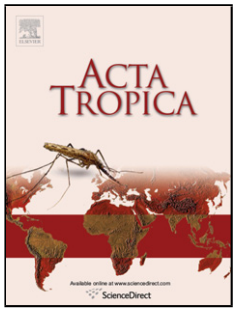

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Leptolegnia chapmanii (Straminipila: Peronosporomycetes) as a future biorational tool for the control of *Aedes aegypti* (L.)

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Abstract

The aim of the present review is to summarize the current knowledge about *Leptolegnia chapmanii* as a pathogen of mosquito larvae. To this end, we present data on its identification, distribution, host range and effects on non-target organisms, effects of environmental factors, in vitro growth, release and persistence in anthropic environments, and effect combined with other insecticides. The data presented allow confirming its potential as a biocontrol agent.

Keywords: *Leptolegnia chapmanii*, biological control, mosquito-vector, *Aedes aegypti*

1. Introduction

Aedes aegypti L. (Diptera: Culicidae) is the main vector of Zika virus, an emerging pathogen which has recently been causing serious epidemics around the world (Ayres, 2016), as well as of other diseases such as yellow fever, dengue, and Chikungunya fever. This mosquito species inhabits anthropic environments, being present in domiciliary and peridomiciliary areas. Therefore, the control of *Ae. aegypti* populations is a must worldwide. Faye and colleagues (2013) have recently reported a list of mosquito species, including *Anopheles coustani L.* (Diptera: Culicidae) and several species of *Aedes*, from which Zika virus strains have been isolated.

The global use of traditional insecticides for mosquito-vector control in recent decades has caused environmental pollution of aquatic ecosystems and has also resulted in insecticide resistance in many mosquito species (Scholte et al., 2004). Therefore, in recent years, there has been an increasing interest in alternative nonchemical strategies. Among these strategies, the most popular one used against larval mosquitoes is the bacterium *Bacillus thuringiensis* var. *israelensis* (*Bti*). Commercial products based on spores and toxins of this bacterium have shown residual effect for more than 7 weeks under laboratory and semi-field conditions (Fansiri et al. 2006; Ritchie et al., 2010). However, under field conditions, the duration of the efficacy of *Bti* is only seven days, so reapplications of *Bti* are required to obtain a prolonged efficacy (Mulla et al., 1990). Nonchemical strategies also include the use of pathogens such as *Coelomomyces* (Blastocladiales: Coelomomycetaceae), *Culicinomyces* (Ascomycota), and *Lagenidium giganteum* (Couch), wich are known to affect mosquito populations and have thus been studied extensively (Scholte et al., 2004). The genus *Lagenidium* belongs to a class of organisms known as Oomycetes and its members are primarily aquatic microorganisms, including saprophytes as well as facultative and obligate parasites (Kerwin 2007). Three formulations of *L. giganteum* have been registered with the United States Environmental Protection Agency (USEPA). This Oomycete recycles in mosquito larvae and is able to persist in larvae for a long time (Kerwin and Washino, 1983). In addition, *L. giganteum* is compatible with *Bti* as well as with *Bacillus sphaericus* (Orduz and Axtell, 1991). Kerwin (2007) considered that the use of this parasite in the field is possible when yields of the sexual stage in liquid culture are increased. However, the use of *L. giganteum* zoospores for operational mosquito control has some disadvantages, including their fragility, limited shelf life, and necessity to store them in water. Thus, their use results in a very high production cost and shipping (Kerwin, 2007). Another

Oomycetepathogen that has been studied in this regard is the genus *Leptolegnia*, but only *Leptolegnia caudata* de Bary (Bisht et al., 1996) and *L. chapmanii* Seymour (McInnis and Zattau, 1982) have been isolated from insects. Although *L. chapmanii* has received limited attention, several authors have agreed that *L. chapmanii* has characteristics that give it potential to act as a biological control agent (Mc Innis and Zattau 1982, Seymour 1984, Lord and Fukuda 1988, Fukuda *et al.* 1997). The objective of the present review is to collate and update the available information about the current state of knowledge of *L. chapmanii* to be considered as a potential agent for the biological control of mosquitoes.

