Journal of Medical Entomology, XX(X), 2018, 1–4
doi: 10.1093/jme/tjy071
Short communication

OXFORD

Effect of UV-B Irradiation on Water-Suspended *Metarhizium anisopliae* s.l. (Hypocreales: Clavicipitaceae) Conidia and Their Larvicidal Activity in *Aedes aegypti* (Diptera: Culicidae)

Marianel L. Falvo,[1,2] Patricia Albornoz Medina,[1,2] Juscelino Rodrigues,[1] Claudia C. López Lastra,[2] Juan J. García,[2] Éverton K.K. Fernandes,[1] and Christian Luz[1,3]

[1]Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás (UFG), Goiânia, Goiás, Brasil, [2]Centro de Estudios Parasitológicos y de Vectores (CEPAVE), Universidad Nacional de La Plata-CONICET, La Plata, Buenos Aires, Argentina, and [3]Corresponding author, e-mail: wchrisluz@hotmail.com

Subject Editor: Lawrence Hribar

Received 22 January 2018; Editorial decision 16 April 2018

Abstract

Ultraviolet (UV) radiation is a key limiting factor for biological pest control with entomopathogenic fungi. While little is known about the impact of UV on *Metarhizium anisopliae* Metchnikoff (Sorokin) (Hypocreales: Clavicipitaceae) conidia in aquatic mosquito-breeding sites, this study determined the effect of UV-B on the viability and virulence of *M. anisopliae* sensu lato (s.l.) strain IP 46 in the laboratory against *Aedes aegypti* (L.) (Diptera: Culicidae) larvae. Conidia were treated in cups under defined water depths (0, 1, 2, and 3 cm) to six different UV-B doses (0, 0.657, 1.971, 3.942, 7.884, 11.826, or 15.768 kJ m^{-2}) at 27 ± 2°C. The ability of treated conidia to germinate up to 24 h postexposure on PDAY + benomyl + chloramphenicol medium at 25 ± 1°C was adversely affected by higher doses of UV-B radiation regardless of the water depth. Germination, however, did not fall below 70% regardless of the test conditions. In fact, conidial virulence against second-instar larvae was not affected by either the water depth ($F_{3,84} = 0.3$, $P = 0.85$) or any tested levels of UV-B radiation ($F_{6,21} \leq 1.2$, $P \geq 0.39$) including those distinctly higher than might be expected for tropical sites. These findings strengthen previous observations that IP 46 has significant potential for use against *A. aegypti* larvae, even when exposed to elevated UV-B irradiance levels in the small breeding sites that are common for this important vector.

Key words: entomopathogenic fungus, mosquito, water level, UV-B tolerance, germination

Introduction

Emerging viral diseases that cause Chikungunya, Zika, and Mayaro fevers are transmitted by mosquitoes and have spread alarmingly across Latin America. Dengue fever remains a serious widespread human threat (Weaver et al. 2018). The aquatic immature stages of the main vector of these arboviruses, *Aedes aegypti* (L.) (Diptera: Culicidae), often develop in small, transient human-made breeding sites (Wong et al. 2011) whose water levels vary according to ambient temperature, humidity, and exposures to rainfall, sunlight, and wind.

The entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) (Hypocreales: Clavicipitaceae) Sorokin is effective against *A. aegypti* eggs, larvae, and adults (Lacey 2017). Ultraviolet (UV) radiation, especially UV-B (280–320 nm), is a key limiting factor for *M. anisopliae* survival and clearly affected in vitro development and virulence of this and other fungi in target pests (Fernandes et al. 2015). However, recent studies confirmed that UV-B does not impair the activity of *M. anisopliae* conidia on *A. aegypti* adults that are subsequently exposed to UV radiation (Falvo et al. 2016). Additionally, in aquatic habitats, water may protect *M. anisopliae* and other microorganisms against UV-B damage, and the impact of UV-B radiation on *M. anisopliae* decreases with deeper water columns (Braga et al. 2001, Häder et al. 2007).

