POPULATION DYNAMICS OF *CULEX QUINQUEFASCIATUS* AND THE FUNGAL PATHOGEN *LAGENIDIUM GIGANTEUM* (OOMYCETES: LAGENIDIALES) IN STAGNANT WATER POOLS[1]

D. R. GUZMAN AND R. C. AXTELL

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613

ABSTRACT. The fungal pathogen *Lagenidium giganteum* (California isolate), cultured on sunflower seed extract (SFE) and agar, was introduced once (May 15) into outdoor caged replicated stagnant water pools containing all instars of larval *Culex quinquefasciatus*. Subsequently, first-instar larvae were added daily (May 15–September 30) to simulate natural oviposition. The fungus persisted for the entire 138-day study period, which corresponded with the season of *Cx. quinquefasciatus* breeding in this region of North Carolina, and recycled in the mosquito larvae producing an 82% reduction of adult mosquitoes produced in comparison to untreated pools. The cycles of fungal activity varied among the pools with 2–4 major epizootics occurring during the study period. Data are presented on the cycling of populations of fungal zoospores, mosquito larvae, pupae and adults during the entire mosquito breeding season.

INTRODUCTION

Several studies have shown that the fungal pathogen *Lagenidium giganteum* Couch can persist in mosquito breeding habitats for prolonged periods (Fetter-Lasko and Washino 1983, Glenn and Chapman 1978, Jaronski and Axtell 1983a). Of interest was the ability of the fungus to persist and overwinter in a variety of environmental situations, including drying and reflooding of the study sites, and varying densities and species of host mosquitoes. However, limited quantitative data were presented on the extent of mosquito population reduction caused by the pathogen, although the three studies reported infection rates of 90–100% in larval samples at various times.

Adequate field evaluation of *L. giganteum* requires data on the impact of the fungus on immature and adult mosquito populations over a long period (an entire mosquito breeding season) following its introduction into the larval habitats. The objective of this study was to experimentally determine the effect of a single inoculative release of *L. giganteum* into stagnant water pools on the population dynamics of the southern house mosquito, *Culex quinquefasciatus* Say, during an entire mosquito breeding season in North Carolina.

MATERIALS AND METHODS

Fungal culture. The isolate used was obtained from J. Lord, Boyce Thompson Institute, Ithica, NY and was derived from a culture provided by J. Kerwin, University of California, Davis, CA. It is referred to as the California (CA) isolate (ATCC, 12301 Parklawn Drive, Rockville, MD 20852, USA, Accession No. 52675). In our laboratory, this isolate was maintained and subcultured weekly in sunflower seed extract media (Jaronski and Axtell 1984, Guzman and Axtell 1986) for about 1 year prior to introduction into the test pools.

Test pools. Nine plastic wading pools (1.6 m diam. x 0.3 m deep) were partially imbedded in the ground inside 3.0 x 3.7 x 1.8 m screened cages (20-mesh) located near Raleigh, North Carolina. The bottom of each pool was covered with a 4-cm layer of sand and a sparse layer of forest litter. Each pool was then filled (May 12, 1985) to a depth of ca. 25 cm with water from an adjacent pond. The day following preparation of these pools, numerous *Culex* egg rafts were observed on the surface of duplicate nearby unscreened pools indicating the attractiveness of the water for mosquito oviposition.

Mosquito larvae (*Cx. quinquefasciatus*) were established in the screened pools by daily (May 13–September 30) addition of newly hatched larvae from 12 egg rafts into each pool (ca. 150 larvae per raft). This daily rate of addition of newly hatched larvae was chosen because it was a reasonable approximation of oviposition by wild *Cx. quinquefasciatus* on uncaged pools in the vicinity. The egg rafts were obtained from a laboratory colony established in March 1985 from larvae collected in the same area. To assure adequate nutrients for the mosquito larvae, starting 33 days after inoculation of the fungus into the ponds and depending on water quality, 100–300 ml of a chicken feed slurry (16% protein laying mash) were added to each pond about once a week. The slurry was prepared by mixing 170 g of chicken feed and tap water to obtain 1 liter of slurry.