2. Identification, distribution, and host range of *L. chapmanii* and its effects on non-target organisms

Leptolegnia chapmanii (Seymour) (Straminipila: Peronosporomycetes) is a facultative pathogen of mosquito larvae. This oomycete was first isolated from *Aedes triseriatus* (Say) larvae in Lake Charles, Louisiana, USA, in 1971 (Seymour, 1984). It is also a virulent pathogen of *Ae.aegypti*, and can kill its hosts with unusual speed, for example an isolate of *L. chapmanii* can lead to 100% mortality of the larvae of this mosquito within 24 h after exposure (Figure 1) (López Lastra et al., 1999). Isolates of this microorganism are restricted to the USA (South Carolina and Florida) and to Argentina and Brazil (Table 1) (Mc Innis and Zattau 1982; Seymour 1984; Lord et al., 1988; Lord and Fukuda, 1990; López Lastra et al., 1999; Rueda Páramo et al., 2015a; Montalva et al., 2016). The life cycle of *L. chapmanii* is typical of saprolegniaceous fungi, as the species is dimorphic, producing biflagellate zoospores. Sexual reproduction is by means of gametangial contact and results in the production of a characteristic papillate oogonium containing a subcentric or eccentric oospore (resistance structures). Larval infection occurs by mobile zoospores (asexual stage) through two methods: one by encystment of secondary zoospores on the cuticle, and the other initiated by germination of ingested primary or secondary zoospore cysts in the larval midgut (Zattau and McInnis, 1987; Lord et al., 1988). Larvae respond with a melanization around the entry point and along the path of hyphal growth within the hemocoele cavity. Subsequently, the rapid proliferation of hyphae within the hemocoele cavity and destruction of tissues result in the death of the larvae (Figure 1) (Zattau and McInnis, 1987).

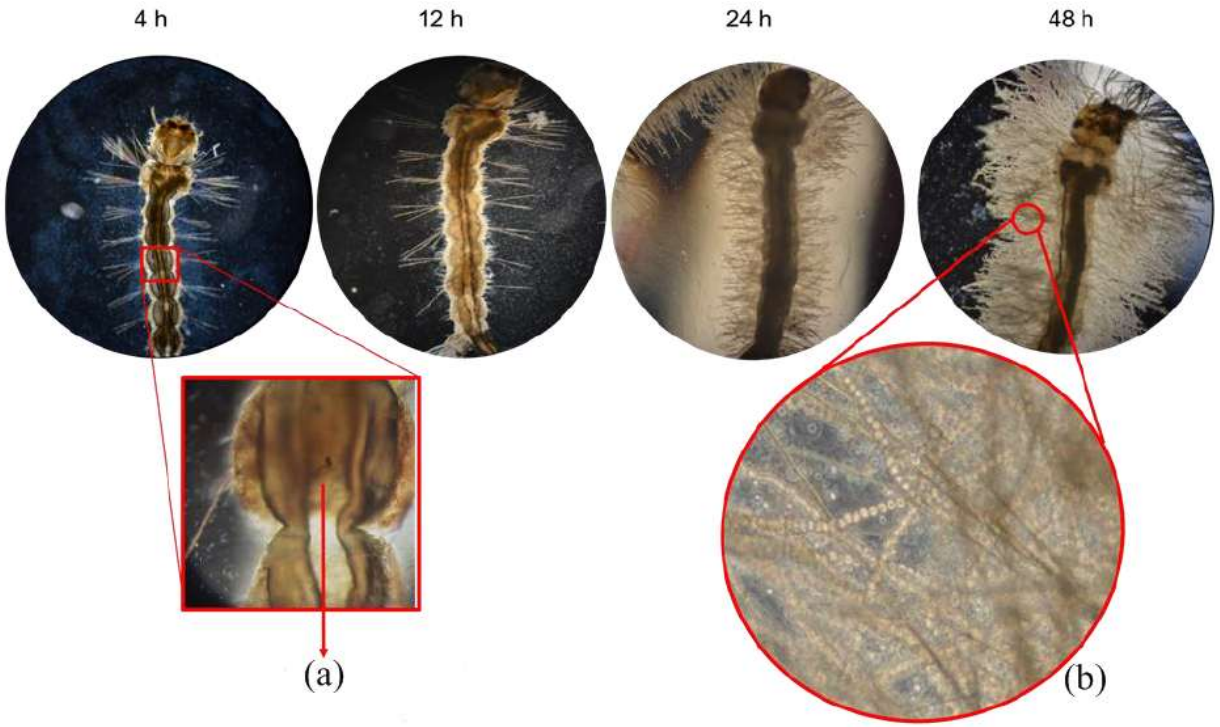

Figure 1. Larva of *Ae. aegypti* infected with *L. chapmanii* in different time, (a) ring of melanin is visible around midgut, (b) sporangia with zoospores.

