EFFECTS OF TEMPERATURE ON INFECTION, GROWTH AND ZOOSPOROGENESIS OF *LAGENIDIUM GIGANTEUM*, A FUNGAL PATHOGEN OF MOSQUITO LARVAE[1]

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ABSTRACT. Isolates of *Lagenidium giganteum* from North Carolina (NC) and Louisiana (LA) optimally infected *Culex quinquefasciatus* larvae at 21 to 29°C. Infection rates below 15°C and above 32°C were markedly reduced. Sporulation by the NC isolate in larvae was optimal from 19 to 32°C, inhibited below 19°C and completely absent at 10°C. The LA isolate sporulated in larval cadavers less successfully; the optimum temperature was 18°C. At temperatures above 21°C the infected larvae were killed too rapidly for the fungus to attain maturation, and the *Lagenidium* perished. When cadavers with fungus of either isolate were subjected to 10°C for 4 days and then returned to 25°C, the fungus successfully sporulated, but after 10 days at 10°C, no sporulation occurred upon return to 25°C.

INTRODUCTION

The oomycete fungus *Lagenidium giganteum* Couch has received much recent attention as a potential biological control agent of mosquitoes. The motile zoospore of the fungus penetrates into the mosquito larva commonly through the buccal and pharyngeal cuticle, less frequently at the base of the anal gills, or randomly on the body. Once the penetration hypha reaches the hemocoel it ramifies throughout the head and body cavity and kills the larva within 48 hr after infection. The mycelium then segments and transforms into chains of sporangia, which produce zoosporogenic vesicles outside the host's body. Zoospores are cleaved from the vesicle cytoplasm and released into the environment. Current information about *L. giganteum* is reviewed by Federici (1981) and Domnas (1981).

Little information exists about the temperature tolerance of this fungus. McInnis[2] reported that vegetative growth in simple liquid media had a narrow optimum around 30°C. Jaronski et al. (1982) examined the temperature optimum for zoosporogenesis from solid media and found it to be 21–27°C. Some data have also been collected concerning the influence of temperature on the success of *L. giganteum* in limited field experiments (Washino 1977, Jaronski and Axtell 1982). The field data indicated that transmission of the fungus was limited to a range of 20–34°C.

Our studies deal with the effect of temperature upon infection, mycelial growth and zoospore production of the fungus in mosquito larvae, and the survival of *L. giganteum* at low temperatures.

MATERIALS AND METHODS

Two isolates of *L. giganteum* were used in this study. The North Carolina isolate (NC) was the same fungus used in earlier studies by our laboratory (Jaronski and Axtell 1982, Jaronski et al. 1982). The second isolate (LA) was a *L. giganteum* from Lake Charles, Louisiana, supplied by Dr. H. C. Chapman. This Louisiana isolate is thought to be the *Lagenidium* reported by Glenn and Chapman (1978).

Both isolates were routinely cultured in "Z-Medium" (Domnas et al. 1982) with weekly passage to fresh medium. The Z Medium consisted of 1.4 g yeast extract/liter, 1.2 g glucose/liter, 1.5 g wheat germ/liter and 250 mg (as soluble protein) hemp seed extract/liter. For experimental work, fungus from 7-day-old liquid cultures was plated onto hemp seed agar (Jaronski et al. 1982) and incubated 5–7 days. Such cultures sporulated when immersed in at least 1 liter distilled water per Petri dish.

Second- and third-instar larvae of *Culex quinquefasciatus* Say were used throughout the study. These larvae were from a continuously breeding colony started from females collected in the vicinity of Raleigh, NC two years previously.

In the initial experiment the percentage of mosquito larvae infected by the fungus at constant temperatures ranging from 15° to 35°C were examined. Groups of 25 larvae were exposed to 9.8 cm² of sporulating fungus culture

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[2] McInnis, T. M., Jr. 1971. A physiological and biochemical investigation of the aquatic phycomycete *Lagenidium* sp., a facultative parasite of certain mosquito larvae. Ph.D. Dissertation, University of North Carolina. 118 p. University Microfilm, Ann Arbor, MI (Diss. Abstr. 72:18,429).

on hemp seed agar (approximately ⅛th of a 10 cm diam Petri dish) in 200 ml distilled water, and fed with a suspension of ground rabbit chow. After 24 hr, the larvae were transferred to fresh distilled water and held at the same temperature. At the end of 3 days the larvae were examined for presence of characteristic hyphae or sporangia. The temperatures were 15, 18, 21, 24, 27, 29, 32 and 35°C, with three replicates per temperature. All temperature regimes were maintained in thermostatically controlled chambers with a range of ± 1.0°C around each temperature setting. Both isolates of *L. giganteum* were tested under these conditions.

