oviposition. The fungus persisted for 10 wk and recycled in the mosquito larvae of both species. The overall reductions of *Cx. quinquefasciatus* and *Ae. aegypti* immatures were higher in tires (55 and 45%, respectively) and wood (67 and 38%) than in concrete containers (17 and 14%). There were low correlations of the numbers of mosquito immatures with measurements of water quality (chemical oxygen demand, ammonia nitrogen and conductivity) in the containers.

INTRODUCTION

The use of biological control, either alone or integrated with other mosquito control strategies, to suppress mosquito populations that develop in artificial and/or natural containers would be advantageous. A potentially useful biological control agent is the fungus *Lagenidium giganteum* Couch (Jaronski and Axtell 1983, 1984; Lacey and Undeen 1986; Guzman and Axtell 1987a, 1987b; Kerwin and Washino 1988). Alginate capsules of presporangial mycelia of the fungus are effective and convenient to store, handle and apply to mosquito breeding habitats (Axtell and Guzman 1987, Patel et al. 1990). These capsules controlled *Culex quinquefasciatus* Say in outdoor plastic wading pools (Axtell and Guzman 1987). The fungus, however, has not been evaluated against mosquitoes in other artificial containers over a long period. In addition, no information has been published on the effects of water quality and temperature in artificial containers on the ability of encapsulated *L. giganteum* to infect mosquito larvae. Therefore, we evaluated encapsulated *L. giganteum* outdoors from July to September 1989 against *Cx. quinquefasciatus* and *Aedes aegypti* (Linn.) in tires, wood and concrete containers.

MATERIALS AND METHODS

Fungal culture: The California isolate (obtained in 1987 from J. L. Kerwin, University of California, Davis, CA) of *L. giganteum* was maintained in our laboratory on extracts of sunflower seeds (SFE) as previously described (Jaronski and Axtell 1984, Guzman and Axtell 1986). Prior to encapsulation, the fungal isolate was passed 22 times through *Cx. quinquefasciatus* larvae (2- to 3-day-old).

Alginate capsules, containing the asexual (presporangia) stage of *L. giganteum*, were prepared following the procedure of Axtell and Guzman (1987) using 1.5% KELGIN HV® (Kelco, Division, Merck & Co., San Diego, CA) sodium alginate. This procedure results in capsules with a 3–4 mm diam and containing 1,500–2,000 sporangia. The encapsulated fungus typically produces zoospores for 7–14 days after immersion in water (Patel et al. 1990). Newly prepared capsules were dried overnight between paper towels at room temperature (ca. 27°C), placed in closed plastic containers and stored for 4 days at 15°C prior to their introduction into the artificial containers.

Test containers: Three types of artificial containers (tires, wood and concrete) were used outdoors during July to September 1989, in the vicinity of Raleigh, NC. The automobile tires (sizes P165/80R13 and P185/80R13) were obtained from a used-tire shop in Raleigh. Each tire was cut in half (along the diameter), and each piece was covered with a black plastic sheet. Wood boxes (inside dimensions: 19.5 × 18.5 × 15 cm height) were made from kiln-dried red oak (2 cm thick) and put together with screws and marine glue. The wood containers were a modification of the fabricated tree holes described by Lewis and Tucker (1978), but without covers. The round concrete containers (diam 18 cm, height 12 cm, thickness 2 cm) were made from Sakrete® mortar mix cement (6 kg/liter water). Like the wood containers they had open tops, but each container was placed in a plastic bucket. One half of each container type were placed outdoors in a wooded, shaded area for either a short (1–2 months) or long (4–9 months) time for aging and weathering prior to the experiments. Containers with the short or long duration outdoors were subsequently referred to as "new" or "old" containers, respectively. At the end of the weathering period, the containers

were rinsed with rain water that was previously collected and stored in plastic drums.

At the start of the experiment, 3 liters of rain water were added to each half-tire and 1.5 liters to each concrete or wood container. The water level in each container was maintained throughout the experiment by adding rain water as needed. Three new and 3 old containers of each type were placed in each of 4 screened cages (1.8 × 1.8 × 1.8 m) outdoors. The cages were used to prevent oviposition in the test containers by wild mosquitoes. Two cages were used for monitoring the populations of *Cx. quinquefasciatus* immatures (larvae and pupae). Containers in one cage were treated with a single addition of encapsulated *L. giganteum* (20 capsules/container on July 18) and those in the second cage were untreated controls. The other 2 cages were similarly used for monitoring *Ae. aegypti* immature populations with treated containers in one cage and untreated containers in the other cage.

