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RESEARCH ARTICLE

Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation

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Abstract Ultraviolet radiation from sunlight is probably the most detrimental environmental factor affecting the viability of entomopathogenic fungi applied to solar-exposed sites (e.g., leaves) for pest control. Most entomopathogenic fungi are sensitive to UV radiation, but there is great inter- and intraspecies variability in susceptibility to UV. This variability may reflect natural adaptations of isolates to their different environmental conditions. Selecting strains with outstanding natural tolerance to UV is considered as an important step to identify promising biological control agents. However, reports on tolerance among the isolates used to date must be analyzed carefully due to considerable variations in the methods used to garner the data. The current review presents tables listing many studies in which different methods were applied to check natural and enhanced tolerance to UV stress of numerous entomopathogenic fungi, including several well-known isolates of these fungi. The assessment of UV tolerance is usually conducted with conidia using dose-response methods, wherein the UV dose is calculated simply by multiplying the total irradiance by the period (time) of exposure. Although irradiation from lamps seldom presents an environmentally realistic spectral distribution, laboratory tests circumvent the uncontrollable circumstances associated with field assays. Most attempts to increase field persistence of microbial agents have included formulating conidia with UV protectants; however, in many cases, field efficacy of formulated fungi is still not fully adequate for dependable pest control.

Keywords *Metarhizium* · *Beauveria* · ultraviolet radiation · fungal tolerance · formulation

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Introduction

Many natural abiotic factors are known to limit the ability of fungal agent to biologically control arthropod pests, but solar ultraviolet (UV) radiation (UV-A and UV-B) is probably the most detrimental environmental factor affecting the viability of fungi applied for pest control (Ignoffo and Garcia 1992; Moore et al. 1993). Most UV-tolerant isolates probably can survive a few hours of direct exposure to solar UV radiation, but UV-susceptible isolates succumb. In addition, the exposure of fungi to UV-B (Fernandes et al. 2007; Fargues et al. 1996; Braga et al. 2001d; Nascimento et al. 2010) or UV-A (Fargues et al. 1997; Braga et al.

2001c) may delay conidial germination of survivors and reduce fungal development, which decreases the persistence and efficacy of infective propagules in the field (Zimmermann 1982; Roberts and Campbell 1977). Attempts to overcome these circumstances have focused on selecting strains with natural UV tolerance and on formulating conidia with adjuvants to absorb or to block solar radiation and, thereby, protect fungi from UV radiation.

The selection of UV-tolerant strains is not simple. Many conditions may interfere with the evaluation of conidial tolerance of entomopathogenic fungi to UV, because many conditions influence their susceptibility or tolerance to UV. For example, tolerance of fungi to UV may vary according to (1) the nutrient supplied for cultivation; (2) the exposure or restriction of fungi to visible light during cultivation or after irradiation; (3) the age of fungal cultures and other conditions surrounding UV exposure. Accordingly, comparisons of UV tolerance among isolates from different studies must be carefully analyzed due to variations in methods. Variability in susceptibility of fungi to UV seems to be related to the habitat or the latitude from where fungi were isolated; no correlation, however, has been found in relation to fungal species, host, or substrate of origin (Braga et al. 2001d; Fernandes et al. 2007, 2011; Bidochka et al. 2001; Fargues et al. 1996).

Formulation of fungal propagules has been investigated as a tool to protect fungi to some extent from UV radiation. Oil-based formulations are reportedly able to significantly enhance the tolerance of conidia to UV radiation, with advanced germination of conidia, in comparison to conidia suspended in conventional water-based formulations (Alves et al. 1998). Accordingly, oil-based formulations prepared with emulsifiable or non-emulsifiable mineral or vegetable oils have been tested. Additional protection of conidia to UV has also been reported when some chemical sunscreens were incorporated into water- or oil-based formulations (Hunt et al. 1994; Moore et al. 1993; Inglis et al. 1995).

Selecting the strains with high virulence to the target pest, and selecting from those candidate isolates with the most outstanding natural tolerance to UV are important steps to identifying promising biological control agents. Moreover, formulating conidia appropriately before considering field application is an important approach for producing efficient biological control products. The current review reports the effort of many studies in searching for entomopathogenic fungi naturally tolerant to UV radiation and in investigating the relationship between their UV tolerance and the origins of isolates. Furthermore, this review also focuses on formulations used to enhance the tolerance of entomopathogenic fungi to UV, as part of a strategy to increase the efficacy of biological control agents.

