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Host range and specificity of an Argentinean isolate of the aquatic fungus *Leptolegnia chapmanii* (Oomycetes: Saprolegniales), a pathogen of mosquito larvae (Diptera: Culicidae)

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Abstract

An isolate from Argentina of the fungal mosquito pathogen *Leptolegnia chapmanii* (ARSEF 5499), was tested against 12 species of mosquito larvae and on species of non-target aquatic invertebrates and vertebrates. The mosquito species tested were *Aedes aegypti*, *Anopheles* sp., *Culex apicinus*, *Cx. castroi*, *Cx. dolosus*, *Cx. pipiens*, *Cx. renatoi*, *Isostomyia paranensis*, *Ochlerotatus albifasciatus*, *Oc. crinifer*, *Psorophora cyanescens*, and *P. ferox*. Mosquito larvae of 10 species were susceptible, with mortality rates from 10–100%. Two mosquito species *Cx. renatoi* and *I. paranensis* were not infected by *Leptolegnia*. None of the non-target fauna treated was infected by *L. chapmanii* with exception of members of the Family Chironomidae which were susceptible at low infection rates.

Key words: fungus, host range, *Leptolegnia chapmanii*, mosquitoes, pathogens, susceptibility

Introduction

Leptolegnia chapmanii Seymour is an aquatic fungal pathogen that has been shown to be pathogenic to larvae of a number of mosquito species (Table 1). *Leptolegnia chapmanii* has been isolated from larvae of *Aedes triseriatus* [9], *Culex quinquefasciatus* [5], *Culex* sp. [3], and *Aedes albopictus* [1], all from sites in the USA. Recently, López Lastra et al. [4] isolated *L. chapmanii* in Argentina, this is the first report of this fungus from the Southern Hemisphere.

Determination of the host range against mosquitoes and safety of *L. chapmanii* for non-target aquatic organisms is required prior to field-testing and is the subject of this paper. The present study is the first report on the host range of mosquito species and non-target fauna for this native isolate of *L. chapmanii*.

Materials and methods

Leptolegnia chapmanii (ARSEF 5499) collected on September 1996 at a permanent pond from M.Romero, La Plata and isolated from *Oc. albifascaitus* larvae, was maintained on Emerson's YpSS agar media (DIFCO) in 60 × 15 mm Petri dishes. Inoculum was prepared cutting cubes of agar (0.5 cm each dimension), mycelial square containing hyphae placing them in 20 ml of sterile distilled water in 90 × 15 mm diameter Petri dishes. When zoospores were observed in the water (after 72 h), third-instar *Aedes aegypti* were placed in the Petri dishes. Dead larvae were removed and examined under phase contrast microscopy to confirm fungal infection.

Twelve mosquito species representing six genera were assayed for susceptibility to *L. chapmanii* (Table 2). Mosquitoes for bioassays were field-collected from the La Plata area. The bioassays were conducted in 250 ml plastic containers containing 100 ml of dechlorinated water. Mosquito larvae from available instars (at least 10 individuals per each replicate) were added to each container. Three *L. chapmanii* infected *Ae. aegypti* larvae, 72 h post-infection were used as fungal inoculum (zoospores/ml/larvae = $1.5 \times 10^5 \pm 0.2$), the number was an average from 12 larvae. Larvae were reared for three days in the same containers to which the inoculum was added

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Table 1. Mosquito species susceptible to different isolates of *Leptolegnia chapmanii*.

Isolate origin	Mosquito host	Reference
South Carolina, USA	*Aedes aegypti*	McInnis and Zattau [5]
	Anopheles albimanus	
	An. quadrimaculatus	
	Culex pipiens quinquefasciatus	
	Ae. taeniorhynchus	McInnis et al. [6]
	Ae. triseriatus	
	Cx. inornata	
	Cx. pipiens	
	Cx. salinarius	
	Ae. aegypti	Nnakumusana [7]
	Ae. africanus	
	Ae. simpsoni	
	An. gambiae	
	Cx. cinereus	
	Cx. decens	
	Cx. duttoni	
	Cx. fatigans	
	Cx. nebulosus	
	Cx. pipiens pipiens	
	Cx. tigripes	
	Eretmopodites sp.	
	Mansonia africana	
	M. uniformis	
	Toxorhynchites brevipalpis	
Ohio, USA	*Ae. triseriatus*	Nnakumusana [8]
	An. quadrimaculatus	
	Cx. pipiens pipiens	
	Cx. restuans	
	Haemagogus equinus	
	T. rutilus	
Florida, USA	*Cx. quinquefasciatus*	Lord and Fukuda [3]
	Ae. albopictus	Fukuda et al. [1]
La Plata, Buenos Aires province, Argentina	*Ae. aegypti*	Present paper
	Anopheles sp.	
	Cx. apicinus	
	Cx. castroi	
	Cx. dolosus	
	Cx. pipiens	
	Ochlerotatus albifasciatus	
	Oc. crinifer	
	Psorophora cyanescens	
	Ps. ferox	

Table 2. Comparison of susceptibility of twelve species of mosquito larvae to *Leptolegnia chapmanii* (ARSEF 5499).

Hosts species	Instar	No. exposed	Replicates	% Infection (±SD)
Aedes aegypti	I	40	4	100
	II	40	4	100
	III	40	4	100
	IV	40	2	85
Anopheles sp.	III	20	2	100
Culex apicinus	II	60	3	13(±4.7)
	III	130	13	0
Cx. castroi	IV	30	2	10
Cx. dolosus	III	40	4	200
Cx. pipiens	II	70	7	92.8(±6.99)
	III	60	3	95(±7.07)
Cx. renatoi	III	20	3	0
Ochlerotatus albifasciatus	II	20	2	100
	III	10	2	80
	IV	100	5	59(±26.1)
Oc. crinifer	II	60	6	68.3(±6.87)
	III	80	3	96(±5.65)
Psorophora cyanescens	III	20	2	90
Ps. ferox	II	80	4	86.2(±9.6)
	III	70	7	77.1(±8.8)
Isostomyia paranensis	II	20	2	0
	III	20	2	0

and fed with finely ground rabbit chow. Exposed larvae were observed daily, and mortality was recorded. Dead larvae were examined microscopically for signs of *Leptolegnia* infection.

