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# Production of Oogonia and Oospores of *Leptolegnia chapmanii* Seymour (Straminipila: Peronosporomycetes) in *Aedes aegypti* (L.) Larvae at Different Temperatures

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**Abstract** The aquatic oomycete fungus *Leptolegnia chapmanii* Seymour is pathogenic to mosquito larvae, but it has been little studied since it was first isolated. Although studies have been performed on different biological isolates of *L. chapmanii* around the world, they were made on zoospores and a very little or even nothing is known about the sexual stage (oogonia and oospores), which allows *L. chapmanii* to remain in the environment when conditions are not favorable. The main objective of this study was to determine the relationship between temperature and time of onset of *L. chapmanii* oogonia and oospores in *Ae. aegypti* larvae. *Leptolegnia chapmanii*-infected IV instar *Ae. aegypti* larvae were incubated at different temperatures between 5 and 45°C and photoperiod-controlled for 90 days. The number of oogonia and oospores was examined daily for each tested temperature. As was expected, low temperatures extended the times of oogonia formation, as much as seven times. Likewise, temperatures significantly affect the number of oogonia produced.

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## Introduction

Although several species of oomycete water molds are parasitic on plants and animals, only *Lagenidium giganteum* (Couch) has been widely recognized to have a significant impact as a natural control agent for mosquitoes [1, 2].

The aquatic oomycete fungus[1] *Leptolegnia chapmanii* Seymour is pathogenic to mosquito larvae, but it has been little studied since it was first isolated [3].

*Leptolegnia chapmanii* was isolated from several mosquito species [4–7]. In 1996, a native isolate of *L. chapmanii* (ARSEF 5499) was found producing an epizootic event in a natural population of the neotropical floodwater mosquito *Ochlerotatus albifasciatus* (Macquart), in the vicinity of La Plata city, Argentina [8], and this was the first report of this pathogen from the Southern Hemisphere.

López Lastra et al. [9] determined the susceptibility of ten species belonging to five genera of mosquitoes to the native isolate of *L. chapmanii*

[1] We acknowledge that all taxa of oomycete water molds are now classified among the kingdom Chromista (= Straminipila) and formally excluded from the true fungi. For the sake of convenience, however, we continue to refer to oomycetes in this paper in the historically broad sense as fungi.

under laboratory conditions. Natural breeding sites for these mosquito species are characterized by a wide variety of biotic and abiotic conditions. A wide range of tolerance of zoospores of the Argentinean isolate to biotic and abiotic factors such as temperature, pH and NaCl concentration was studied [10, 11]. Additional research conducted by Pelizza et al. [12] examined production, survival and infectivity of zoospores of *L. chapmanii* in determined parameters that affect survival and epizootics under natural conditions.

Although studies have been performed on different biological isolates of *L. chapmanii* around the world, they were made on zoospores and a very little or even nothing is known about the sexual stage (oogonia and oospores), which allows *L. chapmanii* to remain in the environment when conditions are not favorable. The main objective of this study was to determine the relationship between temperature and time of onset of *L. chapmanii* oogonia and oospores in *Ae. aegypti* larvae.

## Materials and Methods

### Fungal Culturing

The Argentinean isolate of *L. chapmanii* (ARSEF 5499-CEP 010) was maintained on Emerson's YpSS agar media (yeast extract 4 g, $HK_2PO_4$ 1 g, $MgSO_4$ 0.5 g, starch 15 g, agar 20 g, distilled water 1,000 ml) in 60 mm × 15 mm sterilized Petri dishes.

Inoculum was prepared by cutting cubes of agar (0.5 cm each dimension); mycelial cubes containing hyphae were placed in 20 ml of sterile distilled water in 90 mm × 15 mm diameter sterilized Petri dishes and incubated at 25°C ± 0.5 for 3 days. When zoospores were observed in the water, IV instar *Ae. aegypti* were added and maintained for 48 h. Later, the larvae were removed and examined under phase contrast microscopy to confirm fungal infection. *L. chapmanii*-infected *Ae. aegypti* larvae 48 h post-infection were used as fungal inoculum ($9.4 \times 10^4$ zoospore/larva).

### Mosquito Larvae

*Aedes aegypti* larvae used in this study were obtained from a colony maintained at Centro de Estudios Parasitológicos y de Vectores-CEPAVE, La Plata, Argentina, following standard mosquito breeding techniques [13].

### Production of Oogonia and Oospores at Different Temperatures

*Leptolegnia chapmanii*-infected IV instar *Ae. aegypti* larvae 48 h post-infection were individually placed in 24 multiwell cell culture plates with 2 ml of distilled water and incubated at temperatures between 5 and 45°C (± 0.5) and photoperiod-controlled (12 h light–12 h darkness). Two multiwell cell culture plates with 24 larvae each were used for each temperature and were maintained for 90 days. The number of oogonia and oospores was examined daily in every infected larva from each temperature tested through wet mount preparation observed in a phase contrast microscope at 40×. After examined from each plate, the larvae were placed back in wells and incubated at the corresponding temperature. Experiments described above were repeated three times on different dates under similar laboratory conditions.