Seeking fungal isolates naturally tolerant to UV

Entomopathogenic fungi are, in general, very sensitive to UV radiation. The UV tolerance of *Beauveria bassiana* s.l. (Inglis et al. 1995; Morley Davies et al. 1996; Fargues et al. 1996; Huang and Feng 2009; Posadas et al. 2012), *Isaria fumosorosea* (Fargues et al. 1996), *Metarhizium acridum* (Morley Davies et al. 1996; Fargues et al. 1996; Braga et al. 2001d), and *Metarhizium. anisopliae* s.l. (Fargues et al. 1996; Braga et al. 2001d) have been extensively researched. Other less investigated species include *Aphanocladium album*, *Lecanicillium lecanii*, *Lecanicillium aphanocladii*, *Simplicillium lanosoniveum* (Braga et al. 2002), *Engyodontium albus*, *Beauveria* spp. other than *B. bassiana* (Fernandes et al. 2007), and *Tolypocladium* spp. (Santos et al. 2011). Great inter- and intraspecies variability in susceptibility to solar irradiation has been reported. For example, the survival rate of *B. bassiana* s.l. to UV radiation ranged from 0 to 100 % [at 2.16 kJ m^{-2} total irradiance (Fargues et al. 1996)], and from 0 to 80 %, with most isolates presenting less than 50 % [at 978 mW m^{-2} Quaite-weighted irradiance, 7.04 kJ m^{-2} dose (Fernandes et al. 2007)]; whereas *M. anisopliae* s.l. ranged from 0 to 42 % (Fargues et al. 1996), and *M. acridum* ranged from 18 to 87 % (Fargues et al. 1996). According to Braga et al. (2001d), the tolerance of 30 isolates of *Metarhizium* spp. to UV (19.9 kJ m^{-2} Quaite-weighted dose) ranged from 0 to approximately 55 %. The intraspecific variability in tolerance to radiation indicates that selection of tolerant fungal isolates may be appropriate for considering the development of products for biological control of pests in insolated environments (Huang and Feng 2009; Fargues et al. 1996; Morley Davies et al. 1996; Fernandes et al. 2007).

Variability in tolerance to UV among isolates may reflect natural adaptation to different environmental conditions. Accordingly, comparison between origin of isolates and their tolerance to UV has shown a positive relationship for some species of entomopathogenic fungi. Isolates of *I. fumosorosea* from warm regions were more tolerant to UV (1 h exposure = 1.08 kJ m^{-2}) than isolates from temperate regions (Fargues et al. 1996). Likewise, a significant inverse correlation was reported between tolerance to UV-B radiation at both 1-h and 2-h exposure to 920 mW m^{-2} (doses of 3.3 and 6.6 kJ m^{-2}) and 1200 mW m^{-2} (doses of 4.3 and 8.6 kJ m^{-2}) Quaite-weighted irradiance and the latitude of origin of 26 *Metarhizium* strains from sites at latitudes from 61°N to 54°S. The higher the latitude origin, the lower the tolerance of isolates to UV-B (Braga et al. 2001d). Similar correlation was reported for *B. bassiana* s.l. isolates, where a latitudinal adaptation to UV-B irradiation was detected, i.e., isolates originating near the equator tended to have the highest UV-B tolerances (Fernandes et al. 2007, 2009). An association of genetic populations

of *M. anisopliae* s.l. with their habitat types was reported as well. Isolates originated from agricultural areas showed marked resilience to UV exposure compared to isolates obtained from forested areas (Bidochka et al. 2001). Conversely, conidia of *Hirsutella thompsonii* obtained from different geographical locations exhibited identical UV (200-300 nm) inactivation kinetics (Tuveson and McCoy 1982). The tolerance of *B. bassiana* s.l. to UV radiation also did not reveal apparent relationship with the geoclimatic origin of the isolates (Fargues et al. 1996). No positive correlation has been reported in consideration with entomopathogenic fungi species, their host, or substrate of origin (Braga et al. 2001d; Fernandes et al. 2007, 2011; Bidochka et al. 2001; Fargues et al. 1996).