About 75 first instars (1- to 2-day-old) of *Cx. quinquefasciatus* or *Ae. aegypti* were initially introduced into each container on July 17, and twice weekly (Monday and Thursday) thereafter until September 27. About 2 ml of a liver slurry (35 mg liver powder/ml water) were added into each container twice weekly for the first 4 wk and as needed thereafter to support larval survival. The larvae of *Cx. quinquefasciatus* and *Ae. aegypti* used in this study were obtained from laboratory colonies established in March 1985 and June 1988, respectively, from larvae collected in the vicinity of Raleigh, NC.

Counting methods: The numbers of live larvae and pupae in each container were determined by absolute counts at weekly intervals (11 dates, July 18-September 27, 1989). The water in each container was poured through a fine mesh screen sieve positioned on top of a plastic bowl. The recovered larvae were counted while the sieve was submerged in clear water and classified as small (first and second instars) or large (third and fourth instars). After counting, the immatures and the water in the bowl were returned to the original container.

The presence of *L. giganteum* in the containers was determined by: 1) collecting and examining dead resident larvae, and 2) testing for zoospore activity in water samples. When available, up to 4 dead resident larvae were recovered from each container and examined microscopically for fungal infection. Zoospore activity was monitored weekly by placing 20 larvae (1- to 2-day-old) of *Cx. quinquefasciatus* into a water sample (50 ml) 3–4 h after it was taken from each container. The samples were held for 6 days (ca. 27°C) to determine the number of larvae infected by *L. giganteum* due to zoospores present in the water samples. Dead larvae were removed daily from these samples to prevent zoospore production and fungal recycling from the cadavers.

Water quality was monitored by obtaining a water sample (100 ml) from a container at weekly intervals and measuring chemical oxygen demand (COD) and ammonia nitrogen (NH_3-N) (Hach Company 1985). High levels of COD and NH_3-N have been shown to reduce infection of mosquito larvae by *L. giganteum* (Jaronski and Axtell 1982). Conductivity and pH of the water were recorded at the site using a portable meter (Model 33, Yellow Springs Instrument Co., OH). Water temperatures were recorded hourly in 4 containers of each type using a 21× Micrologger (Campbell Scientific, Inc., UT) with wire probes placed ca. 4 cm below the water surface.

To further verify the suitability of the container water for fungal survival and zoosporogenesis, 10 alginate capsules of *L. giganteum* were immersed in 25 ml of water from each of the samples taken weekly for water quality analysis and 20 larvae (1- to 2-day-old) of *Cx. quinquefasciatus* were added. The capsules and the dead larvae were observed microscopically for vesicle formation and infection, respectively.

Statistics: Split plot analysis of variance with container age as a main plot factor and counting interval as the subplot factor was used to test the significance of differences in numbers of mosquito immatures in new (1- to 2-month weathering) versus old (4- to 9-month weathering) containers. Similar analyses with species as replicate, container age and container type as main plot factors, and counting interval as the subplot factor were used to test the significance of differences in the reduction of the total number of mosquito immatures among the three types of containers. Regression analyses were applied to the data from both new and old containers treated with encapsulated *L. giganteum* to evaluate relationships between the total number of immatures of *Cx. quinquefasciatus* or *Ae. aegypti* and COD, NH_3-N and conductivity. The fitted models included effects for replicates, container type, water quality parameters and water quality by container type interactions. Nonsignificant ($P \geq 0.10$) effects were eliminated and reduced models were run to get prediction equations using PR0C GLM (SAS Institute 1985). The Statistical Analysis System (SAS Institute 1985) was used for all statistical analyses.

RESULTS

For each container type, those treated one time with the encapsulated fungus had a significantly ($P \leq 0.05$) lower mean number of live

immatures (larvae and pupae) of a species than did untreated containers of the same type except for *Ae. aegypti* immatures in concrete containers (Table 1). Overall, there were significantly fewer mean numbers of live immatures of *Cx. quinquefasciatus* than of *Ae. aegypti* in each type of treated container. In the treated containers there was no significant difference in the mean number of immatures in old versus new containers except for *Cx. quinquefasciatus* in tires and *Ae. aegypti* in wood. The mean number of immatures of *Cx. quinquefasciatus* differed significantly among the types of containers treated with fungus; the number was highest in concrete, intermediate in tires and lowest in wood. The mean number of *Ae. aegypti* immatures was not significantly different among the types of treated containers.

