LABORATORY EVALUATION OF *LAGENIDIUM GIGANTEUM* (OOMYCETES: LAGENIDIALES) IN WATER FROM CONTRA COSTA COUNTY, CALIFORNIA, MOSQUITO SOURCES

VICKI I. KRAMER

Contra Costa Mosquito Abatement District, 155 Mason Circle, Concord, CA 94520

ABSTRACT. Six bioassays were performed to evaluate the efficacy of the fungus, *Lagenidium giganteum*, against mosquitoes in water collected from 75 sources. The fungus infected larvae of 4 genera and produced >90% mortality in water from some of the creeks, artificial containers and the wild rice field tested during 4 of the assays. There was no larval mortality due to the fungus in water from irrigated pastures or marshes. Water quality parameters associated with *L. giganteum* infection varied among the bioassays; low measurements of total dissolved solids (TDS), hardness ($CaCO_3$), conductivity, chemical oxygen demand (COD), ammonium nitrogen (NH_3-N), phosphate (PO_4) and salinity were significantly ($P < 0.05$) correlated with fungal efficacy in one or more of the assays. Regression analyses selected TDS, $CaCO_3$, COD, NH_3-N and/or PO_4 as the best predictors of larval mortality due to *L. giganteum*. Turbidity and pH were not correlated with fungal efficacy.

INTRODUCTION

The oomycetous fungus, *Lagenidium giganteum* Couch, is a promising microbial control agent for mosquito larvae (Federici 1981, Lacey and Undeen 1986). It is a virulent pathogen of several genera of Culicidae (McCray et al. 1973a, 1973b) and of most Chaoboridae (Brown and Washino 1977, 1979), but is apparently safe for other organisms (McCray et al. 1973b, Siegel and Shadduck 1987, Kerwin et al. 1988). *Lagenidium giganteum* has been evaluated in a variety of habitats including rice fields, seepage ditches, and irrigated pastures and fields (McCray et al. 1973b; Kerwin and Washino 1986, 1987, 1988), and has demonstrated the ability to recycle in nature (Fetter-Lasko and Washino 1983, Jaronski and Axtell 1983a). Studies have, however, indicated that *L. giganteum* has some restrictive environmental limitations, such as a low tolerance for organic water pollution (Jaronski and Axtell 1982) and salinity (Merriam and Axtell 1982).

Given the environmental constraints of *L. giganteum*, the purpose of this study was to determine under laboratory conditions the Contra Costa County, California, habitats in which the fungus could potentially control mosquito larvae. A second objective was to correlate the efficacy of the fungus with certain water quality parameters.

MATERIALS AND METHODS

The efficacy of *L. giganteum* was evaluated in 6 separate laboratory bioassays between August 2 and October 18, 1988. Water and mosquito larvae were collected in clean plastic containers from creeks, tidal marshes, irrigated pastures, artificial containers and a wild rice field. All of the 75 collection sources, except the Lake County wild rice field, were located in Contra Costa County.

For each bioassay, 6 plastic cups were filled with 250 ml of water from each source and another 6 with 250 ml of distilled water. Ten second- or third-instar larvae, usually from the specified source, were added to each cup. Three cups of each water type were inoculated with from 2 to 10 ml of the asexual (presporangial) stage of *L. giganteum*; the remaining 3 cups without inoculum served as controls. The California strain of *L. giganteum*, grown in yeast extract based fermentation media, was used for all tests and was obtained from the University of California at Davis. The estimated viability of these cells was at least 95% (J. L. Kerwin, personal communication). Prior to inoculation, the number of presporangial cells per ml was counted. The estimated number of cells of inoculum per cup for bioassays 1 through 6, respectively, was 6.0×10^6, 3.0×10^6, 14.0×10^6, 5.0×10^6, 1.8×10^6 and 0.18×10^6. A pinch of food (ground rabbit pellets mixed with liver powder) was added to each cup.

One day postinoculation, a sample of water from each source was examined for the release of zoospores by the presporangial stage of the fungus. Three days postinoculation, dead larvae were examined individually under a compound microscope for fungal infection, and the mortality due to infection by *L. giganteum* calculated for each source. Because the fungus develops rapidly and usually kills host larvae within 72 h, live larvae, unless moribund, were not examined (Umphlett and Huang 1972). Dead larvae from the controls were examined for infection due to contamination by *L. giganteum*. Water temperature during each assay was monitored daily with a maximum/minimum thermometer.

