Temperature-Dependent Development of the Fungal Pathogen *Lagenidium giganteum* (Oomycetes: Lagenidiales) in Larvae of *Culex quinquefasciatus* (Diptera: Culicidae)

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J. Med. Entomol. 28(1): 95–100 (1991)

ABSTRACT The rates of development of *Lagenidium giganteum* were determined in the four larval instars of *Culex quinquefasciatus* Say held at 15, 20, 25, 27, 30, and 34°C. The fastest development was in second instars held at 34°; vesicles and oospores occurred in 50% of the larvae (the median development time) 19.7 and 25.0 h, respectively, after infection. The greatest median time to the formation of vesicles was in third instars at 15°C (185.6 h) and for oospores was in second instars at 15°C (152.3 h). The fungus did not form oospores in fourth instars at 15°C. The median developmental rates of vesicles and oospores in each instar were fit to the Sharpe & DeMichele model, which may be used to predict the effects of different temperatures on the in-vivo developmental rate of the fungus.

KEY WORDS Insecta, *Lagenidium giganteum*, *Culex quinquefasciatus*, temperature-dependent development

Lagenidium giganteum Couch is a fungal pathogen potentially useful for biological control of mosquito larvae in a variety of situations (Jaronski & Axtell 1983a, 1984; McCray 1985; Lacey & Undeen 1986; Axtell & Guzman 1987; Guzman & Axtell 1987a,b; Kerwin & Washino 1987, 1988). The pathogen's developmental rate in an infected larva affects the rate of recycling and amplification in the host population. Temperature, as well as host species, strain, age, and other factors, affect the rate of development of an insect pathogen (e.g., Johnson et al. 1982, Carruthers et al. 1985). Jaronski & Axtell (1983b) reported on the effect of temperature on the rate of infection and reproductive success of *L. giganteum* in second and third instars of *Culex quinquefasciatus* Say, but not on the rates of development of the fungus in the larvae at different temperatures.

We determined the effect of temperature and host age at infection on the rates of development of *L. giganteum* to the vesicle and oospore stages in the four larval instars of *Cx. quinquefasciatus*. The Sharpe & DeMichele (1977) model was used to describe these rates. Brey (1985) presents a detailed description and illustrations of the stages in the formation of vesicles (asexual cycle) and oospores (sexual cycle).

Materials and Methods

Because Jaronski & Axtell (1983b) showed that infection of *Cx. quinquefasciatus* larvae by *L. giganteum* was inhibited at temperatures outside the 15–35°C range, we measured the rate of development of the fungus in *Cx. quinquefasciatus* larvae held in incubators at constant temperatures (±1°C) of 15, 20, 25, 30, and 34°C and in a constant temperature room at 27°C (±1°C). Continuous lighting was provided in all experiments.

Culturing. The California isolate of *L. giganteum* (obtained in 1987 from J. Kerwin, University of California, Davis) was maintained and subcultured routinely in our laboratory on a medium of water extract of ground sunflower seed (SFE) as previously described (Jaronski & Axtell 1984, Guzman & Axtell 1986). Cultures were grown in 100 ml of medium in 500-ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 7 d at 26 ± 3°C. Aliquots (0.5 ml) of the 7-d-old fungus culture were used to inoculate SFE agar Petri plates (10 mm diameter), which were incubated at 26 ± 3°C for 3 d and then stored at 15 ± 1°C until needed for the experiments.

Zoospore Production. When zoospores were needed for infecting mosquito larvae, four SFE agar plates were removed from storage, examined to determine that the fungus was well developed, and placed in shallow enamel pans (40 by 25 by 7 cm) containing 3 liters of deionized water to induce vesicle and zoospore formation. At 26°C, motile zoospores were observed about 12 h after the plates were placed in water and were most abundant at 14–18 h after immersion.

Infection Procedure. Larvae of *Cx. quinquefasciatus* were from a laboratory colony established about 1 yr previously from egg rafts collected near Raleigh, N.C. Newly molted mosquito larvae (about 1,000 per pan) of the desired instar were put in the pan with the fungus at the time of peak zoospore production (about 10^5 zoospores per ml) and removed 2 h later. This procedure resulted in 100% of the larvae being infected. Because the objective

0022-2585/91/0095–0100$02.00/0

Table 1. Maximum percentage (%) of first to fourth instars of *Cx. quinquefasciatus* with *L. giganteum* vesicles and oospores, hours required for the fungus to develop to those stages in the maximum number of larvae, and the hours (observed and predicted from Sharpe & DeMichele model) for vesicle formation, VT_{50}, and oospore formation, OT_{50}, in 50% of the maximum number of larvae in which the fungus developed to those stages after the larvae were held at six constant temperatures