The overall percentage reduction in the numbers of immatures of *Cx. quinquefasciatus* in untreated containers compared to fungus-treated containers of the same type was not significantly ($P \leq 0.05$) different for tires (55%) and wood (67%) and was lower for concrete (17%) containers in the 10 wk following the addition of capsules. Small (first and second instar) larvae, large (third and fourth instar) larvae and pupae were reduced by 59, 52 and 47%, respectively, in treated tires; 59, 73 and 74% in treated wood containers. In concrete containers, no reduction of the number of large larvae and pupae was observed, but there was a 28% reduction of small larvae. Similarly, the overall reduction of the total number of *Ae. aegypti* immatures (larvae and pupae) averaged 45, 38 and 14% in treated tires, wood and concrete containers, respectively. Except for large larvae in concrete containers, small and large larvae and pupae of *Ae. aegypti* were reduced in the treated containers of all three types.

The weekly mean numbers of immatures in fungal-treatments for both species in tires, wood and concrete containers are shown in Fig. 1 (A–F). For both species, the numbers of immatures fluctuated similarly in the treated and untreated tires and wood containers throughout the study period. There were significantly fewer *Cx. quinquefasciatus* immatures in the treated tires than in the untreated tires in all 10 wk. *Aedes aegypti* immatures were also lower in tires in all 10 wk although not significantly so during the last 2 wk. *Culex quinquefasciatus* immatures were also significantly lower in the fungus-treated wood containers except in the last week. In wood, *Ae. aegypti* immatures were significantly fewer in number in the treated containers compared with the untreated containers in the first 5 wk. The poorest control of larvae with the one time fungus treatment was in the concrete containers. Numbers of *Cx. quinquefasciatus* were significantly reduced in the treated concrete containers only during weeks 1 and 10. Numbers of *Ae. aegypti* immatures were also significantly lower in treated concrete containers only in these 2 weeks and in week 7.

The presence of *L. giganteum* in the treated tires, wood or concrete containers was routinely confirmed by microscopic examination of samples of resident dead larvae, when available. In tires, 128 of 138 dead resident larvae examined were infected with *L. giganteum*; 103 of 112 dead larvae in wood containers, and 46 of 59 dead larvae in concrete containers.

The data on water quality (COD, NH_3-N, conductivity and temperature) for the tires, wood and concrete containers are presented in

Table 1. Mean number of immatures per container per week and overall percent reduction of *Culex quinquefasciatus* and *Aedes aegypti* in tires and wood and concrete containers during the 10-wk period following treatment with encapsulated *Lagenidium giganteum* on July 18, 1989, and weekly additions of first instar larvae.

		Cx. quinquefasciatus			*Ae. aegypti*		
Container	Mosquito stage	Untreated $n = 60$	Treated $n = 60$	Percent reduction	Untreated $n = 60$	Treated $n = 60$	Percent reduction
Tire	Small larvae	53.5	22.0	58.9	53.3	33.7	36.8
	Large larvae	48.9	23.5	51.9	67.6	30.8	54.4
	Pupae	10.9	5.8	46.8	19.1	13.1	31.4
	Total immatures	113.4	51.1	54.9	140.0	77.7	44.5
Wood	Small larvae	48.4	20.0	58.7	55.4	35.6	35.7
	Large larvae	50.4	13.7	72.8	52.6	33.0	37.3
	Pupae	9.3	2.4	74.2	15.7	8.0	49.0
	Total immatures	108.0	36.0	66.7	123.7	76.6	38.1
Concrete	Small larvae	51.5	37.2	27.8	41.7	30.2	27.6
	Large larvae	22.2	22.5	0.0	28.4	30.4	−7.0
	Pupae	6.0	7.8	−30.0	16.2	12.6	22.2
	Total immatures	81.2	67.4	17.0	85.1	73.2	14.0