Fungus application. The fungus was added May 15 into the screened pools as a liquid suspension of mycelium and particles of the SFE-

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agar medium. To prepare the inoculum, culture agar-disks (100 mm diam.) were blended into 100 ml deionized water per disk at high speed for 20 seconds in a commercial blender (Jaronski and Axtell 1984). A subsample of the inoculum was held in the laboratory with mosquito larvae to confirm infectivity of the fungus. The fungus was applied to 6 pools at a rate of 6 disks per pool and 3 pools were left untreated. To determine the number of zoospores that could be released from each agar culture plate, three agar disks (200 mm^2) were each immersed in 30 ml deionized water and the number of vesicles formed per 3.14 mm^2 microscopic field in each disk were counted at 8, 12, 18, 24 and 36 hours postimmersion. These vesicle counts were plotted versus time after immersion and the total number of vesicles produced per field was estimated by calculating the area under the resultant curve.

Sampling methods. Larval and pupal abundance were determined by standard dipping (350 ml/dip). Larvae in each dip were classified as small (first- and second-instars) or large (third- and fourth-instars) and returned to the pools after counting. Six dips were taken per pool on Monday, Wednesday and Friday of each week. The number of adults resting inside on the sides and the top of the cages were counted (without removal) at the same intervals. There were 54 sampling dates between May 15 and September 30.

The presence and relative abundance of *L. giganteum* were determined by: a) sentinel larvae, b) collection of resident larvae, and 3) zoospore activity in water samples. From May 15 to August 30 (31 sampling dates) 30–40 second-instar *Cx. quinquefasciatus* larvae confined in screened floating cages were placed in each pool (2 cages per pool) for 2–3 days after which the sentinel larvae were returned to the laboratory and held (ca. 25°C) for 4 days to determine percent mortality. The dead larvae were removed daily and observed microscopically to determine fungal infection. When available, ca. 20 resident larvae from each pool were returned to the laboratory and inspected for fungal infection to confirm the presence of the fungus in the pools. Zoospore activity was monitored by placing 25 second-instar *Cx. quinquefasciatus* larvae into a water sample (120 ml) shortly after it was taken from the center of each pool. The samples were held for 6 days (ca. 25°C) to determine the percentage of larvae infected as a result of exposure to zoospores present in the water samples at the time of collection. Dead larvae were removed daily from these samples to assure that there was no zoospore production from the cadavers. These water samples were taken 3 times a week (Monday, Wednesday, Friday) from July 9 through September 30.

Water quality was monitored by obtaining a water sample (100 ml) from each pool at 6–10 day intervals and measuring chemical oxygen demand (COD), ammonium nitrogen (NH_3-N), turbidity and conductivity (Hach Co. 1985). Previous research has shown inhibition of zoosporogenesis corresponding to high COD and NH_3-N values (Jaronski and Axtell 1982). Turbidity was measured by the absorptometric method in formazin turbidity units (FTU). As an additional assay for the suitability of the pool water for the survival and sporulation of the fungus, agar culture disks (200 mm^2) of *L. giganteum* were observed microscopically for vesicle formation after immersion in 30 ml of the water from each sample taken for water quality analysis. Water temperature was recorded continuously in one of the pools by a Foxboro® recorder (Foxboro Co., Foxboro, MA 02035) with the probe 3–5 cm below the water surface.

RESULTS

The number of *Culex quinquefasciatus* adults emerging during the 138-day period was reduced by 82% (compared to the untreated pools) in pools in which *L. giganteum* was introduced once on May 15. Likewise, overall reduction of pupae in the treated pools averaged 82% while the large (third- and fourth-instar) and small (first- and second-instar) larvae were reduced 73 and 50%, respectively (Table 1). Data for the number of immatures (larvae and pupae) per dip throughout the study, and mean number of resting adults per side of the cage are presented in Figs. 1 and 2. These figures show that the fluctuations in the numbers of mosquito adults and immatures in the treated and the untreated pools were

Table 1. Overall percent reduction of *Culex quinquefasciatus* in pools during the 138-day period following addition of the fungus, *Lagenidium giganteum*, on May 15, 1985.

Mosquito stage	Mean no. (±SE) per dip or per cage side per sampling date[1]		Percent reduction
	Untreated	Treated	
Small larvae	24.6 (0.8)	12.3 (0.6)	50.0
Large larvae	14.7 (0.6)	3.9 (0.3)	73.5
Pupae	3.9 (0.3)	0.7 (0.1)	82.0
Adults[2]	110.1 (7.5)	19.8 (2.2)	82.0

[1] Mean of 3 untreated pools and 6 treated pools, based on 6 dips per pool sampled 54 times during 138-day study period.