Variation in susceptibility of entomopathogenic fungi to UV was also related to the color of conidia, since conidial pigmentation seems to be very efficient in protecting against UV radiation, directly (Braga et al. 2006; Rangel et al. 2006b; Nascimento et al. 2010), or indirectly because enzymes involved in pigment-synthesis pathways are also required for tolerance to abiotic stresses (Fang et al. 2010). A study conducted with simulated ultraviolet sunlight reported that conidial color can influence the inactivation of conidia by the radiation, where the UV radiation may be blocked on black conidia whereas penetration of UV may vary for other pigmented conidia (Ignoffo and Garcia 1992). Accordingly, the black conidia of *Aspergillus niger* presented a remarkably higher mean half-life of 14.8 h when exposed to simulated sunlight than those of all other species investigated, which included less-pigmented conidia of *Beauveria*, *Nomuraea*, *Metarhizium*, and a tan-colored mutant of *A. niger*, with a half-life ranging from 1.1 h for *Nomuraea rileyi* to 2 h for the tan-colored mutant *A. niger* (Ignoffo and Garcia 1992). Color mutants of the darkly pigmented conidia *M. robertsii* wild-type ARSEF 23 were, in general, significantly more tolerant to UV than the less-pigmented conidia of its mutants (Braga et al. 2006; Rangel et al. 2006b; Nascimento et al. 2010). Conversely, other studies report that the hyaline conidia of *B. bassiana* s.l. isolates were generally more tolerant to UV than the darkly pigmented conidia of *M. anisopliae* s.l. or the lightly pigmented conidia of *I. fumosorosea*, with 61 % of the *B. bassiana* s.l. isolates exhibiting over 50 % survival after 1 h of irradiation (1.08 kJ m^{-2}), whereas 26 % of the *M. anisopliae* s.l. and only 3 % of the *I. fumosorosea* exhibited over 50 % survival (Fargues et al. 1996). This indicates that conidial pigmentation may be important but not crucial in protecting conidia from UV radiation.

Variability of entomopathogenic fungi to UV tolerance is mostly assessed by dose-response methods, where the samples usually are exposed to a single dose of radiation. Recent studies, however, have proposed a multiple-dose bioassay system, which estimates a lethal UV-B dose (e.g., LD_{50}) from the survival–dose relationship for an isolate. This system was based on modeling of the inverted sigmoid dose-survival trend to generate absolute estimates of lethal UV doses to compare the tested isolates (Huang and Feng 2009; Yao et al. 2010). In dose-response methods for evaluating the susceptibility of fungi to UV radiation, the UV dose is calculated by multiplying the total irradiance by the period of exposure. However, not only the total irradiance, but the distribution of spectral irradiance is also an important consideration for evaluating the response of fungi to irradiation or for comparing the tolerance of isolates based on data generated from different studies, because the fungal responses to UV are strongly wavelength dependent. While short wavelengths of UV radiation (especially UV-B = 280–315 nm) are detrimental to entomopathogenic fungi, longer wavelengths (375–425 nm) may promote photoreactivation and stimulate recovery of damaged conidia (Fargues et al. 1997; Braga et al. 2002), because photolyase enzymes respond to even very low doses of UV-A or white light. Therefore, conidia exposed to simulated sunlight (short and long wavelengths combined) may benefit from long wavelengths in comparison to conidia exposed to UV-B only. Among the fractions of the UV spectrum that reach the surface of the Earth, UV-B (290–315 nm) is the most harmful to biological systems, as reviewed by Braga *et al.* (2001d). Fargues et al. (1997) showed that the detrimental effects of exposure to simulated solar radiation (approximately 290–2200 nm) depend mainly on the quantity of UV-B received by the inoculum. These results suggest that the dose of UV-B is the most efficient variable to express the effect of sunlight on the persistence of entomopathogenic fungi in irradiated environments.

Laboratory tests investigating the tolerance of fungi to solar radiation should not be assumed to represent real field conditions. Under natural conditions, the scenario is very dynamic and the angle of incidence of radiation and its spectrum which are constantly changing (Fargues et al. 1996), and the fungal infection processes also are dynamic. Nevertheless, most studies on the effect of solar radiation on biological systems are conducted in the laboratory due to the uncontrollable circumstances associated with variation of intensity and spectral content from sunlight, such as time of the day, season, location, altitude, and atmospheric conditions.