The success of a mycoinsecticide against *A. aegypti* larvae will depend on the survival and virulence of applied fungal propagules; this concern is especially acute for small breeding sites with low water levels. The effects of UV-B exposures on the larvicidal activity of *M. anisopliae* conidia in small mosquito-breeding sites still remain

unknown. This study's goals were to evaluate, under laboratory conditions, whether the depth of water covering *M. anisopliae* conidia might protect conidia from UV-B exposure, and the impact of water depth on the germination and virulence of conidia for *A. aegypti* larvae.

Materials and Methods

Origin and Rearing of *A. aegypti* and Preparation of Larvae

The colony originated from larvae collected in 2012 in Goiânia, Brazil, and maintained as noted by Falvo et al. (2016). Recently molted (12–24 h) second-instar larvae (L2) were separated and kept overnight without feeding before use in the experiments.

Origin and Culture of the Fungus

M. anisopliae s.l. strain IP 46 was isolated in 2001 from central Brazilian soil (Rocha et al. 2013) and cultured on potato dextrose agar in a Petri dish (90 × 10 mm) for 15 d at 25 ± 1°C and 12 h photophase (Falvo et al. 2016) before harvesting conidia with a spatula for use here.

Exposure of Conidia to UV-B at Differing Water Depths

Plastic cups (3.9 cm height × 4 cm diameter) were sterilized by exposure to UV radiation (UV-C Lamp G30T8, Philips, Amsterdam, Netherlands) for 20 min. One ml of an aqueous suspension containing 6×10^8 conidia/ml was placed into each cup. One cup was kept overnight in a desiccation chamber with silica gel at 4°C to dry a layer of conidia onto the cup's bottom (water level 0 cm). Three other cups were filled with sterile distilled water (SDW) to final water depths in the cups of 1, 2, and 3 cm, respectively. All cups were covered with a 0.13-mm thick cellulose diacetate film (JCS Industries, La Mirada, CA) that blocked radiation below 290 nm (excluding UV-C, which is absent in the biosphere) while passing UV-B (290–320 nm) (Braga et al. 2001). Cups were then incubated for 16 h at 25°C in darkness to allow complete settling of conidia (at a final 8.5×10^7 conidia/cm^2) onto the bottom. Eight cups were prepared for each water level to test six UV-B radiation doses and with two control cups prepared as presented below. Cups with settled conidia were exposed in a UV-B chamber (133 × 86 × 50 cm) to radiation with four UV lamps (UVB-313 EL, Q-Lab Corporation, Cleveland, OH). The Quaite weighted UV-B irradiance (1,152 mW m^{-2}) was determined as previously described (Quaite et al. 1992, Braga et al. 2001). UV-B irradiance was measured with a USB 2000 + Rad Spectroradiometer (Ocean Optics, Dunedin, FL). The distance between the UV lamps and the bottom of the cups was 28 cm. Conidia in the cups were exposed for 10 min, 30 min, 1 h, 2 h, 3 h, or 4 h, and received total doses of 0.657, 1.971, 3.942, 7.884, 11.826, and 15.768 kJ m^{-2}, respectively. Ambient conditions inside the chamber were 27 ± 2°C and 50 ± 10% RH. For each of the four water levels (0–3 cm), one positive control cup with conidia was completely wrapped with aluminum foil and exposed to UV-B for the longest (4 h) period. Other unwrapped positive control cups containing conidia under each of these water levels were left outside the chamber at 27 ± 2°C for 4 h.

After UV-B exposure, cups with water levels of 0, 1, and 2 cm were filled with SDW to a final volume of 30 ml (the 3-cm height of the highest water level tested). The conidia in each cup were re-suspended with a pipette to standardize the suspension. A final concentration of 2×10^7 conidia/ml was adjusted in all cups that corresponded to the lethal concentration LC_{90} at an 8-d exposure (calculated according to Preisler and Robertson 1989, Supplementary Table S1).

In Vitro Assays on Germination

After irradiation, 50 µl of suspended conidia were taken from each cup and diluted in 950 µl of Tween 80, 0.01% (v/v) to a final 10^6 conidia/ml. Germination tests were performed as reported by Fernandes et al. (2007).

