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Short Communication

Effect of ultraviolet-A radiation on the production of *Leptolegnia chapmanii* (Saprolegniales: Saprolegniaceae) zoospores on dead *Aedes aegypti* (Diptera: Culicidae) larvae and their larvicidal activity

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ABSTRACT

Impact of UV-radiation in entomopathogens in aquatic environments remains little investigated. The present study reports on the effect of UV-A on the larvicidal activity of *Leptolegnia chapmanii* zoospores in *Aedes aegypti*; on the production of zoospores in larvae killed by the pathogen and then exposed to UV-A; and on the activity of these zoospores against healthy larvae. Whereas the virulence of free zoospores in *A. aegypti* larvae was affected by a UV-A exposure time longer than 10 min, production of zoospores in larvae and their virulence were not hampered at a maximal 8 h exposure of dead larvae to UV-A. Findings suggest that dead larvae and zoosporangia provide a certain protection to zoospores against UV-A and emphasize the susceptibility of free encysted zoospores to such radiation.

1. Introduction

Entomopathogens face challenging and complex biotic and abiotic stress scenarios in their natural environments. Sunlight, temperature and humidity are among the most critical factors that affect the persistence of these microorganisms (Benz, 1987). Solar radiation consists of visible light, infrared and ultraviolet (UV) radiation. UV radiation is divided into UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm), and its deadly and particularly mutagenic power in terrestrial and aquatic organisms is attenuated during its passage through the atmosphere. Penetration of solar radiation into aquatic ecosystems depends on the concentration of dissolved or particulate material, and any harmful impact on organisms is related to the total dose (Häder et al., 2007).

Aedes aegypti is the main vector of virus infections in the tropics. Aquatic larval stages of this species develop in smaller water collections eventually exposed to sunlight. A successful control of larvae is often hampered by the difficulty to contact all larvae with a control agent, and by the increasing resistance to synthetic insecticides (Morrison et al., 2008). Specific microbial insecticides present a powerful additional option for integrate control strategies. The aquatic oomycete *Leptolegnia chapmanii* (Saprolegniales) has recently gained considerable attention for mosquito control due to its high virulence against larval stages (Scholte et al., 2004; Pelizza et al., 2011, 2013; Leles et al., 2013). Interest about the impact of abiotic factors on *L. chapmanii* has focused on the influence of temperature, pH and salinity on the infectivity of zoospores (Pelizza et al., 2007), and there is initial evidence about the impact of solar radiation on the activity of this pathogen against *A. aegypti* larvae (Rueda Páramo et al., 2015). A better understanding of the influence of solar radiation on *L. chapmanii* is highly important for the development of an innovative biolarvicide based on this pathogen. The present study reports on the impact of UV-A radiation on *L. chapmanii* and its activity against *A. aegypti* larvae under laboratory conditions.

2. Materials and methods

2.1. Origin, rearing and preparation of A. aegypti

The *A. aegypti* colony tested here derived from larvae collected in 2012 in Goiânia, Brazil. The mosquitoes were reared under

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laboratory conditions at the IPTSP-UFG as described by Lima et al. (2009). Second (L2) and third instar larvae (L3), about 72 h after molting and fed with small amounts of cat food pellets (Black Jack; Alisul Alimentos S.A., São Leopoldo, Rio Grande do Sul, Brazil), were used for the tests.

2.2. Origin of L. chapmanii and preparation of zoospores

L. chapmanii CEP 010 (Collection of Entomopathogenic Fungi of Insects and other Arthropods, CEPAVE) also deposited as ARSEF 5499 used in the present study was isolated from an *Ochlerotatus albifasciatus* larva collected in La Plata, Buenos Aires, Argentina, in 1996 (López Lastra et al., 1999).

For each repetition, zoospores were produced with 300 L2/L3 previously exposed for 48 h to five dead larvae killed by ARSEF 5499. Zoospores were suspended in 1000 ml of distilled water at a final 10^3 zoospores/ml and immediately used in the assays.