[2] Mean number of adults resting on a side of screen cage, based on 5 counts (4 sides and top) per cage, samples 54 times during study period.

similar throughout the season but overall abundance was much lower in the treated pools.

Weekly moving averages of mean number of adults per cage and percent reduction in treated pools relative to untreated pools are presented in Table 2. The percent reduction ranged from 0 to 100% during the weekly intervals except for the last interval. No reduction was observed during the last week of the study, although the populations of larvae in 4 of the 6 treated pools at this time were heavily infected (>90%). The low temperatures during this week slowed larval development, and prolonged the period of fungal development and decreased zoospore activity. During the warmer period of the study (33–113 days posttreatment), weekly percent reduction ranged from 67.8 to 100%. The overall reduction of adult mosquitoes increased from about 48.0% during the first month of the study to 91.8% at 107 days posttreatment and decreased thereafter to 83.0% at 134 days posttreatment. The latter was most likely due to decreasing water temperature causing reduction of fungal activity.

Since there was considerable variability over time among the treated pools, data on the number of larvae per dip are presented for each pool in Fig. 3, along with the results of the water sample assays (percent infection of larvae added to aliquots of water from each pool) which were started on day 55 posttreatment. When the data (number of larvae, pupae and adults) from each pool were averaged over the entire period, there were no significant differences ($P = 0.05$) among the treated pools or among the untreated pools (Table 3). The number of immature mosquitoes per dip increased during the first 2 days posttreatment. Thereafter, the numbers of immatures per dip decreased and after 6 days posttreatment less than 2 larvae per dip were collected in the treated pools while there were 37 larvae per dip in the untreated pools. The number of larvae per dip in the untreated pools decreased gradually up to day 19, probably as a result of depletion of nutrients in the pools. Addition of the chicken feed slurry was started 33 days posttreatment and thereafter the number of larvae per dip increased in all the pools (Fig. 3). Apparently, the increase in larval density favored epizootics of the fungus in the treated pools.

The inserts in Fig. 3, which show the percent infection of *Culex* larvae in samples of water taken from the 6 treated pools, indicate that the presence and relative abundance of fungal zoospores was highly variable. Zoospore activity (mean % infection of challenged larvae ± SD) during the experiment was highest in pool 6 (36.4 ± 43.2% infection of *Culex* larvae) and lowest in pool 2 (4.4 ± 16.0%). Zoospore activity persisted in 4 of the treated pools for at least 120 days, (pools 3, 4, 5 and 6) and for at least 100 days in pool 1 as determined from water samples.

Data for the sentinel larval samples were com-

Table 2. Time-series analysis of mean numbers of *Culex quinquefasciatus* adults emerged from stagnant water pools with (Trt.) and without (Untrt.) a single addition of *Lagenidium giganteum* on May 15.

Interval posttreatment[1] (days)	Mean no. adults per cage-side during interval[2]		Percent reduction during interval	Mean no. adults per cage-side from day 0[3]		Percent reduction from day 0
	Untrt.	Trt.		Untrt.	Trt.	
0–32	23.0	12.0	47.8	23.0	12.0	47.8
33–37	3.7	0	100.0	17.7	8.6	51.0
40–44	188.7	41.0	78.3	54.3	15.5	71.5
47–51	304.3	42.0	86.2	98.4	20.2	79.5
54–58	167.6	0	100.0	108.8	17.2	84.2
61–65	256.7	0	100.0	128.0	14.9	88.4
68–72	346.0	8.0	97.7	153.2	14.1	90.8
75–79	48.0	9.0	81.3	142.1	13.6	90.4
82–86	131.0	0	100.0	141.0	12.3	91.3
89–93	43.0	0	100.0	132.6	11.3	91.5
96–100	11.0	1.0	90.9	123.0	10.4	91.5
103–107	109.0	5.0	95.4	122.0	10.0	91.8
109–113	121.0	39.0	67.8	122.0	12.0	90.2
116–120	51.0	40.0	21.6	117.5	13.8	88.2
123–127	38.5	14.0	63.6	112.8	13.8	87.8
130–134	56.0	98.0	0	109.6	18.6	83.0

[1] Pools were sampled 8 times during first interval (0–32) and 3 times during each of the remaining intervals.
[2] Means based on 3 sampling dates per interval (8 dates for first interval); 6 treated and 3 untreated pools with 5 cage-sides per pool for each sampling date.
[3] Means based on sampling dates from day 0 up to last day of given interval; 6 treated and 3 untreated pools; 5 cage-sides per pool for each sampling date.