## Results and Discussion

It was observed that the number and time of production of *L. chapmanii* oogonia and oospores were directly affected by temperature (Table 1). Oogonia (Fig. 1a) were produced in *Ae. aegypti* larvae exposed from 5 to 40°C. No oogonia was observed in larvae maintained

**Table 1** Time (days) needed to start production of oogonia and oospores of *Leptolegnia chapmanii* in *Aedes aegypti*-infected larvae

| Temperature (°C) | Days for oogonia to appear | Days for oospores to appear | Total cycle time |
|---|---|---|---|
| 5 | 36 days | 32 days | 68 days |
| 10 | 30 days | 24 days | 54 days |
| 15 | 19 days | 22 days | 41 days |
| 20 | 9 days | 18 days | 27 days |
| 25 | 5 days | 17 days | 22 days |
| 30 | 5 days | 10 days | 15 days |
| 35 | 5 days | 6 days | 11 days |
| 40 | 5 days | 4 days | 9 days |
| 45 | – | – | – |

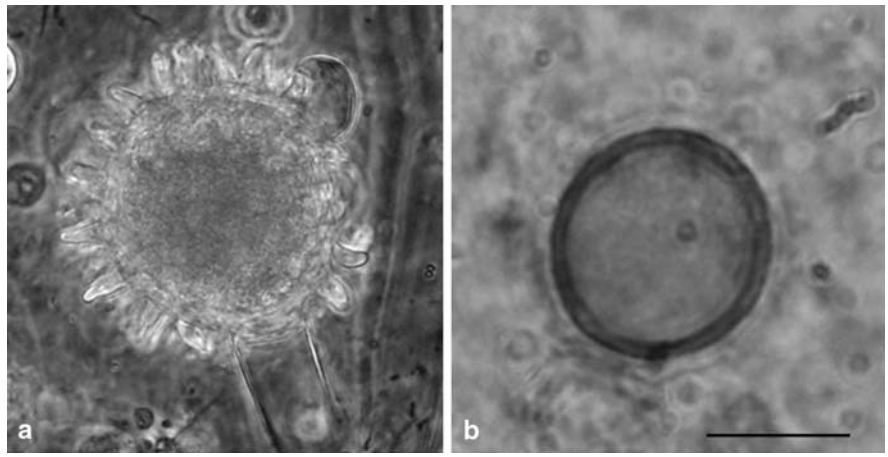


**Fig. 1** *Leptolegnia chapmanii*, oogonium (**a**) and oospore (**b**) obtained from infected mosquito larvae. *Bar*: **a** 23 μm, **b** 20 μm

at 45°C during the 90 days or when these larvae were incubated at 25°C after the 45°C incubation.

When *Ae. aegypti*-infected larvae were placed at 5°C, first oogonia appeared after 36 days, while oospores (Fig. 1b) appeared 32 days after that; thus the time that *L. chapmanii* first produced oospores at 5°C was 68 days. However, when *Ae. aegypti*-infected larvae were placed at 40°C, the time required by the entomopathogenic fungus to develop first oogonia and oospores fell sharply to 5 and 4 days, respectively, requiring 9 days to develop oospores (Table 1).

The number of oogonia formed was influenced by temperature. This behavior has also been observed in the production of oospores by larvae whose higher number was produced in those *Ae. aegypti* larvae that were incubated at 25°C (Table 2).

**Table 2** Mean number of oogonia and oospores of *Leptolegnia chapmanii* formed at different temperatures in *Aedes aegypti* larvae

| Temperature (°C) | Mean number of oogonia (± SD) | Mean number of oospores (± SD) |
|---|---|---|
| 5 | 12 (± 1.3) | 1 (± 0) |
| 10 | 57 (± 2.4) | 5 (± 1.2) |
| 15 | 120 (± 3.9) | 9 (± 1.2) |
| 20 | 131 (± 5.2) | 7 (± 1.7) |
| 25 | 263 (± 8.2) | 10 (± 1.6) |
| 30 | 527 (± 17.5) | 4 (± 0.4) |
| 35 | 843 (± 23.2) | 8 (± 1.6) |
| 40 | 1,030 (± 12.2) | 7 (± 1.1) |
| 45 | 0 | 0 |

The life cycle of *L. chapmanii* is typical of saprolegniaceous fungi [14]. The asexual propagules are the infective agents. Larval infection occurred by two methods, one initiated by encystment of motile zoospores on the larval cuticle and the other by germination of ingested zoospore cysts in the host. The sexually produced oospores are dormant, long-lived and resistant to environmental conditions unfavorable for vegetative growth. Functional *L. chapmanii* oospores serve as a useful source of inoculum and enhance the potential of this fungus as a mosquito larvicide [14]. As was expected, low temperatures extended the times of oogonia formation as much as seven times from 5 days (at 40°C) to 36 days (at 5°C). In the same way, temperature significantly affects oogonia production. Almost 100 times more oogonia were produced at 40°C than at 5°C. Low production of oospores of *L. chapmanii* from oogonia was not well understood although oospore formation was affected by temperature as has been demonstrated in this study.

Mycelium and zoospores, both of which could serve as inoculum, are usually delicate and cannot be stored for more than a few days [12]. Any serious consideration of using *L. chapmanii* as a mosquito larvicide must await demonstration of the feasibility of producing, storing, transporting and dispersing large quantities of the fungus in an infective form. A potentially more useful form of inoculum, the oospore may be the key [14]. *Leptolegnia chapmanii* has potential to become an important addition to mosquito larvicides, both chemical and biological, currently available or under development [15]. The development of this potential must await further investigations

into its effectiveness in the field, its impact on the environment and the role of the oospore in the fungus pathogenic cycle. Because of this, this study provides important knowledge about the biological aspects of this potential biological control fungus.

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