2.3. UV-A radiation assays

White plastic containers (25 ml) with 10 ml of zoospore suspension (10^3 zoospores/ml) at a 1 cm water depth each were arrayed in eight groups. Each group contained six containers (five replicates and one control). Containers were then exposed to UV-A in a photostability Chamber CARON™ 6545-2 (Marietta, OH, USA) with total irradiance of 31.514 mW m^{-2} at 25 °C at increasing exposure time periods: 0 (control), 0.17, 0.5, 1, 2, 4, 6 and 8 h, that corresponded to a total dose of 0 (control), 19.29, 56.72, 113.45, 226.90, 453.80, 680.70, 907.60 kJ m^{-2}, respectively. The spectral distribution of the lamps in the Caron photostability chamber is given in Fig. 1. Spectral irradiance was measured with an Ocean Optics USB 2000 Spectroradiometer (Dunedin, FL, USA). After irradiation, containers were covered completely with aluminum foil up to 8 h. Subsequently, ten L2/L3 were set in each container and maintained at 25 °C and 12 h photophase. Mortality of larvae was registered 48 h afterwards. Larvae were not fed during the assays. Each treatment consisted of three independent repetitions.

In another test series, a single dead L2/L3, that had been exposed to *L. chapmanii* zoospores 24 h earlier, was set in a plastic container (2 ml) with 1.5 ml of distilled water. It was then exposed to UV-A as mentioned and subsequently transferred to a container (20 ml) with 10 ml of distilled water and maintained at 25 °C and 12 h photophase for 24 h. The concentration of zoospores in each container was then determined with a Neubauer chamber. Five healthy L2/L3 were set in each container, and mortality recorded 48 h later.

Mortality data were transformed to arsine square-roots and analyzed as numbers of zoospores by analysis of variance and the Student–Newman–Keuls test for multiple comparison (IBM SPSS 12.0).

3. Results

The highest cumulated mortality (93.3%) at a 48 h incubation time of larvae was obtained with either zoospores that were previously not exposed to UV-A (positive control) or exposed at a shortest 10 min to UV-A. Survival of larvae increased significantly within the next hours of exposure of zoospores to UV-A (0 h and 10 min > 30 min > 1 h up to 8 h; $F_{7,64} = 19.9$; $P < 0.001$), and mortality dropped to a minimal 18.9% at an 8 h exposure (Fig. 2). There was no reduction at all of larvicidal activity of zoospores in the first 10 min of exposure to UV-A. Activity declined then to highest (0.8%) rate of loss of larval mortality per minute of exposure (related to maximal 93.3% attained at 0 and 10 min of exposure) between a 10 and 60 min exposure to UV-A, and dropped finally to a mean loss of 0.08% per min in the following 7 h (Fig. 2).

In the second test series, the total number of zoospores produced by *L. chapmanii* on larvae, 48 h after death and subsequent exposure to UV-A up to 8 h, varied between 10^4 zoospores/larva (control and 30 min of exposure to UV-A) and 5×10^4 zoospores/larva (10 min and 8 h of exposure to UV-A). There was no significant effect of the time of exposure on the number of zoospores/larva ($F_{7,16} = 0.6$; $P = 0.75$; Table). Cumulative mortality of new healthy L3 applied with the zoospores that derived from the previous tests and incubated for 48 h varied between 49.3% (2 h exposure to UV-A) and 94.7% (10 min exposure to UV-A), and likewise there was no significant effect of the time of exposure of dead larvae to UV-A (0 up to 8 h) on the mortality caused by zoospores on new larvae ($F_{7,16} = 1$; $P = 0.5$; Table 1).

In both test series, all control larvae had survived for the same length of time.

4. Discussion

Results clearly showed that free, encysted *L. chapmanii* zoospores suspended in distilled water are susceptible to UV-A radiation at 25 °C, and that their susceptibility was related to the time of exposure and corresponding dose. Virulence of zoospores obviously was not affected by a unique short exposure time up

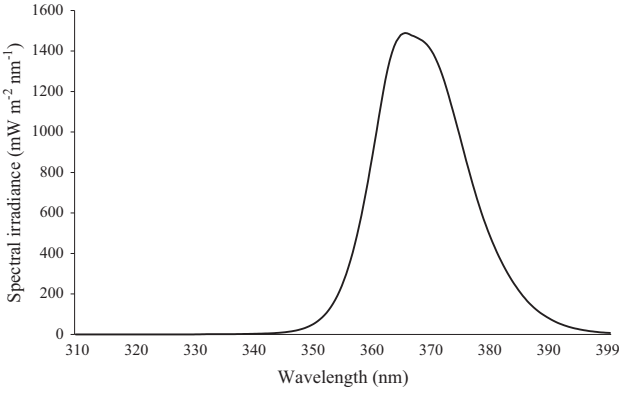

Fig. 1. Spectral irradiances of lamps of the photostability chamber CARON 6545-2. The chamber provided UV-A radiation at a total irradiance of 31.514 mW m^{-2}.