Table 1. Susceptibility of *Aedes aegypti* larvae to different isolates of *Leptolegnia chapmanii*

Isolate origin	Hosts species	Instar	No. exposed	Replicates	% Infection	Concentration Zoospores (ml)	Reference	ARSEF*
La Plata, Buenos Aires province, Argentina	*Aedes aegypti*	I	40	4	100	1.5 × 105 ± 0.2	López Lastra et al. 2004	5499
		II	40	4	100			
		III	40	4	100			
		IV	40	4	85			
Goiânia, Goiás state, in central Brazil	*Ae. aegypti*	II	10	3	100	(**)	Montalva et al. 2016	12829/12831/ 12835/12847
		III	10	3	100			
		II	10	3	< 50			12817/12819/ 12840/12845
		III	10	3	< 50			
South Carolina, USA	*Ae. aegypti*	I	25	6	100	(**)	McInnis and Zattau 1982	2681
		II	25	6	100			
		III	25	6	31			
		IV	25	6	12			

(*)ARSEF: USDA-Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (Ithaca, New York). (**) Larvae were exposed to zoospores released from 0.5-cm disks cut from cultures of *L . chapmanii*

Leptolegnia chapmanii shows a high degree of specificity for mosquito larvae. Several mosquito species from different genera are susceptible to *L. chapmanii* (Table 2). Natural infected larvae have been found in distinctive habitats including tree holes, artificial containers, freshwater/brackish floodplains , woodland ponds, etc. (Fukuda et al., 1997; López Lastra et al., 1999, 2004; Scholte et al., 2004; Montalva et al. 2016).

Table 2. Mosquito species susceptible to different isolates of *Leptolegnia chapmanii*

Isolate origin	Mosquito host	Reference
South Carolina, USA	*Aedes aegypti*	McInnis and Zattau, 1982
	Anopheles albimanus	
	An. quadrimaculatus	
	Culex pipiens quinquefasciatus	
	Ae. taeniorhynchus	McInnis et al., 1985
Florida, USA	*Cx. quinquefasciatus*	Lord and Fukuda, 1988
La Plata, Buenos Aires province, Argentina	*Ae. aegypti*	López Lastra et al., 2004
	Anopheles sp.	
	Cx. apicinus	
	Cx. castroi	
	Cx. dolosus	
	Cx. pipiens	
	Ae. albifasciatus	
	Ae. crinifer	
	Psorophora cyanescens	
	Ps. ferox	
Posadas, Misiones province, Argentina	*Ae. aegypti*	Rueda Páramo et al. 2015a
Goiânia, Goiás state, in central Brazil	*Ae. aegypti*	Montalva et al. 2016

Leptolegnia chapmanii is apparently safe for non-mosquito fauna. Several studies have shown that *L. chapmanii* does not affect non-target organisms. Different species of Insects (Odonata, Trichoptera, Plecoptera, Coleoptera, Diptera), Crustaceans (Cladocera, Amphipoda, Cyclopoida), Nematoda and Vertebrata (Pisces, Amphibia) have been exposed to zoospores of *L. chapmanii* with no consequences for them (Mc Innis et al., 1985; López Lastra et al., 2004).

Horizontal gene transfer (HGT) is the nonvertical inheritance of genetic material by transfer between different species. HGT is an important evolutionary mechanism for prokaryotes. Genome analysis has shown that examples of HGT are not as frequent in eukaryotes, but when they do occur they may have important evolutionary consequences (McCarthy and Fitzpatrick, 2016). The light of some new studies indicate that HGT in Oomycetes is rare; however, when present, these HGT genes give these microorganisms the capacity of acquiring new pathogenicity genes, switching of hosts and even colonizing new hosts (McCarthy and Fitzpatrick, 2016). For example, HGT genes have been detected in the Oomycetes *Phytophthora* and *Pythium* as well as in *L. giganteum*, (Judelson, 2012; Vilela et al., 2015). However, up to the present time, there are no reports about the presence of these genes in *Leptolegnia* sp.