Because irradiation from lamps seldom provide environmentally realistic spectral distributions, it is important to quantify the biological effectiveness of irradiances used for the UV exposure experiments (Braga et al. 2001c). The BSWF (Biological Spectral Weighting Functions) weighs the effectiveness of each wavelength. Although there are no specific action spectra for the inactivation of fungal conidia, many studies have used the BSWF based on action spectra developed for other biological systems

(Braga et al. 2001c; Caldwell and Flint 1997). A frequently used spectrum for fungi is a DNA-damage function developed by Quaite et al. (1992), which attributes most of the effectiveness to UV-B and short UV-A wavelengths (Braga et al. 2001c). Accordingly, many studies that evaluated the effect of UV radiation on entomopathogenic fungi have calculated the dose based on the Quaite-weighted irradiance rather than on the total irradiance (Fernandes et al. 2007; Braga et al. 2001a, d; Rangel et al. 2004; Nascimento et al. 2010). For a more detailed discussion on this subject, see Braga et al. (2015) in this issue of Current Genetics.

Most tolerance tests of entomopathogenic fungi are conducted with dormant conidia (Braga et al. 2015). However, the effects of irradiance on various life-cycle stages of the fungus may differ. For example, the final phase of germination of *Metarhizium*, during or after germ tube emergence, is the most sensitive stage to UV-B (Braga et al. 2001b). In nature, the high tolerance of dormant conidia to UV may increase their persistence in the field; but if the conidium reaches the arthropod cuticle and germinates, and if at that point the fungus is exposed to UV radiation, then the fungal infection may be strongly compromised. Blastospores (short hyphal bodies produced *in vitro*) of entomopathogenic fungi were also very sensitive to UV (Ottati-de-Lima et al. 2012), and in preliminary laboratory tests with two *B. bassiana* s.l. isolates, these blastospores were as sensitive to UV as were conidia (at 839 mW m^{-2} Quaite-weighted irradiance and dose of 1.51 kJ m^{-2}) (Bernardo and Fernandes, unpublished data). Although hyphal bodies form naturally, they are protected from sunlight because they are in the hemocoel of their infected hosts. Blastospores also are produced in liquid media for use in commercialized biocontrol formulations (Faria and Wraight 2007), but their efficacy may be reduced by direct exposure to solar radiation during field applications.

M. acridum is reported to be one of the most tolerant entomopathogenic fungi to UV radiation (Fargues et al. 1996). ARSEF 324, a *M. acridum* isolate from Queensland, Australia, is one of the most UV-tolerant strains yet tested (Rangel et al. 2005b; Braga et al. 2001d). This marked tolerance of ARSEF 324 may be correlated with its ecological conditions during its evolution, which was an area that exerted strong selective pressure for tolerance to high insolation (Rangel et al. 2005a). Among the *Metarhizium* spp. from the *anisopliae* complex, *M. robertsii* ARSEF 2575 is a strain with marked UV-B tolerance. For example, in a comparison of 24 non-*M. acridum* isolates, it was the only one that had some conidial germination after exposure to UV-B [19.9 kJ m^{-2} (Quaite-weighted dose)] (Braga et al. 2001d). The susceptibilities/tolerances to UV of some well-known strains of entomopathogenic fungi are listed in Table 1.

Conditions influencing the tolerance of entomopathogenic fungi to UV

Many studies have aimed to select entomopathogenic fungi with marked natural tolerance to UV stress. The tolerance of fungal isolates, however, may be masked by laboratory methods. Accordingly, a single fungal isolate may demonstrate different responses due to the methods applied during investigation. The methods may be very diverse, for example, in regard to 1) the culture media for growing the fungi, 2) the age of cultures, 3) the water activity of the inoculum, 4) the surface used to support the inoculum during irradiation, 5) the culture medium used to evaluate the survivals, 6) the exposure of irradiated inoculum to visible light or darkness, etc. Table 2 lists many studies in which different methods were applied to check fungal tolerance to UV stress, as well as several studies using formulated fungi to attempt enhancement of UV tolerance of conidia.