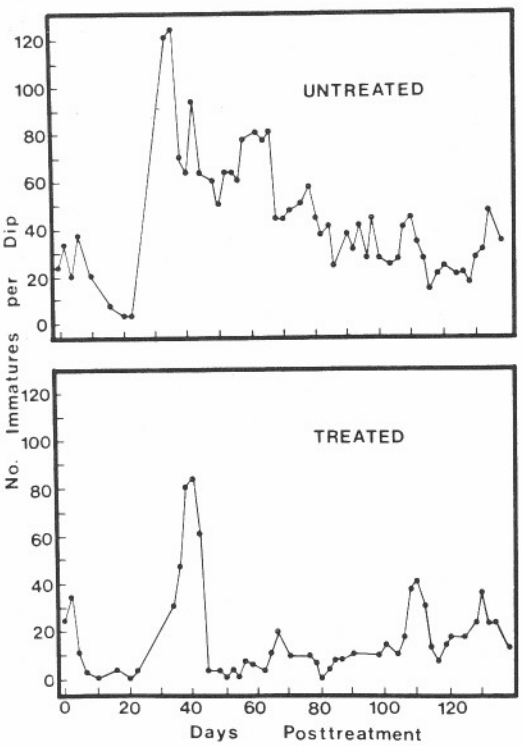

Fig. 1. Mean number of immatures (larvae and pupae) of *Culex quinquefasciatus* per sample time posttreatment in outdoor caged stagnant water pools with (treated) and without (untreated) a single addition of the fungus *Lagenidium giganteum* on May 15.

plicated by the presence of a saprophytic species of fungus (*Saprolegnia* sp.) which rapidly colonized and developed in dead *Lagenidium*-infected sentinel larvae. This made observation and identification of *L. giganteum* in the cadavers very difficult and unreliable. Since identification of *Lagenidium* infection in the sentinel larvae was impractical, data were compared to untreated pools and based on percent mortality and on percent infection. Percent mortality of sentinel larvae from the untreated pools was significantly higher (ANOVA; $P = 0.05$) than in the untreated pools. Mean mortality of sentinel larvae over the 31 sampling dates in the treated pools was 26.1% and ranged from 3.0 to 66.2%. Mortality in the untreated sentinel larvae averaged 15.1% and ranged from 1.2 to 48.4%. The presence of *L. giganteum* in the treated pools was routinely confirmed by inspection of samples of resident larvae taken from the pools at various times. When very few larvae were observed in the dip counts, at least 24 first-instar larvae were collected, held in the laboratory for ca. 24 hours, and inspected for infection. In those cases, nearly all of the larvae were infected with *L. giganteum*.

The data on water quality (turbidity, conductivity and COD), temperature, and vesicle formation are presented in Table 4. Ammonium nitrogen levels remained very low (0.07–1.40

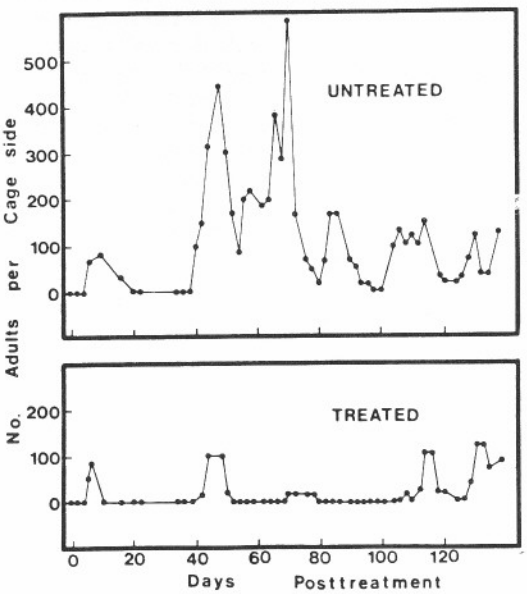

Fig. 2. Mean number of adults of *Culex quinquefasciatus* per cage side per sample time posttreatment produced from outdoor caged stagnant water pools with (treated) and without (untreated) a single addition of the fungus *Lagenidium giganteum* on May 15.

Table 3. Overall mean number of *Culex quinquefasciatus* (larvae and pupae per dip and adults per cage side) throughout the 138 day study-period in each pool with *Lagenidium giganteum* added (treated) and not added (untreated).