In Vivo Assays on Mortality

Ten L2 were added into each cup with suspended nonirradiated (positive control) or irradiated conidia at 2×10^7 conidia/ml and incubated at 25 ± 1°C and 12 h photophase. Ten L2 were kept in 30 ml of SDW without conidia (negative control). Every 2 d, larvae were fed as mentioned by Falvo et al. (2016). Mortality was recorded daily for up to 10 d. Dead larvae were transferred to Petri dishes (55 × 15 mm) containing 0.6% water-agar amended with 0.025% chloramphenicol, 0.0002% thiabendazole, 0.0005% crystal violet (all w/v), and the pH adjusted to 5.5. Dishes were then incubated at 25 ± 1°C and 12 h photophase. The development of mycelium and new conidia was monitored for up to 10 d.

Data Analyses

The percent germination of conidia and larval mortality were calculated for four independent repetitions and then arcsine-square root transformed. Then the effects of water level and UV-B radiation on germination and mortality were examined with analysis of variance and SNK test (Statistica) at $P < 0.05$. The values of mean germination presented here followed by different letters (A–B) were significantly different. The lethal times to kill 50 and 90% (LT_{50} and LT_{90}) of the larvae were calculated with Probit analysis for dependent values, and values compared based on their confidence intervals (Throne et al. 1995).

Results

Effect of UV-B Radiation on Germination

The percent germination of water-suspended conidia after exposure at different water levels (0, 1, 2, and 3 cm) and UV-B radiation doses (0, 0.657, 1.971, 3.942, 7.884, 11.826, and 15.768 kJ m^{-2}), 24 h after incubation on medium appear in Fig. 1a. There was a significant general effect of the water level tested on germination ($F_{3,84} = 6.7$, $P < 0.001$; 1 cm [A]; 3 cm [AB]; 0 and 2 cm [B]), and also a significant general effect of the irradiation dose regardless of the water level on germination ($F_{6,84} = 9.5$, $P < 0.001$; 0, 0.657; 1.971, 3.942; 7.884 and 11.826 kJ m^{-2} [A] and 15.768 kJ m^{-2}[B]). At specific water levels, germination of conidia tested without water (level 0 cm) after 24-h incubation varied from 82.1 ± 1.5% (15.7 kJ m^{-2}) to 97.6 ± 1.2% at 0 kJ m^{-2} (in the aluminum foil covered control). At a 1-cm water level, germination varied from 96.5 ± 0.6% (1.97 kJ m^{-2}) to 99.7 ± 0.1% (0.657 kJ m^{-2}). At a 2-cm water level, germination ranged from 72.8 ± 10.8% (15.7 kJ m^{-2}) to 97.3 ± 0.9% (3.942 kJ m^{-2}). Finally, at the highest water level tested at 3 cm, germination varied from 89.5 ± 2% (15.7 kJ m^{-2}) to 99.1 ± 0.1% (in the aluminum foil covered control).

Effect of Irradiated Conidia on Larval Mortality

Larvae treated with conidia that were previously irradiated with UV-B or not (positive control) started to die within the first 24 h after treatment. Mean cumulative mortalities after the 10-d exposure period ranged from 60 ± 14.1% (7.884 kJ m^{-2}) at a 2-cm water level to 90 ± 5.1% (7.884 kJ m^{-2} without water and 15.7 kJ m^{-2} at a 2-cm water level and for the positive control at a 1-cm water level;