Temp, °C	Instar															
	I				II				III				IV			
	%	Hours	VT_{50} or OT_{50}		%	Hours	VT_{50} or OT_{50}		%	Hours	VT_{50} or OT_{50}		%	Hours	VT_{50} or OT_{50}	
			Observed	Predicted			Observed	Predicted			Observed	Predicted			Observed	Predicted
							Vesicle formation									
15.0	84.4	103.8	99.0	81.3	78.1	116.3	64.0	62.3	53.1	201.0	185.6	130.5	43.8	251.5	164.8	116.0
20.0	100.0	49.3	44.3	50.7	84.4	46.0	38.7	44.2	87.5	69.8	57.3	63.9	75.0	91.8	66.3	78.6
25.0	100.0	45.8	36.8	32.9	96.9	45.5	31.9	31.7	96.9	52.5	35.3	37.0	75.0	71.5	58.0	53.9
27.2	96.9	36.0	26.5	27.9	100.0	43.0	30.8	27.5	90.6	44.0	34.0	32.3	56.0	56.5	41.0	45.9
30.0	100.0	31.0	24.4	24.5	84.4	27.0	23.5	23.0	87.5	45.8	33.3	30.6	68.8	50.0	39.8	37.5
34.0	100.0	27.5	25.4	26.0	84.4	24.5	19.7	19.7	65.6	35.5	32.5	35.6	40.6	31.5	29.0	29.5
							Oospore formation									
15.0	90.6	127.0	117.4	114.5	37.5	163.8	152.3	94.3	12.5	152.0	147.5	112.8	0.0	—[a]	—[a]	279.8
20.0	100.0	68.5	57.8	70.8	84.4	83.5	43.8	61.6	46.9	90.5	68.6	83.2	50.0	119.5	99.4	147.4
25.0	100.0	55.3	53.5	44.4	100.0	58.5	43.8	40.8	59.4	76.5	63.3	62.0	53.1	91.0	87.2	79.3
27.2	93.8	47.0	37.2	36.4	90.6	47.0	38.0	34.1	40.1	56.5	50.0	54.6	31.3	80.5	77.6	60.8
30.0	100.0	42.0	27.0	28.9	90.6	31.5	26.0	27.4	59.4	51.0	56.7	46.6	12.5	55.0	39.8	43.6
34.0	37.5	38.5	29.1	29.7	93.3	28.5	25.0	24.5	28.1	48.0	39.0	39.0	15.6	67.5	29.0	29.0

[a] No oospores formed.

Table 2. Mean ± SD of ratings for *L. giganteum* vesicle abundance in first to fourth instars of *Cx. quinquefasciatus* larvae held at six temperatures

Temp, °C	Instar			
	I	II	III	IV
15.0	1.76 ± 0.72 (25)	1.84 ± 0.85 (25)	1.76 ± 0.75 (17)	1.79 ± 0.70 (14)
20.0	2.16 ± 0.85 (32)	2.00 ± 0.62 (27)	2.18 ± 1.29 (28)	3.75 ± 1.14 (24)
25.0	2.28 ± 0.73 (32)	2.19 ± 1.09 (31)	3.13 ± 1.43 (30)	3.46 ± 1.38 (26)
27.2	1.90 ± 1.09 (31)	2.25 ± 0.71 (32)	2.45 ± 1.18 (29)	3.00 ± 1.25 (18)
30.0	2.03 ± 1.15 (32)	1.67 ± 0.97 (27)	2.79 ± 0.99 (28)	2.64 ± 1.54 (22)
34.0	2.13 ± 0.92 (32)	1.89 ± 1.22 (27)	2.52 ± 1.10 (21)	3.23 ± 0.51 (13)

Values in parentheses are numbers of vesicle-bearing larvae at observation times when they were most abundant.

of our study was to observe postinfection development, this method for infecting hosts was acceptable, even though each larva was infected by several zoospores.