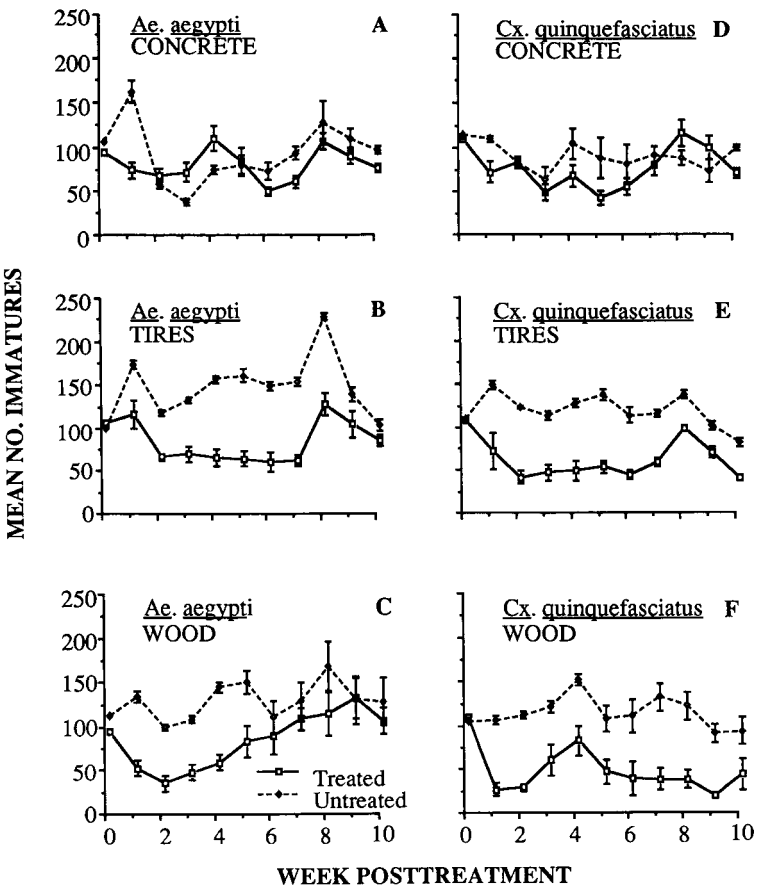

Fig. 1. Mean numbers (±SE) of *Aedes aegypti* and *Culex quinquefasciatus* immatures (larvae and pupae) per container per week in concrete, tire and wood containers after a single treatment with encapsulated *Lagenidium giganteum* on July 18, 1989.

Table 2. The pH values had a narrow range, 6.9–7.3, in all containers for both species throughout the study period and, therefore, the data are not presented. Water temperature ranges were 17.0–32.6, 16.4–31.6 and 16.6–33.0°C in tires, wood and concrete containers, respectively, in the study period, except in the last week with cooler temperatures. There were only minor differences in the hourly temperatures among the 3 types of containers at any time during the 10 wk of the study. The temperatures in all containers, except during the last week, should have been appropriate for fungal growth and zoosporogenesis (Jaronski and Axtell 1983, Guzman and Axtell 1987b).

Of the several regression analyses tested, including univariate, multivariate and multivariate with higher order interactions, no single model showed a strong correlation between any of the water quality parameters measured and the number of immatures in the fungus-treated containers. All models tested had low r^2 values (generally less than 0.5). Relationships at these low r^2 values, if significant, were considered weak. There was no consistent pattern in relationships. For example, in a multiple linear regression model to relate total immatures and all 3 water quality parameters, the numbers of *Ae. aegypti* immatures in all fungus-treated containers were significantly and positively correlated with COD ($P \leq 0.05$, $r^2 = 0.13$), but the numbers of *Cx. quinquefasciatus* were negatively correlated with COD ($P \leq 0.05$, $r^2 = 0.09$).

Throughout the study period, quality of water from tires and wood containers, and to a lesser extent from concrete containers, was apparently appropriate for vesicle formation since alginate capsules of *L. giganteum* that were added to samples of water collected from the containers were observed microscopically to produce vesicles. Vesicles were produced from the capsules in 97 of 112 samples in tires, 87 of 112 samples in wood containers and 74 of 111 samples in concrete containers. Furthermore, fungal infec-

Table 2. Mean (±SE) water quality parameters (COD, NH_3-N, conductivity and temperature for 10 wk in tires and wood and concrete containers with *Aedes aegypti* or *Culex quinquefasciatus* following introduction of encapsulated *Lagenidium giganteum* on July 18, 1989, and weekly additions of first instars.