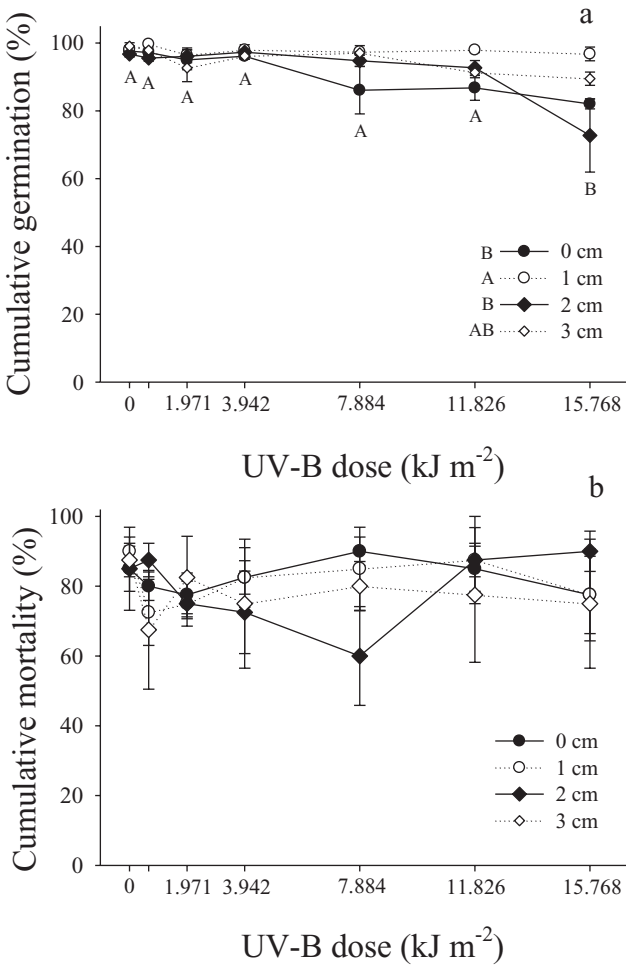

Fig. 1. (a) Cumulative mean germination (±SE) of *Metarhizium anisopliae* s.l. IP 46 conidia, in different water levels (0–3 cm) and then exposed to UV-B doses from 0 (controls) to 15.768 kJ m^{-2} and subsequently inoculated on potato dextrose agar medium supplemented with 1 gl^{-1} yeast extract, 0.002% (w/v) benomyl and chloramphenicol (0.05%, w/v) and incubated for 24 h at 25 ± 1°C and 12 h photophase and (b) cumulative mean mortality (±SE) of *Aedes aegypti* second-instar larvae treated with 2 × 10^7 conidia/ml, prepared as mentioned, 10 d after incubation at 25 ± 1°C and 12 h photophase. Different capital letters (A–B) in panel a indicate significant differences found for germination of conidia exposed to UV-B doses and water levels ($P < 0.001$).

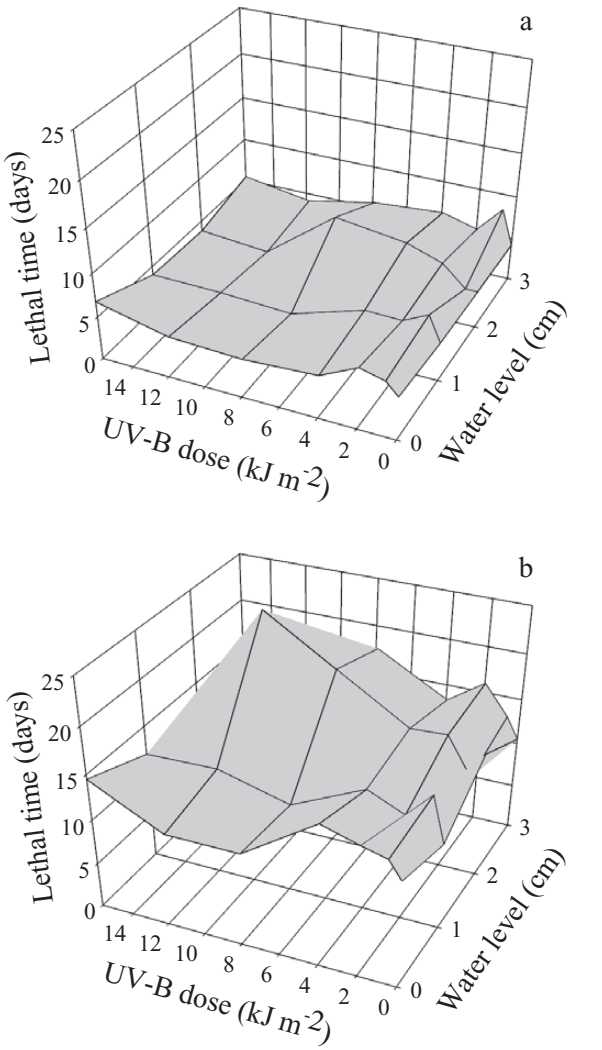

Fig. 2. Lethal times (days) to kill 50% (a) or 90% (b) of *Aedes aegypti* second-instar larvae treated with *Metarhizium anisopliae* s.l. IP 46 (2 × 10^7 conidia/ml) adjusted previously at water levels up to 3 cm and exposed to UV-B doses up to 15.768 kJ m^{-2}.