Data Collection. Forty-five 12-well tissue culture plates, with each 4-cc well containing a newly infected first instar, were placed in each incubator and in the constant-temperature room. Each well contained 3 ml of deionized water and 0.25 ml of a 35-mg/ml liver powder slurry. For each of the other instars, 61 plates were prepared for each temperature. For each instar and temperature, five plates were used for monitoring and not for data collection. These plates were removed periodically; larvae were examined under a microscope to determine the stage of fungal development and returned to the incubator. As the fungus in these monitoring plates progressed from the vegetative mycelial, presporangial, and discharge tube stages to the vesicle formation stage, the intervals at which those plates were removed for observation were shortened from 24 h to as little as 0.5 h, depending on the temperature. These observations of the monitoring plates allowed us to remove the remaining plates at appropriate times so that there were at least five data collection times before and after peak vesicle formation for each instar and temperature. At each observation time, four plates were removed from each incubator and the constant-temperature room, and eight larvae, randomly selected from each plate, were examined microscopically. With this procedure, data were collected using larvae that were removed only once from the holding temperature. The abundance of vesicles or oospores or both were rated on a scale of 0 to 5 as follows: 0, none present; 1, 1–5; 2, 6–10; 3, 11–50; 4, 51–100; and 5, >100 vesicles or oospores per larva.

The midpoint between two observation times (hours after infection) was defined as the median developmental time (VT_{50} and OT_{50} for vesicles and oospores, respectively) if the number of larvae containing vesicles or oospores increased during the interval to at least 50% of the maximum number of larvae that eventually possessed those stages. The VT_{50} and OT_{50} values obtained from our constant-temperature experiments, as well as those obtained in previous experiments (unpublished data) at 35°C, were used to calculate the parameters in the Sharpe & DeMichele (1977) nonlinear model of poikilotherm processes to describe the temperature-dependent development rate of the fungus to the vesicle and oospore stages in each larval instar of the host. The Sharpe & DeMichele four-parameter model with high-temperature inhibition of development has the form:

$$r(K) = \frac{RH025 \times \frac{K}{298.15} \times \exp\left[\frac{HA}{1.987}\left(\frac{1}{298.15} - \frac{1}{K}\right)\right]}{1 + \exp\left[\frac{HH}{1.987}\left(\frac{1}{TH} - \frac{1}{K}\right)\right]}$$

where r(K) is the median rate of development ($days^{-1}$) at temperature K (Kelvin, °C + 273.15). RH025, HA, TH, and HH are parameters estimated by the nonlinear regression routine outlined by Wagner et al. (1984) for use in the NLIN procedure of SAS (SAS Institute 1982). Because the observed data on development at 30 and 34°C indicated high-temperature inhibition, an additional point between those temperatures was graphically estimated to refine the final estimates of the parameters for each instar to include the effects of high temperature inhibition more accurately. Once the parameters are determined, the equation can be used to calculate development rates at any temperature, including variable temperatures under field conditions, as required by population simulation models. The biological significance of the parameters is discussed by Schoolfield et al. (1981).

To test the model using variable temperatures, the rate of development of the fungus was determined, using the procedures described above, un-

Table 3. Duration (hours) of vesicle formation by *L. giganteum* in first to fourth instars of *Cx. quinquefasciatus* larvae at six temperatures

Temp, °C	Instar			
	I	II	III	IV
15.0	38.0	165.3	81.5	132.8
20.0	41.0	36.5	78.5	78.5
25.0	24.0	24.8	55.0	81.1
27.2	23.5	37.5	44.5	68.0
30.0	22.3	20.5	45.5	48.0
34.0	15.5	26.0	32.5	41.0