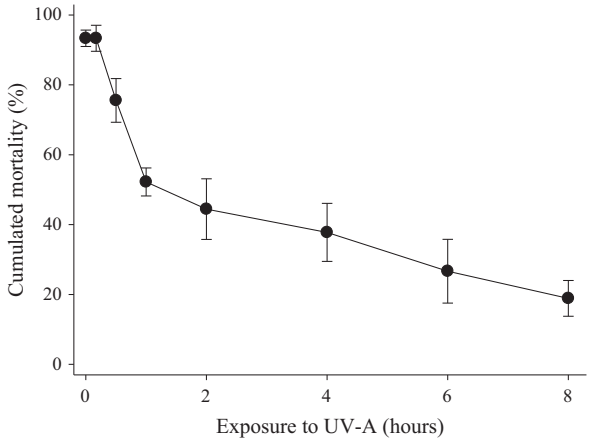

Fig. 2. Cumulative mortality (% ± standard error) of *Aedes aegypti* third instar larvae after a 48 h incubation with *Leptolegnia chapmanii* zoospores previously exposed to UV-A radiation up to 8 h.

Table 1
Mean number of zoospores (± standard error, SE) produced by *Leptolegnia chapmanii* on dead *Aedes aegypti* third instar larvae after exposure to increasing UV-A radiation (31.514 mW m^{-2}) and cumulative mortality (± SE) of new larvae exposed to radiated cadavers with zoospores.

Time of exposure (h)	UV-A	Zoospores/larva	Cumulative
	Dose (kJ m^{-2})	($\times 10^4$)[a]	Mortality (%)[b]
0	0	1 ± 0.3	86.3 ± 7.3
0.17	19.3	1.5 ± 0.9	94.7 ± 3.5
0.5	56.7	1 ± 0.4	68.7 ± 7.7
1	113.5	1.3 ± 0.5	69.3 ± 16.4
2	226.9	1.1 ± 0.2	49.3 ± 16.2
4	453.8	1.1 ± 0.4	71.3 ± 16.2
6	680.7	1.3 ± 0.6	73.3 ± 13.3
8	907.6	1.5 ± 0.3	84 ± 14.1
		$F_{7,16} = 0.6$	$F_{7,16} = 1$
		$P = 0.75$	$P = 0.5$

[a] Values based on individual tests (3 repetitions with 5 replicates each), larvae were exposed to UV-A, 12 h after death, and the numbers of zoospores were quantified 36 h after initiating radiation.
[b] Each dead larva was tested with 5 new L3; mortality presented at a 48 h exposure at 25 °C.

to 10 min but a longer exposure of zoospores to UV-A instantly affected their larvicidal activity in *A. aegypti*.

Further findings suggested that dead larvae in which the oomycete is actively producing zoosporangia and zoospores, provided, to a certain extent, protection against UV-A radiation and maintained virulence against the larvae during the test period. This suggests that a direct application of dead larvae previously infected with *L. chapmanii* to disseminate zoospores in breeding sites seems to be strategy worth being evaluated for biological control of mosquitoes with a longer residual effect than zoospores alone.

The outcomes of the present study together with previous results (Rueda Páramo et al., 2015) corroborate presumptions about the susceptibility of *L. chapmanii* zoospores to UV radiation. However, at this stage nothing is known about any adaptation or recovery capacities of *L. chapmanii* after exposure to UV-radiation or of specific photo-repair mechanisms of DNA damages or oxidative stress caused as was reported for other entomopathogens (Chelico and Khachatourians, 2008; Rangel et al., 2011; Fang and St. Leger, 2012).

Females of *A. aegypti* preferably oviposit in breeding sites protected from continuous exposure to sunlight (Reiter, 2007). Depending on the diurnal course of the sun and properties of the breeding site and its immediate surroundings, the exposure time to solar radiation and its intensity on the aquatic microbiota will vary (Häder et al., 2007).

The highest activity of *L. chapmanii* against *A. aegypti* can be expected in breeding sites that are exposed to little or no sunlight (e.g., tree holes and other shaded habitats). Interestingly, the first mosquito larva (*A. triseriatus*) infected with this entomopathogen was detected in a tree hole (Seymour, 1984). In conclusion, we emphasize a need to strengthen investigations about the general impacts of solar radiation and its more specific impacts on the effectiveness of *L. chapmanii* in *A. aegypti*.

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