Fig. 1b). There was no significant effect of UV-B dose regardless of the water level (0 up to 3 cm) tested ($F_{6,21} \leq 1.2$, $P \geq 0.39$) nor any effect of water level on larval mortality ($F_{3,84} = 0.3$, $P = 0.85$). Lethal times to kill 50 and 90% of the larvae treated with UV-B irradiated conidia at different doses and kept at different water levels varied from 3.1 d (11.826 kJ m^{-2} at a 2 cm level) to 8.15 d (0.657 kJ m^{-2} at a 3-cm level) for values of LT_{50} and from 8.8 d (11.826 kJ m^{-2}) to 19.7 d (3.942 kJ m^{-2}) at a 2-cm level for values of LT_{90} without significant difference among either the values of LT_{50} and LT_{90} (Fig. 2a and b).

Discussion

The efficacy of *M. anisopliae* species against different developmental stages of *A. aegypti* is well known (Scholte et al. 2004, Lacey 2017). *M. anisopliae* s.l. strain IP 46 was isolated from central Brazil and belongs in the *M. anisopliae* complex (Rocha et al. 2013). This isolate is particularly well known for its ovicidal activity against *A. aegypti* (Luz et al. 2007). There is, however, also evidence of larvicidal and adulticidal activity of IP 46 (Silva et al. 2004, Leles et al. 2010, Falvo et al. 2016) that makes this fungus a promising candidate for mosquito control. Our findings about the impact of conidial concentrations on this strain's larvicidal activity represented an important basis for testing the impact of UV-B on conidia and, in turn, on their virulence for larvae (Supplementary Table 1S). Posterior in vivo tests were run with a conidial concentration of IP 46 that, without any previous irradiation, was expected to kill most larvae (LC_{90}) during the tests, and permitted us to determine any harmful effect of UV-B on conidial virulence against these larvae.

The viabilities of IP 46 conidia or conidia suspended in water and subsequently exposed to UV-B irradiation was clearly affected by increasing UV-B doses. However, in vitro germination continued to be elevated at all test conditions and was only notably diminished at the highest UV-B levels tested (15.768 kJ m^{-2}). This highest irradiation level distinctly exceeded those measured previously in field

sites in metropolitan Goiânia on midday during August (706 mW m^{-2} corresponding to 2.55 kJ m^{-2} h^{-1}) and October 2015 (911 mW m^{-2} corresponding to 3.28 kJ m^{-2} h^{-1}) where and when *A. aegypti* occurs commonly (Falvo et al. 2016). Our findings, however, made clear that an overlying water layer up to 3 cm deep provided no clear protection against UV-B or detrimental effect of UV-B for sedimented conidia.

A. aegypti larvae are bottom feeders and frequently develop in small breeding sites with low water volumes where they may be periodically exposed to intense solar radiation (Wong et al. 2011). In these habitats, larvae can be infected by sedimented conidia from mycoinsecticial *Metarhizium* granular formulations whether those infections occur by direct cuticular contact of after conidial ingestion. Interestingly, *A. aegypti* adults treated with IP 46 conidia and subsequently exposed to the same UV-B doses used in this study were no less susceptible to fungal infection than fungus-treated adults that were not exposed to UV-B (Falvo et al. 2016); this high efficacy of conidia even after exposure to UV-B against adults and larvae strengthens our belief in the potential of this fungus for multistage control of *A. aegypti*.

This is the first study to show the remarkable larvicidal outcome of terrestrial *M. anisopliae* s.l. conidia sedimented in small water volumes and stressed by UV-B against *A. aegypti* larvae.