Additional experiments were conducted to determine the effect of alternating high (35°C) and low (25°C) temperatures on zoospore production from agar cultures. Similar alternating temperatures may occur in shallow, sun-lit mosquito-breeding habitats in the field. In the first experiment, a 9.8 cm^2 portion of NC culture on hemp seed agar was immersed in 1 liter distilled water at 35°C for 4, 8 or 12 hr, then transferred to 200 ml at 25°C containing 25 larvae. These exposure times corresponded with the initial third, two-thirds and entire induction period for zoospore production at 35°C. In the second experiment, the fungal culture was immersed in water at 25°C for 12 hr, to induce zoospore production, then transferred to 200 ml at 35°C with 25 larvae for another 1 hr to determine whether *Lagenidium* would produce zoospores at the higher temperature once it had been induced to do so at 25°C. The control in both experiments consisted of fungus held at 25°C during the entire induction and zoospore production period. Both experiments were replicated three times. In these experiments infected larvae were determined by microscopic examination 3 days after exposure. A decrease in the percentage of larvae infected after any particular temperature treatment of the fungus, in comparison to the control, indicated that zoospore production had been inhibited.

The rate of successful development and zoospore production by the fungus at different temperatures was determined by transferring newly infected larvae to water at 10, 15, 18, 21, 25, 29 or 32°C. The larvae were observed during the next 8 days for the appearance of zoosporogenic vesicles and zoospores. Both NC and LA isolates were studied in this manner.

Tolerance of *L. giganteum* to 10°C was further examined by storing cadavers containing fungus at the early sporangial phase, characterized by the transformation of hyphae into swollen sporangial sub-thalli, at 10°C for varying periods of time. The cadavers were then transferred to 25–27°C water and monitored for zoosporogenesis during the subsequent 3 days.

RESULTS AND DISCUSSION

Highest larval infection rates occurred at 21–29°C for both isolates (Fig. 1). There was a marked decrease in the infection rates above 29°C and below 21°C. This temperature range corresponds to the range for zoosporogenesis (Jaronski et al. 1982).

Zoospore production was inhibited only when the fungus was subjected to 35°C for at least the entire first 12 hr after immersion in water, i.e., during the entire zoospore induction period (Table 1, Regimes A and D). Shorter exposures to 35°C had no effect (Table 1, Regimes B and C); *Lagenidium* was able to recover when it was subsequently transferred to 25°C. If *Lagenidium* was exposed to 25°C during zoospore induction, i.e., the first 12 hr post-immersion, exposure to 35°C during the subsequent 12 hr did not inhibit zoospore production (Table 1, Regime F). Evidently, the high temperature did not interfere with zoospore production once *Lagenidium* had been induced to produce zoospores.

Successful development of the fungus was measured by the proportion of infected larvae in which *Lagenidium* produced zoospores at different temperatures. Data are presented in Tables 2 and 3. Below 19°C the proportion of larval cadavers containing sporulated NC fungus decreased markedly with lower temperatures and the appearance of vesicles and zoospores was delayed progressively. At 10°C no zoospore production occurred; the fungus developed slowly until the early sporangial phase, then stopped. This arrested development was reversed when infected cadavers were returned to 27°C after 2 or 4, but not 10 days at 10°C. The NC isolate thus was able to survive at the low temperature for only a short time. At temperatures above 29°C the numbers of sporangia and vesicles per cadaver were low even though most cadavers contained sporulating fungus. At these higher temperatures the larvae often died before the fungus had grown very much, and the sporangia were restricted to the heads and, sometimes, thoraces of the larvae rather than being spread throughout the bodies as at lower temperatures. At 35°C the fungus sporulated in very few cadavers; fungus that did reproduce produced very few zoospores.

The Louisiana isolate responded differently to temperature (Table 3). At 21°C and above, the LA isolate killed larvae rapidly, often before it had time to penetrate into the hemocoel to any great extent. In such cases (and in other experiments in which newly infected larvae