The tolerance to UV radiation can be improved by physiological manipulations (Rangel et al. 2004; Rangel and Roberts 2007). Accordingly, variation of UV tolerance has been reported in relation to the culture medium on which conidia were produced (Rangel et al. 2006a). A considerable increase in UV tolerance, for example, was observed in conidia of *M. robertsii* (ARSEF 2575) produced on minimal medium [Czapek medium (inorganic nitrogen without saccharose), or minimal medium supplemented with non-preferred carbon sources]. This is in comparison to conidia produced on the most commonly used medium, PDAY. Enhanced UV tolerance of conidia produced on low-nutrient medium may be because the growth on the minimal medium represents a stress condition that requires the fungi to resist damage (Rangel et al. 2006a, 2008; Rangel and Roberts 2007). This subject is broadly discussed in a review published in this issue (Rangel et al. 2015).

Responses of *M. robertsii* ARSEF 2575 conidia to UV-B were altered when the fungus was grown on the same medium type (PDAY or PDBY, potato dextrose broth supplemented with 1 g l^{-1} yeast extract) with pHs varying from 4.59 to 9.45. Aerial conidia produced under alkaline conditions were two times more tolerant than conidia produced on PDAY or PDBY adjusted to neutral or acidic pH (Rangel and Roberts 2007). Many other culture media have been considered for obtaining fungal inocula for UV tolerance tests, such as Molish's agar (Speare 1920), Sabouraud Maltose Agar (SMA), Maltose Agar (MA), Malt Extract Agar (MEA), rice grains, etc (see Table 2).

The tolerance of fungi also may vary according to the age of the cultures. Air-dried conidia of *B. bassiana* s.l. and *M. anisopliae* s.l. cultured for 14 d were more tolerant to UV (mostly UV-B, at 2.3 W m^{-2}) than conidia from isolates cultured for 7 d. Conversely, air-dried conidia of *Lecanicillium muscarium* and *L. lecanii* cultured for 14 d

Table 1 Susceptibility/tolerance to UV of some well-known strains of entomopathogenic fungi is listed below