Pool no.	Mean no. larvae/dip[1]	Mean no. pupae/dip[1]	Mean no. adults/cage side[2]
	Untreated[3]		
1	36.9	3.9	111.4
2	37.4	4.4	121.7
3	40.1	3.5	101.3
	Treated[3]		
1	11.4	0.5	11.9
2	22.4	1.2	20.5
3	14.4	0.9	16.8
4	12.5	0.6	20.1
5	15.2	0.7	40.1
6	21.0	0.5	9.3

[1] Mean of 54 sampling dates, 6 dips per pool per sampling date.

[2] Mean of 54 sampling dates; 5 counts per cage per sampling date.

[3] There were no significant differences in the mean no. larvae, pupae or adults among the untreated pools or among the treated pools (Duncan's new multiple range test, $P = 0.05$).

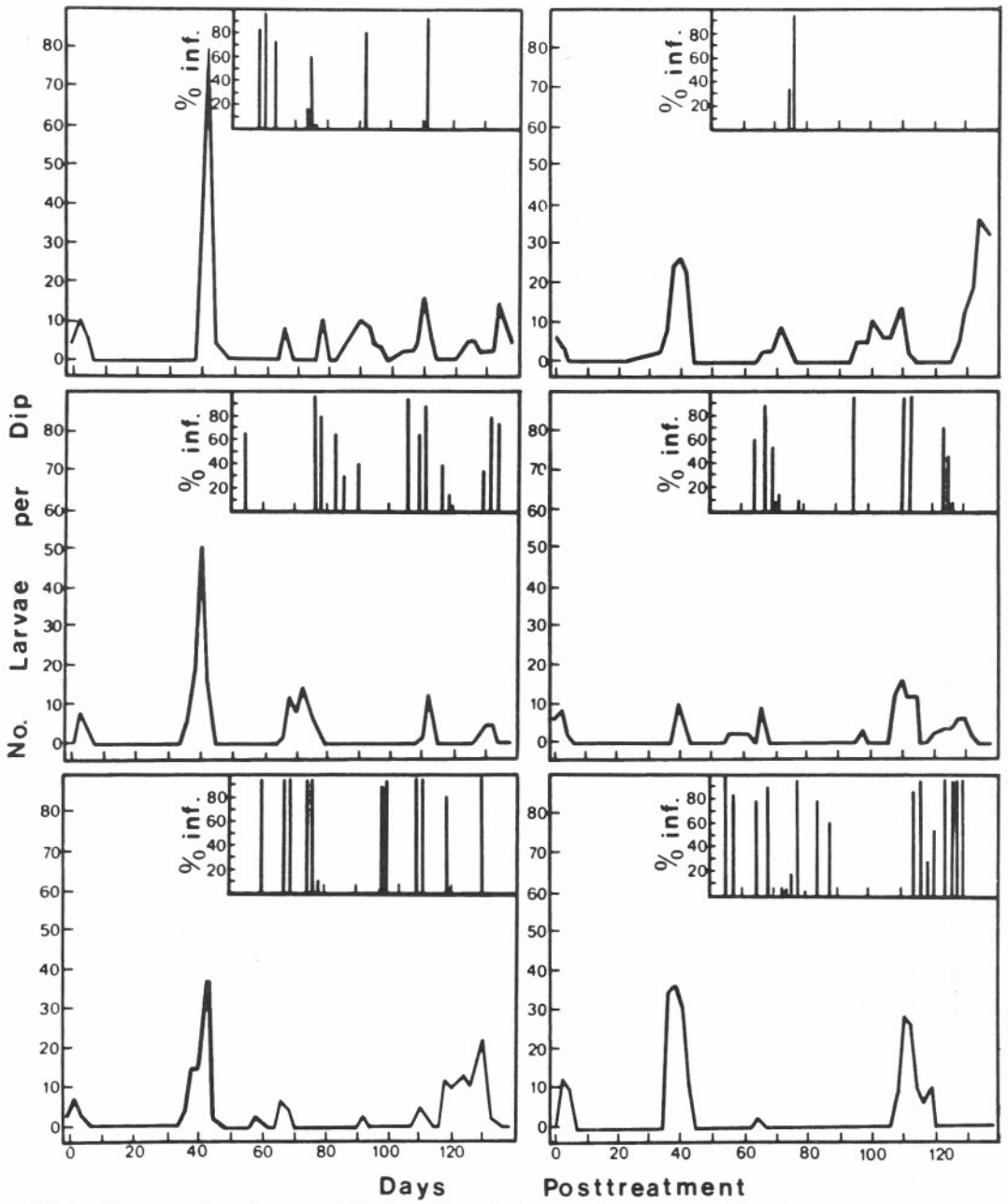

Fig. 3. Mean number of larvae of *Culex quinquefasciatus* and zoospore activity (inserts showing percent infection vs days posttreatment of larvae added to water samples) of the fungus *Lagenidium giganteum* in 6 outdoor caged stagnant water pools after a single addition of the fungus on May 15.