Species	Container	Water quality parameters			Temp °C	
		COD mg/liter	NH_3-N mg/liter	Conductivity umhos/cm	Maximum	Minimum
Ae. aegypti	Tires	67.7 ± 10.5	1.7 ± 0.2	52.6 ± 2.8	29.1 ± 0.4	18.6 ± 0.9
	Wood	145.2 ± 39.6	1.1 ± 0.2	29.2 ± 1.5	29.9 ± 0.5	18.5 ± 1.0
	Concrete	105.8 ± 17.7	0.4 ± 0.0	196.1 ± 8.3	30.8 ± 0.5	18.2 ± 1.0
Cx. quinquefasciatus	Tires	47.6 ± 9.3	1.5 ± 0.2	50.6 ± 2.9	30.2 ± 0.5	18.2 ± 1.0
	Wood	144.3 ± 36.4	1.0 ± 0.2	30.1 ± 1.2	25.9 ± 1.2	18.3 ± 0.9
	Concrete	88.8 ± 15.2	0.5 ± 0.1	220.9 ± 11.6	30.3 ± 0.5	18.0 ± 1.0

tion was observed in *Cx. quinquefasciatus* larvae added to the water samples along with alginate capsules of *L. giganteum* which produced vesicles. Zoospore activity, as indicated by mean percent infection of *Cx. quinquefasciatus* larvae assayed in water samples removed weekly from different containers, was relatively low (tires, 18%; wood, 11%; concrete, 5%), but confirmed fungal activity throughout the study period in tires and wood containers and to a lesser extent in the concrete containers.

Following termination of the experiment in September due to cold weather, the containers were left outdoors in the cages over the winter. During the following early summer (July 20 to July 4, 1990), first instars of *Cx. quinquefasciatus* were added to each container at 2- to 5-day intervals. Larvae were removed 2–5 days after adding to the containers, and the dead larvae were examined for infection. Four infected larvae were recovered on two different dates from one concrete container but none from any other container. Thus, there was evidence, although only slight, that the fungus overwintered, probably in the oospore stage.

DISCUSSION

The tires and wood and concrete containers exhibited different water quality characteristics that apparently affected *L. giganteum* activity and thus the density of immature mosquito populations in those containers. In this study, water quality, particularly in tires and wood containers, was suitable for the establishment of *L. giganteum* in the immature populations of both mosquito species. The overall reduction in immature populations of both species was higher in wood containers and tires than in concrete containers. Although COD levels greater than 250 mg/liter occurred, particularly in wood containers, *L. giganteum* zoosporogenesis persisted. In previous studies (Jaronski and Axtell 1982), the infectivity of *L. giganteum* (NC isolate) against *Cx. quinquefasciatus* was lost in water with the COD = 119–186 mg/liter. In another study (Guzman and Axtell 1987b), the fungus (CA isolate) caused 80–92% larval infection in the same mosquito species in water with the COD = 97–214 mg/liter. The inoculum used in our experiment was obtained after 22 serial passages of the fungus (CA isolate) through *Cx. quinquefasciatus* larvae, which may have increased its vigor and tolerance of higher COD levels. Lord and Roberts (1986) reported that 15 serial passages through mosquito larvae restored the vigor of *L. giganteum* (CA isolate) in terms of zoosporogenesis, oosporogenesis and infectivity.

In concrete containers, the slight decline in the number of immatures of *Ae. aegypti* could be attributed to factors other than those measured by us. Kramer (1990) showed a strong negative correlation between water hardness (as measured by mg/ml $CaCO_3$) and mortality of mosquito larvae due to *L. giganteum*. Lord and Roberts (1985a, 1985b) observed that various species of bacteria and other microorganisms affected zoosporogenesis and zoospore survival. Jaronski and Axtell (1982) showed that TKN and P affected infection of mosquito larvae by the fungus. Guzman and Axtell (1987a) reported adverse effects of turbidity on zoosporogenesis. Other factors could have contributed to the fluctuations in the numbers of immatures.