Strain	Tolerance of fungi to UV*
ARSEF 324, *Metarhizium acridum* (Morley Davies et al. 1996)	Oil formulation, 4-h exposure, percent germination = 76.0 % Oil formulation, 8-h exposure, percent germination = 53.6 % Oil formulation, 16-h exposure, percent germination = 17.3 %
ARSEF 324, *Metarhizium acridum* (Fargues et al. 1996)	Dry conidia, 1-h exposure, percent surviving (CFU) = 80 % Dry conidia, 2-h exposure, percent surviving (CFU) = 81 % Dry conidia, 4-h exposure, percent surviving (CFU) = 35.3 % Dry conidia, 8-h exposure, percent surviving (CFU) = 11.1 %
ARSEF 324, *Metarhizium acridum* (Braga et al. 2001d)	Water suspension, 1-h exposure (920 mW m^{-2}), culturability (CFU) ~100 % Water suspension, 1-h exposure (1200 mW m^{-2}), culturability (CFU) ~100 % Water suspension, 1-h exposure (1200 mW m^{-2}), relative percent germination, 24-h incubation = 97 %, 48 h incubation = 100 % Water suspension, 4-h exposure (920 mW m^{-2}), culturability (CFU) ~80 % Water suspension, 4-h exposure (1200 mW m^{-2}), culturability (CFU) ~50 % Water suspension, 4-h exposure (1200 mW m^{-2}), relative percent germination, 24-h incubation = 5 %, 48-h incubation = 96 %
ARSEF 324, *Metarhizium acridum* (Braga et al. 2001c)	Water suspension, 2-h exposure (full-spectrum sunlight), culturability (CFU) ~100 % Water suspension, 2-h exposure (UV-A sunlight), culturability (CFU) ~100 % Water suspension, 2-h exposure (full-spectrum sunlight), relative percent germination, 12-h incubation ~45 %, 24 h incubation ~100 % Water suspension, 2-h exposure (UV-A sunlight), relative percent germination, 12 h incubation ~80 %, 24-h incubation ~100 % Water suspension, 4-h exposure (full-spectrum sunlight), culturability (CFU) ~70 % Water suspension, 4-h exposure (UV-A sunlight), culturability (CFU) ~90 % Water suspension, 4-h exposure (full-spectrum sunlight), relative percent germination, 12-h incubation ~10 %, 24 h incubation ~100 % Water suspension, 4-h exposure (UV-A sunlight), relative percent germination, 12-h incubation ~40 %, 24-h incubation ~100 %
ARSEF 324, *Metarhizium robertsii* (Rangel et al. 2005a).	Water suspension, 3-h exposure (8.3 kJ m^{-2}), PDAY, culturability (CFU) ~90 % Water suspension, 3-h exposure (8.3 kJ m^{-2}), insect, culturability (CFU) ~85 % Water suspension, 4-h exposure (11.1 kJ m^{-2}), PDAY, culturability (CFU) ~85 % Water suspension, 4-h exposure (11.1 kJ m^{-2}), insect, culturability (CFU) ~80 %
ARSEF 2575, *Metarhizium robertsii* (Braga et al. 2001d).	Water suspension, 1-h exposure (920 mW m^{-2}), culturability (CFU) ~100 % Water suspension, 1-h exposure (1200 mW m^{-2}), culturability (CFU) ~85 % Water suspension, 1-h exposure (1200 mW m^{-2}), relative percent germination, 24-h incubation = 95 %, 48-h incubation = 100 % Water suspension, 4-h exposure (920 mW m^{-2}), culturability (CFU) ~30 % Water suspension, 4-h exposure (1200 mW m^{-2}), culturability (CFU) <5 % Water suspension, 4-h exposure (1200 mW m^{-2}), relative percent germination, 24-h incubation = 28 %, 48-h incubation = 63 %
ARSEF 2575, *Metarhizium robertsii* (Braga et al. 2001c)	Water suspension, 2-h exposure (full-spectrum sunlight), culturability (CFU) ~75 % Water suspension, 2-h exposure (UV-A sunlight), culturability (CFU) ~100 % Water suspension, 2-h exposure (full-spectrum sunlight), relative percent germination, 12-h incubation ~70 %, 24-h incubation ~100 % Water suspension, 2-h exposure (UV-A sunlight), relative percent germination, 12-h incubation ~95 %, 24-h incubation ~100 % Water suspension, 4-h exposure (full-spectrum sunlight), culturability (CFU) ~0 % Water suspension, 4-h exposure (UV-A sunlight), culturability (CFU) ~30 % Water suspension, 4-h exposure (full-spectrum sunlight), relative percent germination, 12-h incubation ~10 %, 24 h incubation ~10 % Water suspension, 4-h exposure (UV-A sunlight), relative percent germination, 12-h incubation ~25 %, 24-h incubation ~60 %
ARSEF 2575, *Metarhizium robertsii* (Rangel et al. 2004)	Water suspension, 3-h exposure (768 mW m^{-2}), PDAY, culturability (CFU) ~40 % Water suspension, 3-h exposure (768 mW m^{-2}), *G. mellonella* larva, culturability (CFU) ~20 % Water suspension, 3-h exposure (768 mW m^{-2}), *Z. morio* larva, culturability (CFU) <5 % Water suspension, 2-h exposure (897 mW m^{-2}), Czapek, culturability (CFU) ~70 % Water suspension, 2-h exposure (897 mW m^{-2}), Emerson, culturability (CFU) ~70 % Water suspension, 2-h exposure (897 mW m^{-2}), Rice, culturability (CFU) ~ 65 % Water suspension, 2-h exposure (897 mW m^{-2}), PDAY, culturability (CFU) ~90 % Water suspension, 2-h exposure (897 mW m^{-2}), PDA, culturability (CFU) ~50 %