During the study, *L. giganteum* was evaluated against 10 mosquito species [*Aedes melanimon* Dyar, *Ae. dorsalis* (Meigen), *Ae. nigromaculis* (Ludlow), *Anopheles freeborni* Aitken, *An. punctipennis* (Say), *Culex tarsalis* Coq., *Cx. apicalis* Adams, *Cx. stigmatosoma* Dyar, *Cx. pipiens*

Linn., and *Culiseta incidens* (Thomson)]. Larvae were assayed in the same source water in which they were collected except for the last 2 bioassays (October 5 and 18), when field collected *Cs. incidens* was the only species available. In the distilled water, the fungus was evaluated against *Cs. incidens* in all the bioassays, and against *Ae. melanimon* and *Ae. dorsalis* in bioassays 1 and 3, respectively.

During the last 5 bioassays, a sample of water from each source was analyzed (Greenberg 1985) within 24 h for pH, turbidity, total dissolved solids (TDS), conductivity, salinity and phosphate (PO_4) concentration. Hardness ($CaCO_3$) and chemical oxygen demand (COD) were also measured except during bioassays 2 and 5, respectively. Ammonium nitrogen (NH_3-N) was measured during the final bioassay.

Data from each bioassay were examined to determine if fungal efficacy was delineated by specific measurements of the water quality parameters. Correlation between larval mortality due to the fungus and water quality was evaluated by constructing Pearson correlation matrices. Regression analyses were used to analyze the multivariate effects of the water quality parameters on fungal efficacy. Stepwise regressions were first performed to determine which water quality parameters should be used to estimate fungal efficacy. These parameters were then used in multiple regression analyses to determine the proportions of variance in larval mortality due to *L. giganteum* explained by the parameters in the regression model. The arcsine transformation of the percentage mortality was used in both the correlation and regression analyses.

RESULTS AND DISCUSSION

The water temperature in the laboratory fluctuated between 20.9 and 23.3°C throughout the study. This is within the optimal range (21–29°C) for infection of larvae by the North Carolina and Louisiana isolates of *L. giganteum* (Jaronski and Axtell 1983b).

Bioassay 1: There was 100% infection of the larvae with *L. giganteum* in water from the creek (*Cx. tarsalis, Cs. incidens)* and in the distilled water (*Ae. melanimon).* There was no mortality due to the fungus in water collected from the irrigated pasture or the 4 marshes, or in the controls. Water from this initial bioassay was not analyzed.

Bioassay 2: More than 90% of the larvae in water collected from Sycamore Creek (*Cs. incidens*), Bollinger Creek (*Cx. tarsalis, Cx. apicalis, An. punctipennis),* the wild rice field (*An. freeborni, Cx. tarsalis*) and the distilled water (*Cs. incidens*) were dead and packed with sporangia 3 days postinoculation (Table 1). Water from each source and the fungus were separately refrigerated for 72 h, and then the bioassay was repeated with the *L. giganteum* dosage doubled to 6.0×10^6 cells of inoculum per cup. There were mortalities of 77, 53 and 80% in water from Sycamore Creek, Bollinger Creek and the distilled water, respectively. There was no mortality due to the fungus in the wild rice field water or, at either inoculation rate, in 3 creeks, 2 irrigated pastures, 5 marshes or the controls. The decrease in the fungal infection rate at the higher dosage may have been due to the refrigeration and age of the *L. giganteum* inoculum.

Infection of larvae by *L. giganteum* corresponded to low measurements of turbidity, conductivity and TDS (Table 1). The 2 creeks and wild rice field water had turbidities between 1.0 and 1.8 NTU ($\bar{x} = 1.4$), conductivities between 550 and 1,000 micromhos/cm ($\bar{x} = 813$), and total dissolved solids between 450 and 610 mg/liter ($\bar{x} = 510$); whereas water from the 10 sources without larval infection ranged between 2.7 and 53.7 NTU ($\bar{x} = 18.3$), 1,300 and 23,500 micromhos/cm ($\bar{x} = 8{,}180$), and 800 and 3,000+ mg/liter, for the 3 water quality parameters, respectively. However, TDS was the only parameter that correlated significantly with fungal efficacy ($r = -0.62$, $P < 0.05$). Stepwise regression identified TDS and PO_4 as the best predictors of larval mortality due to *L. giganteum.* A multiple regression using only these 2 parameters explained 60% (adjusted R^2) of the variance and was significant ($P < 0.01$). The regression analysis yielded the equation, percent larval infection = 74.6 − 0.02 (mg/liter TDS) − 9.04 (mg/liter PO_4); the mortality due to the fungus decreased 0.02% for each additional mg/liter TDS and 9.04% for each additional mg/liter PO_4 in the water.

Bioassays 3 and 4: There was no larval mortality due to *L. giganteum* infection in water from any of the 8 creeks, 2 irrigated pastures, 2 marshes, 1 artificial container or in the distilled water tested during bioassay 3, nor in water collected from 16 sources for bioassay 4. Water quality parameters were evaluated from these sources and were similar to the quantities measured from the same habitats in other bioassays.