The encapsulated fungus was effective against *Cx. quinquefasciatus* and *Ae. aegypti* in tires and wood containers, and to a lesser extent in concrete containers. The fungus was able to recycle for 10 wk in the artificial containers and perhaps would recycle longer, but the experiment was terminated due to cool weather. Although our study demonstrated the ability of the encapsulated fungus to reduce the immature populations of *Cx. quinquefasciatus* and *Ae. aegypti* in wood containers, similar trials should be done in actual tree holes and tires containing organic debris. Tree holes are complex mosquito breeding habitats, with leaf litter content and water quality parameters varying enormously (Carpenter

1982), which could affect fungal activity. In concrete containers, encapsulated *L. giganteum* may not be efficacious as evidenced by the low reductions in immature mosquito populations in treated containers in this study. However, this should be tested further in large concrete water storage containers.

Encapsulated *L. giganteum* can be conveniently applied to natural and artificial containers. Ideally, the application should be done when the mosquito larvae are in early instars and the water quality is suitable. Changes in water quality, host density and age-structure influence the reduction of mosquito larval population over a period of time (Guzman and Axtell 1987a). Although all species of mosquitoes can be infected by *L. giganteum*, differences in species susceptibilities may cause variations in persistence and cycling of the fungus. There should be further evaluations of encapsulated fungus against other species of mosquitoes in tires, wood and concrete containers as well as in other types of natural and artificial containers.

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REFERENCES CITED

Axtell, R. C. and D. R. Guzman. 1987. Encapsulation of the mosquito fungal pathogen *Lagenidium giganteum* (Oomycetes: Lagenidiales) in calcium alginate. J. Am. Mosq. Control Assoc. 3:450–459.

Carpenter, S. R. 1982. Stemflow chemistry: effects on population dynamics of detritivourous mosquitoes in tree-hole ecosystems. Oecologia 53:1–6.

Guzman, D. R. and R. C. Axtell. 1986. Effect of nutrient concentration in culturing three isolates of the mosquito fungal pathogen, *Lagenidium giganteum* Couch, on sunflower seed extract. J. Am. Mosq. Control Assoc. 2:196–200.

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

Guzman, D. R. and R. C. Axtell. 1987b. Temperature and water quality effects in simulated woodland pools on the infection of *Culex* mosquito larvae by *Lagenidium giganteum* (Oomycetes: Lagenidiales) in North Carolina. J. Am. Mosq. Control Assoc. 3:211–218.

Hach Company. 1985. Water analysis handbook, 1985 edition. Loveland, CO.

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

Jaronski, S. T. and R. C. Axtell. 1983. Effects of temperature on infection, growth and zoosporogenesis of *Lagenidium giganteum*, a fungal pathogen of mosquito larvae. Mosq. News 43:42–45.

Jaronski, S. T. and R. C. Axtell. 1984. Simplified production system for the fungus *Lagenidium giganteum* for operational mosquito control. Mosq. News 44:377–381.

Kerwin, J. L. and R. K. Washino. 1988. Field evaluation of *Lagenidium giganteum* (Oomycetes: Lagenidiales) and description of a natural epizootic involving a new isolate of the fungus. J. Med. Entomol. 25:452–460.

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

Lacey, L. A. and A. H. Undeen. 1986. Microbial control of black flies and mosquitoes. Annu. Rev. Entomol. 31:265–296.

Lewis, L. F. and T. W. Tucker. 1978. Fabrication of artificial tree holes and their performance in field tests with *Aedes sierrensis* and *Orthopodomyia signifera*. Mosq. News 38:132–135.

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

Lord, J. C. and D. W. Roberts. 1985b. Solute effects on *Lagenidium giganteum:* zoospore motility and bioassay reproductibility. J. Invertebr. Pathol. 46:160–165.

Lord, J. C. and D. W. Roberts. 1986. The effects of culture medium quality and host passage on zoosporogenesis, oosporogenesis and infectivity of *Lagenidium giganteum* (Oomycetes: Lagenidiales). J. Invertebr. Pathol. 48:355–361.

Patel, K. J., L. M. Rueda and R. C. Axtell. 1990. Comparisons of different types and concentrations of alginates for encapsulation of *Lagenidium giganteum* (Oomycetes: Lagenidiales), a fungal pathogen of mosquito larvae. J. Am. Mosq. Control Assoc. 6:101–104.

SAS Institute. 1985. SAS user's guide: statistics. SAS Institute, Cary, NC.