Table 1 continued

Strain	Tolerance of fungi to UV*
GHA, *Beauveria bassiana* (Commercialized by Mycotech Corp., USA) (Inglis et al. 1995)	Water formulation, 15-min exposure, on glass, percentage reduction = 96.0 % Water formulation, 60-min exposure, on glass, percentage reduction = 99.4 % Water formulation, 60-min exposure, on leaves, percentage reduction = 99.7 % Oil formulation, 15-min exposure, on glass, percentage reduction = 22.4 % Oil formulation, 60-min exposure, on glass, percentage reduction = 74.4 % Oil formulation, 60-min exposure, on leaves, percentage reduction = 97.4 %
GHA, *Beauveria bassiana* (Fargues et al. 1996)	Dry conidia, 1-h exposure, percent surviving (CFU) = 37 % Dry conidia, 2-h exposure, percent surviving (CFU) = 0.3 %
GHA, *Beauveria bassiana* (Commercialized by Mycotrol, Emerald BioAgriculture Corp., USA) (Leland and Behle 2005).	Water formulation, Regression Time (RT_{50}) = 2.8 h; RT_{75} = 3.7 h Lignin-coated conidia in water, RT_{50} = 3.0 h; RT_{75} = 4.3 h Cross-linked lignin-coated conidia in water, RT_{50} = 28.3 h; RT_{75} = 58.1 h Corn oil formulation, Regression Time (RT_{50}) = 4.1 h; RT_{75} = 5.9 h Lignin-coated conidia in corn oil, RT_{50} = 5.7 h; RT_{75} = 11.1 h Cross-linked lignin-coated conidia in corn oil, RT_{50} = 8.0 h; RT_{75} = 19.9 h
GHA, *Beauveria bassiana* (Fernandes et al. 2007)	Water suspension, 2-h exposure (7.04 kJ m^{-2}), relative germination ~60 %
IMI 330189, *Metarhizium acridum* (LUBILOSA product's active ingredient; holotype for *M. acridum*) (Moore et al. 1993)	Water formulation, cultures 7-10 d old, 1-h exposure, germination rate = 3.7 % Water formulation, 14-20 d or 30-42 d old, 1-h exposure, germination rate = 5.2 % Oil formulation, cultures 7-10 d old, 1-h exposure, germination rate = 27.1 % Oil formulation, cultures 14-20 d old, 1-h exposure, germination rate = 28.9 % Oil formulation, cultures 30-42 d old, 1-h exposure, germination rate = 53.4 %
IMI 330189, *Metarhizium acridum* (Morley Davies et al. 1996)	Oil formulation, 4-h exposure, percent germination = 67.4 % Oil formulation, 8-h exposure, percent germination = 39.4 % Oil formulation, 16-h exposure, percent germination = 45.4 % Oil formulation, 24-h exposure, percent germination = 28.6 %
IMI 3300189, *Metarhizium acridum* (Fargues et al. 1996)	Dry conidia, 1-h exposure, percent surviving (CFU) = 46 % Dry conidia, 2-h exposure, percent surviving (CFU) = 23 % Dry conidia, 4-h exposure, percent surviving (CFU) = 7.5 % Dry conidia, 8-h exposure, percent surviving (CFU) = 5.2 %

The laboratory methods used for testing the fungal tolerance to UV are briefly mentioned in Table 2; please note that for orientation, the citation of each study is listed in the first column of both Tables 1 and 2. Studies are listed according to the isolate, which are sorted alphabetically, and then by the year of publication

* *GR* germination rates, *CFU* colony-forming units

were more sensitive to UV radiation than conidia from isolates cultured for 7 d (Le Grand and Cliquet 2013). Conidia from older cultures of *M. acridum* were also more tolerant to UV than conidia from the younger ones (Moore et al. 1993) (see Table 1). Therefore, screening for UV tolerance among isolates utilized cultures with approximately the same age to avoid biased results. Despite its importance, mention of the age of cultures is lacking in several studies.

Studies have considered exposure of fresh or dried fungal conidia, formulated or not, to UV radiation (see Table 2). In fact, variable responses in tolerance to UV (mostly UV-B, at 2.3 W m^{-2}) were reported for a single isolate of *B. bassiana* s.l., where an increased viability of air-dried conidia was demonstrated in relation to freshly harvested conidia (Le Grand and Cliquet 2013). Conidia usually have been irradiated on glass, cellulose filter membranes, plant leaves, culture media, etc. The culture media used have often varied in nutrient content and supplementations with different antibiotics or fungicides. The most used fungicides, for example, include 1) chloramphenicol, to avoid bacterial contamination (Leland and Behle 2005); 2) benomyl, due to its minimal effect on conidial germination even though benomyl severely inhibits the growth of germ tubes without adversely affecting germination, thereby preventing overgrowth of mycelium and allowing germination to be monitored for up to 72 h (Milner et al. 1991); and 3) dodine (n-dodecylguanidine acetate), as an ingredient of a semi-selective medium (Chase et al. 1986; Rangel et al. 2010) as reported by Inglis et al. (1995) to isolate entomopathogenic fungi from material possibly contaminated with undesired fungi. No variation in tolerance to UV, however, has been reported in consideration to these chemicals.