mg/liter) in all the pools throughout the study and, therefore, the data are not presented. Significant differences in COD, turbidity and conductivity occurred over time (ANOVA; $P = 0.01$). Water temperature was appropriate for fungal activity throughout all except the last 2 weeks of the study period, with cooler temperatures in May and September. Except for the last 2 weeks the temperature range was 16.7–30.6°C which should have been suitable for fungal growth and zoosporogenesis (Jaronski and Axtell 1983b). Throughout the study period, water quality appeared suitable for vesicle formation as evidenced by inspection of agar culture disks of the fungus which were added to samples of water removed from the pools.

Table 4. Water quality, temperature, and vesicle formation by *Lagenidium giganteum* added to water samples from experimental pools at intervals posttreatment.

Days Post-treatment	Water quality parameter[1]			Temp. (°C)		Vesicle formation[2]
	Turbidity (FTU)	Conductivity (μmhos/cm)	COD (mg/liter)	Max	Min	
0	38.9	75.7	79.9	26.1	21.1	+
16	15.0	67.8	35.6	27.8	16.7	
33	13.8	42.1	33.0	30.6	17.2	+
40	21.9	73.9	66.9	29.4	20.0	
44	25.9	85.6	56.7	28.3	19.4	+
55	21.6	50.8	35.0	28.3	21.1	
65	46.9	84.2	97.2	30.6	21.7	+
70	22.8	79.8	72.8	31.1	22.8	
78	17.0	48.6	74.4	29.4	22.8	+
90	20.9	45.8	48.1	30.6	19.4	
99	25.9	23.3	80.9	30.6	22.2	+
106	21.3	38.2	38.2	27.8	18.9	
112	22.2	41.9	53.9	28.9	21.7	+
119	30.4	83.9	45.0	30.0	23.9	
126	25.4	68.7	78.9	22.2	13.3	+
133	33.8	66.4	81.1	22.8	14.4	

[1] Values are means of 9 samples taken from all of the pools (1 sample per pool).

[2] Vesicles formed (+) by agar disk of *L. giganteum* culture placed in samples of water removed from the pools and held in the laboratory.

Although vesicle formation from agar cultures occurred in aliquots of pool water taken on day 0, the number of vesicles formed was less than in deionized water. Approximately 4.4 ± 0.7 x 10^5 vesicles per agar plate were produced in deionized water. Assuming 11 zoospores were released per vesicle (unpublished data), ca. 4.8 x 10^6 zoospores per plate were produced. In contrast, 0.7 x 10^6 zoospores per plate were produced in water samples from the pools. Therefore, overall zoospore activity in the pools resulting from the initial inoculum (6 plates/pool) probably averaged ca. 10.4 zoospores per ml. Moreover, the number of vesicles produced in each water sample on the day of treatment had a significant correlation with its respective turbidity measurement ($F = 9.58$ with 1,8 df, $r = 0.76$, $P = 0.02$). No correlation existed between number of vesicles produced and the conductivity or COD of the water.

DISCUSSION

Turbidity was the only water quality parameter of those measured which correlated with the ability of the fungus to produce vesicles in pool water at the time of inoculation. Turbidity may be caused by suspended organic and inorganic matter, soluble compounds, and microscopic organisms. In this case, it appeared that most of the turbidity was caused by soluble organic and inorganic compounds. After the samples were filtered through filter paper (Whatman no. 2) to eliminate suspended particles from the samples, there were no significant differences in turbidity between filtered and unfiltered samples. Soluble organic compounds, such as tannins, may have originated from the woodland detritus which was placed in each pool and experiments should be conducted to determine the effects of tannins on vesicle formation and zoosporogenesis. After day 10 posttreatment, the turbidity was reduced by the addition of water pumped from the nearby pond and by a considerable amount of rain during the 10–16 days posttreatment. No correlation was found between turbidity and the level of vesicle formation in pool water samples at this time or for the rest of the study period.