Bioassay 5: Mortality due to *L. giganteum* was evident in larvae (*Cs. incidens*) from 3 of the 7 creeks tested (90, 87 and 10% infection), 2 of the 3 artificial containers assayed (used tires 97%, a metal tub 100%) and the distilled water (100%) (Table 1). There was no fungal infection in larvae from the irrigated pasture or marsh, or in the controls.

Table 1. Bioassays 2, 5 and 6. Percentage of larvae infected by *Lagenidium giganteum* and water quality parameters for each habitat.[1]

Bio-assay no.	Habitat	Number of sources	Infected larvae (%)	Turbidity (NTU)	Conductivity (micromhos/cm)	TDS (mg/liter)	Salinity (ppt)	PO_4 (mg/liter)	COD (mg/liter)	Hardness (mg/liter)
2	Creek	2	94.0 (91–97)	1.2 (1.0–1.3)	945 (890–1,000)	530 (450–610)	0.33 (0.30–0.35)	0.83 (0.68–0.97)	72 (10–134)	N/A
	Creek	3	0	19.7 (4.8–48.3)	1,733 (1,500–2,100)	1,177 (1,100–1,310)	0.73 (0.50–0.90)	2.69 (0.57–5.60)	14 (14)	N/A
	Wild Rice	1	94.0 (94)	1.8 (1.8)	550 (550)	470 (470)	0.60 (0.60)	0.55 (0.55)	192 (39–395)	N/A
	Pasture	2	0	3.3 (2.7–3.9)	3,150 (1,300–5,000)	1,103 (800–1,405)	2.15 (0.80–3.50)	5.20 (4.60–5.80)	602 (170–1,033)	N/A
	Marsh	5	0	23.6 (3.7–53.7)	14,060 (3,000–23,500)	3,000+ (3,000+)	20.60 (2.50–43.0)	1.43 (0.45–2.80)	1,026 (57–1,620)	N/A
5	Creek	3	62.3 (10–90)	1.9 (1.2–2.7)	991 (516–1,236)	517 (260–650)	0.28 (0.10–0.45)	1.83 (0.46–3.40)	N/A	267 (200–380)
	Creek	5	0	1.2 (0.9–1.7)	1,303 (976–1,995)	678 (520–1,030)	0.26 (0.08–0.80)	0.57 (0.23–1.18)	N/A	460 (420–500)
	Container	2	98.5 (97–100)	1.9 (1.0–2.8)	746 (181–1,310)	370 (80–660)	0.29 (0.07–0.50)	0.68 (0.11–1.25)	N/A	150 (60–240)
	Container	1	0	1.6 (1.6)	1,064 (1,064)	550 (550)	0.13 (0.13)	4.40 (4.40)	N/A	460 (460)
	Pasture	1	0	1.1 (1.1)	2,550 (2,550)	1,370 (1,370)	1.05 (1.05)	4.20 (4.20)	N/A	520 (520)
	Marsh	1	0	7.1 (7.1)	3,990 (3,990)	2,300 (2,300)	2.50 (2.50)	0.49 (0.49)	N/A	520 (520)
6	Creek	9	97.4 (87–100)	5.4 (2.0–18.0)	907 (553–1,185)	480 (270–670)	0.18 (0.07–0.38)	1.06 (0.21–2.60)	50 (22–88)	313 (160–560)
	Creek	1	0	11.0 (11.0)	2,510 (2,510)	1,400 (1,400)	0.65 (0.65)	1.30 (1.30)	150+ (150+)	660 (660)
	Container	2	100.0 (100)	2.7 (1.9–3.4)	735 (224–1,245)	375 (70–680)	0.26 (0.05–0.47)	0.58 (0.15–1.00)	67 (38–95)	150 (60–240)
	Container	1	0	2.0 (2.0)	1,180 (1,180)	650 (650)	0.10 (0.10)	6.00 (6.00)	300+ (300+)	560 (560)
	Pasture	1	0	8.1 (8.1)	4,510 (4,510)	2,980 (2,980)	1.80 (1.80)	8.00 (8.00)	300+ (300+)	980 (980)

[1] Mean and range of values given for each habitat [$\bar{x}$ (range)].