Pelizza et al. (2013) studied the sublethal effects of *L. chapmanii* infections on *Ae. aegypti* and found that females that survived infection with *L. chapmanii* laid fewer eggs, had a smaller number of gonotrophic cycles, had shorter wings, and had lower fecundity than controls. The fact that larval mortality occurs within less than 24 h post-challenge, the relatively little or no risk for non-target organisms and the fact that sublethal effects hamper *Ae. aegypti* reproduction are excellent characteristics for considering *L. chapmanii* as a new potential candidate for the biological control of *Ae. aegypti*.

3. Environmental factors affecting *L. chapmanii* infection

To determine the potential *L. chapmanii* as a biological control agent, Pelizza et al. (2007a, b) studied biotic and abiotic factors affecting *L. chapmanii* infection in *Ae. aegypti*. Natural epizootics of *L. chapmanii* require successful contact between zoospores and a large portion of the mosquito population. These authors demonstrated that the ability of the motile zoospores to find and infect larvae of *Ae. aegypti* is affected by the surface area and/or density of mosquitoes within the breeding sites, and that one *Ae.*

aegypti larva produces $6.1 \pm 0.2 \times 10^4$ zoospores during the 48 h after infection (Pelizza et al., 2007a). These authors also studied the effects of other factors such as salinity, temperature and pH, on the longevity and virulence of zoospores of *L. chapmanii* under laboratory conditions (Pelizza et al., 2007b). They demonstrated that variations in pH between 4 and 10 at 25 ºC did not affect the pathogenicity of *L. chapmanii* on *Ae. aegypti*, resulting in 100% infection. These authors also found that the mortality rates of larvae of *Culex pipiens* exposed to similar pH values and zoospore concentrations increased from 62% to 99% when pH increased from 4 to 7, and then decreased to 71% at pH 10. Pelizza et al. (2007b) also demonstrated that NaCl reduced mycelial growth of *L. chapmanii* with complete inhibition at 15 parts per thousand (ppt). Pelizza et al. (2007b) reported that the mortality of *Ae. aegypti* larvae exposed to *L. chapmanii* zoospores in NaCl concentrations ranging from 0 to 7 ppt was of 100%. However, when larvae of *Cx. pipiens* were exposed to *L. chapmanii*, the authors found that mortality decreased from 96% in distilled water to 31.5% in water with 6 ppt of NaCl. Control larvae of *Cx. pipiens* could not tolerate NaCl concentrations higher than 7 ppt (Pelizza et al., 2007b). Other authors (Lord et al., 1988) determined that concentrations of up to 5 ppt of NaCl enhanced the mycelial growth of an isolate of *L. chapmanii* from an unidentified *Culex* larva collected from a ground pool at the edge of a salt marsh in Florida, USA.

Regarding the effect of temperature on the virulence and longevity of *L. chapmanii*, Pelizza et al. (2008a) showed that zoospores from infected larvae were infective to *Ae. aegypti* larvae for 51, 12, and 5 consecutive days when maintained at 25, 35, and 10 ºC, respectively. They also showed that zoospores of *L. chapmanii* were infectious at temperatures between 10 and 35 ºC but not at 5 or 40 ºC (Pelizza et al., 2007b). They concluded that temperature directly affects the infectivity and production of zoospores *in vivo* and *in vitro*, although *L. chapmanii* zoospores tolerate a wide range of temperatures (Pelizza et al., 2007a, b, 2008a).