The ability of mosquito larvae to develop in the pools between 16 and 33 days posttreatment may have been limited by low nutrient levels. The addition of nutrients starting on day 33 posttreatment resulted in a rapid increase in the number of larvae per dip in all of the pools. The water quality measurements 16 and 33 days posttreatment indicated that turbidity, conductivity, and COD decreased relative to measurements made at the beginning of the experiment, probably as a result of dilution. These water quality measurements were higher 40 days posttreatment, as a result of the addition of nutrients and the resulting increases in populations of microscopic organisms. Thereafter, water quality measurements fluctuated as a result of varying amounts of rain, and growth of bacteria, protozoans, crustaceans and algae in the pools. The screen cages prevented the introduction of larger organisms and natural oviposition by mosquitoes.

Under the experimental conditions, with suitable water quality and temperatures, a single application of *L. giganteum* mycelia at the beginning of the breeding season in North Carolina resulted in 82% reduction of adult *Cx. quinquefasciatus* production from pools of water during the entire mosquito breeding season. Epizootic cycles of the fungus varied among the pools, and zoospore activity, as detected by adding larvae to water samples, generally corresponded with an increased number of larvae per dip (see Fig. 3). These larvae became infected and further recycled the fungus. Some zoospore activity was detected even when very few larvae were present in the pools. However, since first-instar larvae were added daily, it is likely that these larvae became infected and promoted further zoospore activity.

The factors which affect epizootics of *L. giganteum* in *Cx. quinquefasciatus* populations appear to have complex interactions. The ability of the fungus to recycle in larval populations at a particular site may be affected by host density, which in turn is affected by mortality factors other than infection by *L. giganteum* and by the rate of addition of new larvae (i.e., oviposition) into the habitat. In the present experiment, the number of new larvae was relatively constant (ca. 1,800 larvae per day per pool). However, the mortality rate due to factors other than *L. giganteum* infection varied throughout the season as evidenced by the fluctuation of population numbers observed in the untreated pools. The overall survival rate of larvae in the untreated pools (mean no. pupae per dip/mean no. of small larvae per dip x 100) was ca. 16.7%. Approximately 248,000 larvae were introduced into each pool during the study period, and ca. 70,000 adults were produced from each control pool, which was a 28% survival rate. The survival rates based on pupal counts and adult counts were based on different methods of population estimates and cannot be compared. However, the data indicated that there was considerable mortality due to unknown factors in the untreated pools. Approximately 13,000 adult mosquitoes were produced from pools containing *L. giganteum*, which is equivalent to an overall survival rate of ca. 5%.

This experiment reinforces previous studies (Jaronski and Axtell 1983a) in which *L. giganteum* was observed to recycle for an entire breeding season in mosquito habitats in North Carolina. The data also indicate that there was a greater reduction of small larvae than large larvae. Initially the application of *L. giganteum* resulted in only about 40% reduction of the larval population. This can be attributed to the size distribution of the population and water quality at the time of application. To achieve the greatest short term control with *L. giganteum*, the application should be made when the target mosquito larvae are in the early stages of development and water quality is appropriate (Jaronski and Axtell 1984). Long term reduction of mosquito larvae in the treated site may thereafter be greatly influenced by changing water quality and host density.

Although inoculative application of *L. giganteum* may result in nearly 100% reduction of mosquito populations in permanent water habitats, the cyclical nature of the pathogen-mosquito relationship and temporal changes in water quality may result in production of unacceptably large numbers of mosquitoes at certain times during the breeding season. Alternate control strategies may be needed during the outbreaks. One possible complementary control during periods of low fungal activity is the use of fast-acting but non-residual *Bacillus thuringiensis israelensis* (*B.t.i.*). Research is being conducted to determine the efficacy of combinations of *L. giganteum* and *B.t.i.* on reduction of mosquito populations and the impact on the persistence of *L. giganteum*. Also, previous research (Merriam and Axtell 1983) indicated that certain pesticides may be compatible with *L. giganteum* mycelial growth and zoosporogenesis and might be used to reduce populations when *L. giganteum* is at enzootic levels. Alternatively, innundative application of *L. giganteum* under appropriate environmental conditions could be attempted when activity of the pathogen is low, thus reducing the larval population and increasing the level of the pathogen in the habitat.

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