Supplementary Material

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

Authors thank Richard A. Humber (USDA-ARS, Ithaca, NY; retired) for the critical review of the manuscript. This research was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) (PPCP 016/2011), and by the Foundation for Research of the State of Goiás (FAPEG, Brazil, 201210267001060). We also thank CNPq for the grants: PQ 308850/2015–7 to É.K.K. Fernandes, and PQ 308189/2013-2 to C. Luz and ANPCyT (PICT 2012-0622) for a doctoral grant to M.L. Falvo.

References Cited

Braga, G. U., S. D. Flint, C. L. Messias, A. J. Anderson, and D. W. Roberts. 2001. Effects of UVB irradiance on conidia and germinants of the entomopathogenic hyphomycete *Metarhizium anisopliae*: a study of reciprocity and recovery. Photochem. Photobiol. 73: 140–146.

Falvo M., R. Pereira-Junior, J. Rodrigues, C. C. López Lastra, J. J. García, É. K. K. Fernandes, and C. Luz. 2016. UV-B radiation reduces in vitro germination of *Metarhizium anisopliae* s.l. but does not affect virulence in fungus-treated *Aedes aegypti* adults and development on dead mosquitoes. J. Appl. Microbiol. 121: 1710–1717.

Fernandes, E. K. K., D. E. N. Rangel, A. M. Moraes, V. R. Bittencourt, and D. W. Roberts. 2007. Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. J. Invertebr. Pathol. 96: 237–243.

Fernandes, E. K. K., D. E. N. Rangel, G. U. L. Braga, and D. W. Roberts. 2015. Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. Curr. Genet. 61: 427–440.

Häder, D. P., H. D. Kumar, R. C. Smith, and R. C. Worrest. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem. Photobiol. 6: 267–285.

Lacey, L. A. 2017. Microbial control of medically important mosquitoes in tropical climates, pp. 409–430. In L. A. Lacey (ed.), Microbial control of insect and mite pests from theory to practice. Academic Press, London, United Kingdom.

Leles, R. N., N. A. Sousa, L. F. N. Rocha, A. H. Santos, H. H. G. Silva, and C. Luz. 2010. Pathogenicity of some hypocrealean fungi to adult *Aedes aegypti* (Diptera: Culicidae). Parasitol. Res. 107: 1271–1274.

Luz, C., M. H. H. Tai, A. H. Santos, L. F. N. Rocha, D. A. S. Albernaz, and H. H. G. Silva. 2007. Ovicidal activity of entomopathogenic Hyphomycetes on *Aedes aegypti* (Diptera: Culicidae) under laboratory conditions. J. Med. Entomol. 44: 799–804.

Preisler, H., and J. Robertson. 1989. Analysis of time-dose-mortality data. J. Econ. Entomol. 82: 1534–1542.

Quaite, F. E., B. M. Sutherland, and J. C. Sutherland. 1992. Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. Nature 358: 576–578.

Rocha, L. F. N., P. W. Inglis, R. A. Humber, A. Kipnis, and C. Luz. 2013. Ocurrence of *Metarhizium* spp. in central Brazilian soils. J. Bas. Microbiol. 53: 251–259.

Scholte, E.-J., B. G. J. Knols, R. A. Samson, and W. Takken. 2004. Entomopathogenic fungi for mosquito control: a review. J. Insect Sci. 4: 19.

Silva, R. O., H. H. G. Silva, and C. Luz. 2004. Effect of *Metarhizium anisopliae* isolated from soil samples of the central Brazilian Cerrado against *Aedes aegypti* larvae under laboratory conditions. Rev. Pat. Trop. 33: 207–216.

Throne, J. E., D. K. Weaker, V. Chew, and J. E. Baker. 1995. Probit analysis of correlated data: multiple observations over time at one concentration. J. Econ. Entomol. 88: 1510–1512.

Weaver, S., C. Scott, C. Charlier, N. Vasilakis, and M. Lecuit. 2018. Zika, Chikungunya, and other emerging vector-borne viral diseases. Annu. Rev. Med. 69: 6.1–6.14.

Wong, J., S. T. Stoddard, H. Astete, A. C. Morrison, and T. W. Scott. 2011. Oviposition site selection by the dengue vector *Aedes aegypti* and its implications for dengue control. PLOS Neglect Trop. D. 5: e1015.