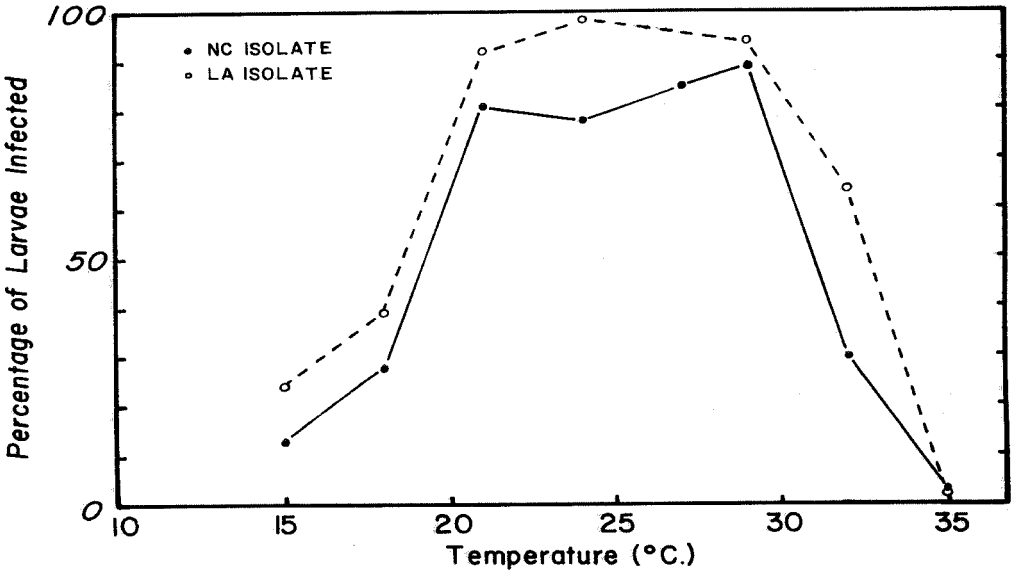

Fig. 1. Infection of *Culex quinquefasciatus* larvae by zoospores of *Lagenidium giganteum* at different temperatures. Three replicates of 25 larvae each per temperature.

were killed by mechanical puncture wounds of the cuticle), if the host died before the fungus could begin to proliferate through its head and thorax, zoosporogenesis did not occur. Instead, the larvae were overwhelmed by bacterial and protozoan saprophytes. The highest reproductive success of the LA isolate was 60% at 18°C, a temperature slightly inhibitory to the reproduction of the NC isolate. At 10°C, the LA isolate ceased to develop at the early sporangial phase and, like the NC isolate, resumed development to successful sporulation when returned at 25° after 2 or 4 but not 10 days at 10°C.

These laboratory data clarify the previous report that fungal transmission in outdoor experimental lagoons ceased as the water cooled to below 20°C but larval infections persisted in a

Table 1. Effects of different high-low temperature regimes during zoospore induction and production by the NC isolate of *Lagenidium giganteum* from agar cultures, expressed as percent infection among *Culex quinquefasciatus* larvae exposed to the sporulating fungus under standard conditions.

Temperature regime	Percent infection[1]
A 35°C Continuous	0 c
B 35°C First 4 hr post-immersion; 25°C thereafter	90.7 a
C 35°C First 8 hr post-immersion; 25°C thereafter	92.0 a
D 35°C First 12 hr post-immersion; 25'C thereafter	69.3 b
E 25°C Continuous	92.0 a
F 25°C 12 hr post-immersion; 35°C 12–24 hr; 25°C thereafter	89.3 a

[1] Means of three replicates of 25 second-instar *Culex quinquefasciatus* each. Mean percents infection followed by the same letter are not significantly different at p = .01 (Duncan New Multiple Range Test).

Table 2. Developmental and reproductive success of the North Carolina isolate of *Lagenidium giganteum* in *Culex quinquefasciatus* larvae at different temperatures.[1]

Temperature (°C)	Percentage cadavers with sporulated fungus		
	After 2 days	After 4 days	After 8 days
10	0	0	0
15	0	42	50
19	16	89	88
23	70	90	90
29	76	92	92
32	68	90	90
35	0	6	6

[1] Fifty infected larvae were used at each temperature.

small portion of the population (Jaronski and Axtell 1982). Low water temperature inhibited sporulation and thus reduced transmission. Si-

Table 3. Developmental and reproductive success of the Louisiana isolate of *Lagenidium giganteum* in *Culex quinquefasciatus* larvae at different temperatures.[1]

	Percentage cadavers with sporulated fungus	
Temperature (°C)	After 3 days	After 8 days
10	0	0
15	0	0
18	10	60
21	26	30
25	8	8
29	4	4
32	0	0

[1] Fifty infected larvae were used at each temperature.

multaneously, both fungal and larval development were slowed so that infected larvae were present long after transmission had ceased. The intolerance of the NC isolate to low temperature limits its use against larvae in the early spring while habitats are still below 20°C. Intolerance of the NC isolate to temperatures above 32°C explains our failure to establish the fungus in experimental lagoons during 1981 when water temperatures daily exceeded 35°C for at least 10 hours and often reached 37–39°C (unpublished observations).

Lagenidium might be used successfully in such habitats by immersing the fungal cultures in water between 21° and 29°C for 12 hr and transferring to the habitats in the evening. With this procedure zoospore production and larval infection would occur at night, when water temperatures should drop below 32°C.

Although the behavior of the LA isolate increases its utility in cooler habitats, its potential as a self-sustaining biological agent is limited in warm water habitats because of its low reproductive success. Nevertheless, the differences between NC and LA isolates suggest that both high- and low-temperature tolerant isolates may exist in nature. If these different isolates can be found and cultured, then possibly a strain tailored for specific environmental tolerances could be selected for appropriate habitats.

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