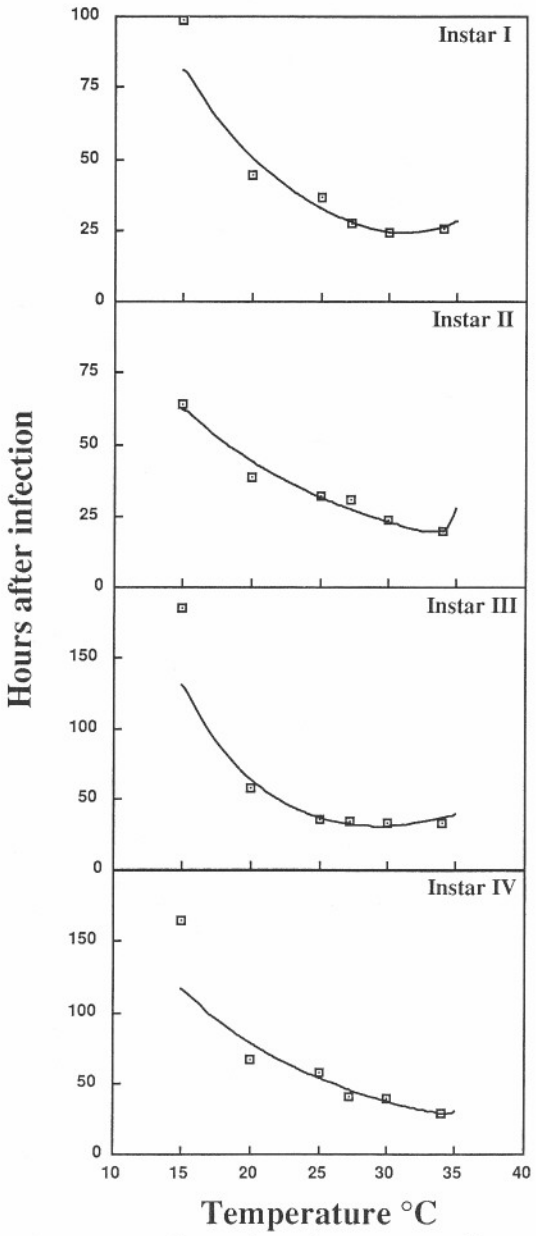

Fig. 1. VT_{50} (hours after infection required for vesicle formation in 50% of the maximum number of larvae containing vesicles) of *L. giganteum* in first to fourth instars of *Cx. quinquefasciatus* held at 15–35°C. Points are observed values and line represents predicted values calculated by the Sharpe & DeMichele (1977) equation (see text).

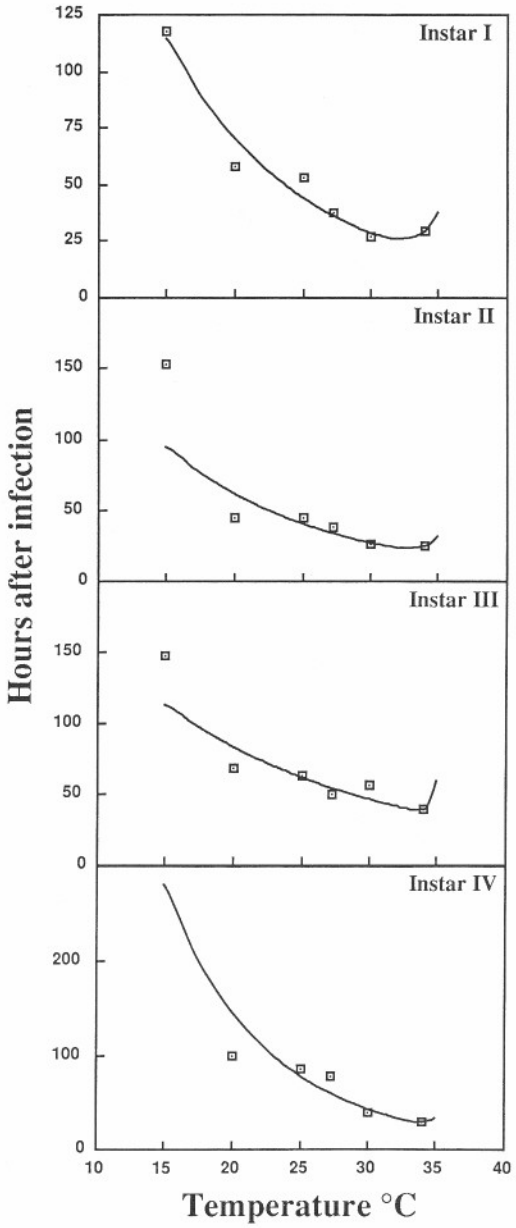

Fig. 2. OT_{50} (hours after infection required for oospore formation in 50% of the maximum number of larvae containing vesicles) of *L. giganteum* in first to fourth instars of *Cx. quinquefasciatus* held at 15–35°C. Points are observed values and line represents predicted values calculated by the Sharpe & DeMichele (1977) equation (see text).

der two sets of alternating temperatures (15 and 25, 30 and 35°C). Incubators were set at the alternate temperature every 12 h to obtain average daily temperatures of 20 and 32.5°C. Eight plates (64 larvae) of fourth instars and four plates (32 larvae) for each of the other instars were examined at each observation time. There were nine observation times for each instar and temperature.