Pelizza et al. (2008b) also studied the effects of the water quality of the mosquito breeding sites on the pathogenicity and infectivity of zoospores from one Neotropical isolate of *L. chapmanii* . The authors found that *L. chapmanii* was successful as a biological control agent in the different water samples obtained from ditches, rain pools, containers and Río de la Plata River pools from Argentina, producing high larval mortality. There were highly significant differences among mortalities in the water from containers (70.2%), rain pools (99.5%), and Río de la Plata pools (95%), whereas there were no significant differences in the larval mortality in the water from ditches, rain pools and Río de la Plata pools (Pelizza et al., 2008b). The results of this study allow us to conclude that this isolate of *L.*

chapmanii was pathogenic and virulent within a wide range of organic matter content and water pollutants (Pelizza et al., 2008b). In contrast, *L. giganteum* does not tolerate certain levels of water pollution, such as low levels of dissolved oxygen, extreme pH and a large number of other microorganisms present in the water, thus making its infectivity and effectiveness scarce or null (Lord and Roberts 1985). Also, increased water temperature (less than 8°C and greater than 34°C), high salinity and high levels of organic load may limit mosquito infection by *L. giganteum* (Kerwin 2007). Jaronski and Axtell (1982) showed that *L. giganteum* produced infection in the range of 27 – 100 % in *Cx. quinquefasciatus* larvae, when the test was performed in water with low levels of contamination and organic load, but observed no infections in water with moderate to high levels of pollution and organic load.

In conclusion, all the results described above demonstrate that the *L. chapmanii* isolates tested tolerate a wide range of organic load, temperatures, pH, water chemistry and salinity, and thus suggest that this oomycete has the potential to adapt to a wide variety of mosquito habitats.

Zattau et al. (1987) showed that functional *L. chapmanii* oospores serve as a useful source of inoculum and enhance the potential of this oomycete as a mosquito larvicide, because functional oospores can remain dormant for long periods of time and resistant to environmental conditions unfavorable for vegetative growth. Pelizza et al. (2010a) further studied the production of oogonia and oospores of *L. chapmanii* in larvae of *Ae. aegypti* at different temperatures and showed that the number of oogonia formed was influenced by temperature, ranging from 12 to 1,030 between 5 and 40°C, respectively. They also observed that the number of oospores in larvae of *Ae. aegypti* was higher when they were incubated at 25°C (10 oospores/larva). However, the authors pointed out that the low production of oospores of *L. chapmanii* from oogonia was not clearly understandable. In addition, it has been recently reported that Brazilian isolates of *L. chapmanii* are extremely poor at producing oospores (Montalva et al., 2016). Kerwin (2007) considered that, for the mass production of the reproductive stages of *L. giganteum*, the strain of parasite used and, consequently, how it is isolated and maintained in vitro are of primary importance. Kerwin showed that oospores are the ideal stage for large-scale production and application of *L. giganteum* since they can be stored in the original culture medium or as a dry powder for months or years (Kerwin 2007). On the other hand, there remain two major impediments to use *L. giganteum* oospores for operational mosquito control. First, oospore yields in agar or liquid culture

106 are 2 to 3 orders of magnitude lower than those obtained for the asexual stage. Secondly, it is still not
107 known how to break oospore dormancy without causing premature abortion (Kerwin 2007).
108 We can preliminarily conclude that these thick-walled spores are a valuable source of inoculum and
109 would improve the potential of this oomycete as a mosquito larvicide. However, further studies are
110 needed to understand the physical, chemical and nutritional conditions that affect the formation,
111 development and germination of oospores in different isolates of this pathogen.
112
113 **4. *In vitro* growth of *L. chapmanii***
114 *Leptolegnia chapmanii* grows readily on PYG and Emerson YPss culture media (Pelizza et al.,
115 2011). However, the use of such culture media for its mass production could be rather expensive. Thus, in
116 our laboratory, we have recently tested a culture medium based on sunflower seeds (SSE), reported by
117 Guzman & Axtell (1986) as an alternative for culture of *L. giganteum* (Couch), as an inexpensive
118 alternative medium for the mass production of *L. chapmanii* (Rueda Páramo et al., 2016).
119 Growth and development of *L. chapmanii* in solid and liquid SSE was compared with the traditional
120 media PYG and Emerson YPss. We found higher production of zoospores as well as mortalities of *Ae.*
121 *aegypti* larvae with *L. chapmanii* by using the alternative SSE medium. *L. chapmanii* also developed a
122 great number of small mycelial masses in SSE liquid culture medium compared with the single large
123 biomass formed in PYG and Emerson YPss. The previous-results suggest that *L. chapmanii* could be an
124 auxotrophic organism that develops vegetatively on poor sterol media, but sterol is required for initiation
125 of the reproductive cycles (sexual / asexual), as described for *L. giganteum* (Domnas et al., 1977; Kerwin
126 & Washino, 1983). An increased production of zoospores by the enrichment of culture medium with
127 sunflower oil has also been demonstrated for *L. giganteum* (Maldonado-Blanco et al., 2011). In
128 conclusion, *L. chapmanii* could be mass produced in an inexpensive medium and maintaining its
129 virulence for mosquito larvae. However, further studies are needed to determine the nutritional
130 requirements of the reproductive stages.
131
132 **5. Release and persistence of *L. chapmanii* in anthropic environments**
133
134 Larval stages of *Ae. aegypti* are aquatic and develop in natural and artificial containers with water
135 located in domestic and peridomestic places. In our laboratory, we performed a series of experiments to