The only water quality parameter that corresponded to *L. giganteum* mortality was water hardness (Table 1). In those sources with > 87% infection, hardness ranged between 60 and 240 mg/liter ($\bar{x}$ = 180). Sources without any infected larvae had $CaCO_3$ values between 420 and 520 mg/liter ($\bar{x}$ = 475). The water hardness of the creek with an infection rate of only 10% was 380 mg/liter. Water hardness correlated significantly with larval mortality due to the fungus ($r = -0.96$, $P < 0.001$); Pearson correlations of other water quality parameters were not significant. A regression using just this parameter explained 92% of the variance ($P < 0.001$). According to the regression equation, percent larval infection = 115.97 − 0.24 (mg/liter $CaCO_3$), the percentage of larvae infected by the fungus decreased 0.24% for each additional mg/liter of $CaCO_3$.

Bioassay 6: Lagenidium giganteum infected *Cs. incidens* larvae from 11 of the 14 sources assayed. Mortality due to the fungus was 100% in water from 8 sources (6 creeks, 2 artificial containers and the distilled water) and 87, 93 and 97% in 3 other creeks (Table 1). There was no mortality due to the fungus in water from one artificial container, one creek, the irrigated pasture or in the controls.

Five water quality parameters ($CaCO_3$, TDS, conductivity, COD and NH_3-N) were lower from sources that had mortality due to *L. giganteum* than in water from sources with no fungal infection (Table 1, NH_3-N averaged 0.27 and 1.25 mg/liter from sources with and without fungal infection, respectively). Two of the 3 sources without infected larvae had high phosphate concentrations, and one source was highly saline. These 7 parameters were significantly ($P < 0.05$) correlated with larval mortality ($r = -0.90$ for COD, −0.84 for $CaCO_3$, −0.80 for NH_3-N, −0.76 for PO_4, −0.75 for conductivity, −0.73 for TDS, and −0.58 for salinity). There was little correlation between turbidity and *L. giganteum* mortality rates ($r = -0.15$); however, Guzman and Axtell (1987) found a significant correlation between turbidity and the ability of the fungus to produce vesicles, which release zoospores. All water samples were within the appropriate pH range (4.5–8.4) for *L. giganteum* infection (Lord and Roberts 1985), and the correlation with larval mortality was not significant ($r = -0.37$).

Stepwise regression identified COD, hardness and NH_3-N as the best predictors of larval mortality due to the fungus. A multiple regression equation based on these 3 parameters accounted for 88% of the variance ($P < 0.001$). According to the regression equation, percent larval infection = 115.07 − 0.14 (mg/liter COD) − 0.06 (mg/liter $CaCO_3$) − 24.13 (mg/liter NH_3-N), the infection rate decreased 0.14% for each additional mg/liter COD, 0.06% for each additional mg/liter $CaCO_3$ and 24.13% for each additional mg/liter NH_3-N in the water.

In the bioassays with successful fungal infection, zoospores were evident in the distilled water and in water from all sources that subsequently had larvae infected by *L. giganteum*; whereas in water without infected larvae, zoospores were not observed 1 day postinoculation. Water quality clearly affected the ability of the fungus to release zoospores and infect mosquito larvae. The water quality parameters associated with the efficacy of *L. giganteum* varied among the bioassays. Low measurements of TDS during the second bioassay, hardness during the fifth assay, and during the sixth assay, hardness, TDS, conductivity, COD, NH_3-N, PO_4 and salinity, were significantly correlated with fungal mortality. This variability and the variable efficacy of the fungus among the bioassays may have been due to the varying quantity and viability of fungal cells used during each bioassay.

Salinities > of 1.5 ppt have been shown to completely inhibit the release of zoospores by the Louisiana and North Carolina strains of *L. giganteum* (Merriam and Axtell 1982). This probably explains the lack of larval infection by the California strain of the fungus in water from the marshes and some of the irrigated pastures. In bioassay 6, the fungus was not effective in water from one creek, a backyard artificial container or an irrigated pasture. These sources apparently had high organic loads, as indicated by the COD, NH_3-N and PO_4 levels (Table 1). Jaronski and Axtell (1982) demonstrated that water with a high organic content inhibits zoospore release.

The dilution rate may also have influenced the efficacy of *L. giganteum*. The addition of too many cells of the fungus to the 250 ml of water in each cup may have inhibited the vegetative cells from releasing zoospores (J. L. Kerwin, personal communication). Sufficient dilution may be the reason for the increased mortality during the last bioassay, when the number of cells inoculated into each cup was at least 10 times less than during any of the other trials. It may also explain, in part, the lack of fungal infection during bioassays 3 and 4, and the decreased infection rate when the dosage of the fungus was doubled in bioassay 2. This dilution factor is unlikely to be important in field situations except when treating small artificial containers.

These bioassays demonstrated that *L. giganteum* has the potential to control a wide range of mosquito species in Contra Costa County mosquito sources with relatively clean water.

The fungus was not an effective mortality agent of mosquitoes assayed in either irrigated pasture or marsh waters from this county.

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