Results and Discussion

Although all larvae were infected, the fungus did not complete the asexual or sexual cycles in some of the larvae. The maximum percentages of larvae of each instar in which the fungus completed development to the vesicle or oospore stages and the hours required at each temperature are given

Table 4. Parameters for the Sharpe & DeMichele (1977) developmental rate model

Fungus stage	Instar	RH025	TH	HA	HH
Vesicle	I	0.75340	307.8	15,390	64,035
	II	0.75630	308.4	10,930	361,441
	III	0.84899	302.4	25,239	49,357
	IV	0.44500	309.7	12,490	259,544
Oospore	I	0.54040	308.1	15,585	147,921
	II	0.58888	308.5	13,733	214,907
	III	0.38710	308.3	9,637	511,946
	IV	0.30250	308.8	20,932	319,697

Parameters are based on median times of vesicle and oospore formation by *L. giganteum* in *Cx. quinquefasciatus* larvae.

in Table 1. The developmental times generally decreased with increasing temperature and were greater in later instars than in early instars. Except for vesicle production at 15°C and oospore formation at 34°C, >90% of the infected first instars had vesicles and oospores at one or more observation times at each temperature. At no temperature did the fungus form vesicles or oospores in >75% of the infected fourth instars.

At the observation times when the numbers of larvae with vesicles were highest, the ratings of vesicle abundance were generally higher in the third and fourth instars than in the earlier instars, except at 15°C (Table 2). Vesicle ratings were low in larvae of all ages at 15°C. The duration of vesicle formation decreased with increasing temperature (Table 3). Vesicle formation occurred during a shorter period of time in the first and second instars than in third and fourth instars.

The mean ratings for oospore abundance did not differ substantially among the various instars and temperatures. At the observation times when the numbers of larvae with oospores were highest, mean oospore ratings ranged from 1.00 to 1.88 in all cases except for fourth instars at 15 and 30°C. At 15°C, oospores did not form in any of the fourth instars. At 30°C, the mean oospore rating was 2.38 in the fourth instars when a maximum of 8 of 32 larvae were observed to have oospores.

The observed and predicted hours required for the fungus to develop vesicles (VT_{50}) or oospores (OT_{50}) in 50% of the maximum number of larvae in which those stages occurred are given in Table 1. The predicted values were calculated with the Sharpe & DeMichele equation using our estimates of the parameters (Table 4). The equation with those parameters gave a curve of predicted values that fit the observed data well overall (r^2 = 0.90–0.97 for vesicles and r^2 = 0.88–0.96 for oospores in the various larval stages) as illustrated in Fig. 1 and 2. The differences between the observed and predicted VT_{50} and OT_{50} values were generally smaller at high temperatures and larger at low temperatures. At low temperatures, the lower percentages of larvae producing vesicles and oospores (Table 1) and low vesicle ratings (Table 2) indicate the slow and erratic development of the fungus at 15°C.

Table 5. Predicted and observed values (hours) of VT_{50} and OT_{50} for *L. giganteum* in *Cx. quinquefasciatus* under variable temperatures

$\bar{x}$ Temp, °C	Instar	VT_{50}		OT_{50}	
		Predicted	Observed	Predicted	Observed
20.0	I	51.0	50.0	70.5	63.2
	II	44.2	50.0	61.5	63.2
	III	64.0	66.7	82.8	70.6
	IV	78.7	74.4	150.0	111.5
32.5	I	23.5	23.5	26.1	35.8
	II	20.3	24.5	23.3	27.5
	III	32.9	41.4	41.4	45.3
	IV	32.9	27.6	34.8	56.0

Data from the variable-temperature experiment were in general agreement with the model predictions (Table 5). Overall, vesicle formation was more predictable than oospore formation. This is consistent with the findings of Kerwin & Washino (1987), which showed that the formation, persistence, germination, and degeneration of oospores is less predictable than vesicle production.

The Sharpe & DeMichele model with our parameter estimates provides reasonable predictions of the developmental rates of *L. giganteum* in *Cx. quinquefasciatus* larvae at any temperature. Thus, it can be used in a simulation model with variable temperature input to predict fungal developmental rates in larvae in natural habitats. The validity of the model and parameter estimates for predicting the temperature-dependent rates of fungal development also should be determined in other species of mosquitoes because minor changes in the parameters may be needed to fit each observed data matrix. This model may be used together with the temperature-dependent development rates of the host mosquito species (e.g., Rueda et al. 1991) in computer models to simulate mosquito–pathogen population dynamics. Obviously, these simulation models should include the effects of other factors, in addition to temperature; e.g., differences in host susceptibility, fungal strain differences in virulence, zoospore survival rates, oospore germination rates, water quality, and the effect of other organisms.

Acknowledgment

This is paper no. 12568 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh,

N.C. This research was supported in part by NIH grant AI 20886 and by financial support from the United Nations Development Program/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

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Received for publication 6 March 1990; accepted 20 June 1990.