136 determine the mortality of *Ae. aegypti* larvae through time, with a single inoculation of zoospores of *L.*
137 *chapmanii* in containers located in three different anthropic areas in domestic and peridomestic
138 environments where *Ae. aegypti* develops naturally. The pathogenicity of zoospore suspensions was
139 evaluated along 6 weeks and the inoculum was of $3.05 \pm 1 \times 10^5$ zoospores for a final concentration of
140 $1.22 \pm 0.4 \times 103$ zoospores/ml. The results showed that the persistence and pathogenicity of a native
141 isolate of *L. chapmanii* decreased over time regardless of the location. However, the mortality of *Ae.*
142 *aegypti* larvae was significantly lower ($p < 0.05$) in containers located outdoors without sun protection
143 (89% in the first week and 9% in the sixth week) compared with the containers located indoors (97% in
144 the first week and 42% in the sixth week) and outdoors with shade (89% in the first week and 29% in the
145 sixth week), possibly because of the exposure to sun radiation (Rueda Páramo et al., 2015b). These data
146 corroborated the presumptions about the susceptibility of *L. chapmanii* zoospores to UV radiation. Solar
147 radiation consists of visible light, infrared and ultraviolet (UV) radiation. UV radiation is divided into
148 UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm), and its deadly and particularly
149 mutagenic power in terrestrial and aquatic organisms is attenuated during its passage through the
150 atmosphere. Penetration of solar radiation into aquatic ecosystems depends on the concentration of
151 dissolved or particulate material, and any harmful impact on organisms is related to the total dose (Häder
152 et al., 2007). Our results showed that free encysted *L. chapmanii* zoospores suspended in distilled water
153 are susceptible to UV-A radiation at 25 ºC, and that their susceptibility is related to the time of exposure
154 and corresponding dose. The virulence of zoospores was not affected by a single short exposure time up
155 to 10 min but a longer exposure of zoospores to UV-A instantly affected their larvicidal activity in *Ae.*
156 *aegypti*. The production of zoospores in larvae and their virulence were not hampered at a maximal 8 h
157 exposure of dead larvae to UV-A (Rueda Páramo et al., 2015c). However, so far, little is known about
158 adaptation or recovery capacities of *L. chapmanii* after exposure to UV-radiation or of specific photo-
159 repair mechanisms of DNA damages or oxidative stress caused, as reported for other entomopathogens
160 (Chelico and Khachatourians, 2008; Rangel et al., 2011; Fang and St. Leger, 2012) .
161 In other studies, mortalities of 95 and 100% were reported for *Ae. aegypti* larvae treated with a
162 suspension of 1.8×10^5 zoospores/ml from *L. chapmanii*, under seminatural conditions, with sunlight and
163 shade, respectively (Pelizza et al., 2010b). It has also been shown that solar radiation affects the stability
164 and persistence of different entomopathogenic fungi (Gardner et al., 1977; Roberts and Campbell, 1977).
165 In conidial fungi, the germination rate is reduced after exposure to UV-B radiation (Le Grand and

Cliquet, 2013). Both solar UV-A and UV-B radiations impair conidial viability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae* (Braga et al., 2001).

In conclusion, *L. chapmanii* could potentially be used as a biological control agent for larval populations of *Ae. aegypti*, in different anthropogenic places. Under these environmental conditions, where *Ae. aegypti* develops naturally, the pathogenic action of *L. chapmanii* persists for several weeks. The results obtained so far also suggest that dead larvae and zoosporangia provide zoospores with a certain protection against UV-A and emphasize the susceptibility of free encysted zoospores to such radiation (Rueda Páramo et al., 2015 b,c).