The non-target aquatic organisms tested are given in Table 3. Non-target organisms for bioassays were collected from temporary and permanent ponds around La Plata and were maintained in aerated water from the collection sites. Non-target organisms were exposed as groups in 100 ml of dechlorinated water to three *Ae. aegypti* larvae infected with *L. chapmanii* (Table 3). Exposed organisms were observed daily during five consecutive days.

The bioassays were conducted at 25±1 °C. Second instar larvae of *Ae. aegypti* were used as positive controls to verify viability of *L. chapmanii* zoospores. The same number of individuals were used as control groups in all tests and handled in a similar manner but without including the fungal inoculum, and no mortality was recorded in any case. For the host range assays a minimum of two replicates were included for each mosquito species and non-target organisms depending on the availability of them, and the data were pooled to determine the overall infection rate for each species.

Results

Ten of the twelve mosquito species tested were susceptible to the native isolate of *L. chapmanii* under laboratory conditions (Table 2). Eight of the species were highly susceptible with mortality rates of 59–100% in a maximum of 3 days. Larvae of *Cx. apicinus* and *Cx. castroi* were less susceptible to infection with this fungal isolate, with a maximum larval mortality of 13% (Table 2). Larvae of *Cx. renatoi* and *Isostomyia paranensis* were not susceptible to *Leptolegnia chapmanii*.

Leptolegnia chapmanii infection in all susceptible mosquito larvae began with the attachment of zoospores to the host cuticle, causing death of the infected hosts. Encysted zoospores were observed melanized at the abdomen region of all infected larvae between 6–24 h p.i. Surviving larvae of the twelve

Table 3. Susceptibility of non-target aquatic fauna to *Leptolegina chapmanii* (ARSEF 5499).

Host species	No. exposed	Replicates	% Infection (±SD)
Crustacea:			
0. Cladocera – *Daphnia* sp.	30	2	0
0. Amphipoda – *Hyalella curvispina*	10	1	0
0. Cyclopoida – *Mesocyclops annulatus*	20	2	0
Insecta:			
Odonata:			
Zygoptera – Fam. Coenagrionidae	15	1	0
Diptera:			
Fam. Psychodidae	85	3	0
Fam. Ceratopogonidae – *Dasyhelea necrophila*	10	1	0
Fam. Chironomidae			
Sp. 1	65	3	16.9(±2.1)
Sp. 2	35	3	5.7(±0.8)
Coleoptera:			
Fam. Hydrophyllidae	8	1	0
Nematoda:			
Fam. Mermithidae – *Strelkovimermis spiculatus*	10	1	0
Vertebrata:			
Pisces – *Cnesterodon decenmaculatus*	40	4	0
Amphibia – *Bufo arenarum* (larvae)	8	1	0

mosquito species tested were not infected with *L. chapmanii* when checked by microscopic examination.

No mortality was recorded for the majority of the non-target aquatic organisms exposed to zoospores of *L. chapmanii* with the exception of the unidentified midges (Diptera: Chironomidae) that were tested which a maximum mortality of 16.9% after 72 h p.i. (Table 3).

Discussion

The native isolate of *L. chapmanii* used in this study was originally found on a natural population of the flood water neotropical mosquito *Ochlerotatus albifasciatus* [4] in an epizootic condition. In addition, *L. chapmanii* infected larvae of *Ae. aegypti* were found in artificial plastic containers in La Plata in October 2000 (unpublished data).

The majority of the twelve mosquito species tested were susceptible to *L. chapmanii* infection following exposure to fungal zoospores discharged from infected *Ae. aegypti* larvae. *Leptolegnia chapmanii* appeared to undergo normal invasion and development in each of the susceptible mosquito species as described in *Ae. aegypti* by Zattau and McInnis [10]. The major difference was the degree of infection, which was considerably less intense in *Cx. apicinus* and *Cx. castroi*. Larvae of *Cx. castroi* and the two other mosquito species refractory to *Leptolegnia* infection, *Cx. renatoi* and *I. paranensis*, live in phytotelmata habitats, subdivision leaf axils, according to Laird's [2] standard system for classifying larval mosquito habitats.

The lack of pathogenicity for a wide range on non-target aquatic invertebrates and vertebrates tested suggests that this isolate may be specific to mosquito larvae with the exception of members of the Family Chironomidae, which were marginally susceptible to the *L. chapmanii* native isolate. A previous study on the susceptibility of non-target organisms to a strain of this fungus species isolated from South Carolina, USA, showed a wide host range among mosquitoes and a lack of pathogenicity to a wide range of aquatic insects including larvae of the Chironomidae [6]. A study by Nnakumusana [7] on the susceptibility of the isolate of *L. chapmanii* (SC-1) in India showed high mortality rates in 15 mosquito species and susceptibility of larvae of five non-target nematoceran dipterans from the Ceratopogonidae, Chaoboridae, Chironomidae, Psychodidae, and Tipulidae.

The results of this study indicate that *L. chapmanii* (ARSEF 5499) is notably host-specific for mosquitoes and poses little risk for non-target aquatic organisms. Because of its specificity this Argentinean isolate has great potential to be used as a biocontrol agent for medically important mosquitoes in Argentina and possibly elsewhere.

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