6. Effect of ***L. chapmanii*** combined with insecticides

Historically, the control of *Ae. aegypti* throughout the world has been achieved through natural, nonchemical methods involving the elimination of breeding sources and by the use of traditional insecticides, typically organophosphates. Since the 1980s, several commercial products with active substances such as the *Bti* endotoxins have been used for the control of *Ae. aegypti* and other culicids of relevance to public health. This bacterium has a high specificity for aquatic Diptera and is safe for both vertebrates and aquatic invertebrates (Lacey, 2007). Another important positive feature for *Bti* is that there are no reports on resistance development under field conditions.

Several authors have demonstrated the advantages of the synergistic interaction between fungi and chemical insecticides when applied simultaneously (Ferron, 1985; Barjan et al., 1995; Pristavko, 1996). The combined effect of sublethal concentrations of *Bti*, temephos, and *L. chapmanii* zoospores indicates not only that this Oomycete is not inhibited by these two agents but also that zoospores, when used together with these other agents, exert a synergistic larvicidal effect on *Ae. aegypti* (Table 3) (Pelizza et al., 2010b). Pelizza et al. (2010b) also observed an enhancement of the larvicidal activity when zoospores were used along with either of the two compounds alone, both in laboratory assays and under seminatural conditions. However, it was not possible to check whether or not there was synergism when using three insecticides together (Table 3). In small-scale treatments, they determined that *L. chapmanii* zoospores were infective for up to 56 days (Pelizza et al., 2010b). These data are consistent with the previous observation that zoospores or their cysts can survive for 51 days under field-like conditions (Pelizza et al.,

2008a). However, little is known about the synergistic interaction between isolates of *L. chapmanii* and other biological or chemical insecticides when applied simultaneously.

Table 3. Different concentrations and combinations of *Bacillus thurigiensis* var. *israelensis*, temephos, and Argentinian isolate of *Leptolegnia chapmanii* zoospores used in laboratory bioassays

Treatment	Concentrations			
	Zoospores (ml)	Temephos (ppm)	*Bti* (ppm)	Mortality % [(a)]
A	$6.1x10^4$			45 (3,4)
B		0.00035		60 (3)
C		0.001		95 (1)
D			0.012	35 (4)
E			0.027	60 (3)
F	$6.1x10^4$	0.00035		90 (1,2)
G	$6.1x10^4$	0.001		100 (1)
H	$6.1x10^4$		0.012	80 (1,2)
I	$6.1x10^4$		0.027	90 (1,2)
J		0.00035	0.012	100 (1)
K		0.001	0.027	100 (1)
L	$6.1x10^4$	0.00035	0.012	100 (1)
M	$6.1x10^4$	0.001	0.027	100 (1)

(a) Percent mortality of third-instar *Ae. aegypti* larvae exposed to different concentrations and combinations of *Bacillus thurigiensis* var. *israelensis*, temephos, and *Leptolegnia chapmanii* zoospores. The percent mortalities followed by the same number are not significantly different according to the Duncan test (P = 0.01). No mortality was recorded in controls in the absence of larvicidal agents. The cumulative larval mortality was recorded at 48 h after the beginning of the assay.

7. Conclusions and future prospects

Leptolegnia chapmanii has been proved to be a potential candidate to develop a mosquito larvicide. This oomycete has a high host specificity at a family level, infects larvae of mosquitoes in different anthropic areas (domestic and peridomestic environments), and shows very low risk of harming non-target aquatic organisms. In addition to these attributes presented above, this pathogen presents a high potential to cause epizootics. Zoospores of *L. chapmanii* remain viable in a wide range of temperature, pH and salinity values in both laboratory and semi-field conditions. Studies on the dispersal of this pathogen have not yet been developed and little is known about the synergistic interaction between isolates of *L. chapmanii* and other larvicides, either biological or chemical, when applied simultaneously. Weaknesses of *L. chapmanii* include the difficulty in producing oospores and activating its germination, as well as the fact that successive transfers of cultures *in vitro* contribute to decreasing the virulence of the isolates. In summary, further studies about oospores and mass scale production as well as formulation of *L. chapmanii* and specific aspects related to this subject should be performed.

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