Many studies kept the irradiated fungi in dark conditions to avoid the influence of long wavelengths on photoreactivation, which may stimulate the recovery of damaged conidia while other studies exposed the fungi to a broad spectrum of irradiance that includes short and long

Table 2 Different laboratory methods used for evaluating the susceptibility/tolerance of entomopathogenic fungi to UV radiation are listed below considering the irradiance, the method used for exposing fungi to radiation, and the method used for evaluation of survivals. Studies are listed according to the year of publication

Irradiance	Exposure of fungi*	Evaluation of survivals**
Simulated sunlight generated from a bank of blacklight fluorescent lamps (Ignoffo and Garcia 1992)	Conidia produced on SMA medium at 25 °C. Light period was not mentioned. Age of cultures: 10-20 d. Water conidial suspension was applied to the bottom of Petri plates and air dried at room temperature before UV exposure	Counts of CFU 2-3 d after conidia being re-suspended in water and plated on SMAY medium. Plates were held in the dark
Radiation from a 1000 W (J s^{-1}) solar simulator with UV-B (1.032 mW cm^{-2}) and UV-A (8.40 mW cm^{-2}) combined (280-400 nm). Most infrared and visible radiation were removed to prevent over-heating (Moore et al. 1993)	Conidia produced on MA medium at 25 °C. Light period was not mentioned. Age of cultures: 7-10 d, 14-20 d, or 30-42 d. Vegetable oil or water conidial suspensions were applied and spread onto the bottom of Petri dishes and exposed to radiation	Conidia were washed out in water + Tween 80 from the oil. Same procedure for water suspensions. Conidia were filtered and transferred to culinary gelatin on Petri plates and incubated at 25 °C. Conidial germination was assessed 24 or 48 h
Radiation from a Sunlight simulator with 1000 J s^{-1} (UV-B, UV-A, and longer wavelengths) with temperature not exceeding 30 °C (Hunt et al. 1994)	Conidia were produced on Molisch's agar (Speare, 1920) at 25 °C in Petri plates. Light period and age of cultures were not mentioned. Conidia were suspended in deodorized kerosene and sonicated. Sunscreen formulations were prepared by dissolving the sunscreen in kerosene to obtain a 2 % concentration, and then adding equal proportion of conidial suspension to form a 1 % solution. Formulations were applied and spread onto the bottom of Petri dishes and exposed to radiation	A few drops of conidial suspensions were transferred directly onto gelatin in Petri plates, and the plates held at 25 °C for 24 and 48 h. Germination was assessed to determine the percentage of germinating conidia
Radiation from fluorescent bulbs ranging from 260-400 nm with a peak near 300–320 nm. Intensity ranged from 601 to 675 µW cm^{-2}) (Inglis et al. 1995)	Dry conidia supplied by Mycotech were suspended in water or paraffinic emulsified oil, and pipetted on coverslips or onto the surface of leaf pieces of wheatgrass and irradiated. Medium, light period, and age of cultures were not mentioned	Conidia were suspended in 0.01M phosphate buffer + Tween 80, and washed free from the oil. Aliquots were spread on a semi-selective oatmeal–dodine agar medium (Chase et al. 1986). Plates were incubated at 25 °C for 6-7 d, and the number of CFUs was enumerated. Percentage reduction of conidial viability was calculated
Sunlight simulator with 1000 J s^{-1} (UV-B, UV-A, and longer wavelengths) at constant 40 °C (Morley Davies et al. 1996)	Conidia were produced on Thai Jasmine rice in plastic bags at 25 °C for 7 d. Light period was not mentioned. Conidial powder was obtained by sieving and dried with silica gel before being suspended in paraffinic oil. Samples were pipetted in a thin layer into Petri plates, and the plates floated on water bath at 40 °C below the radiation source	A small drop of oil formulation was spread directly onto fresh SDA plates, and the plates held at 25 °C for 22 h. Germination was assessed to determine the percentage of germinating conidia
Controlled-environment chamber with artificial sunlight device with radiation spectrum ranging from 270 to 1100 nm, and UV-B irradiance of 0.3 W m^{-2} at 25 °C and 40 % mean RH (Fargues et al. 1996)	Conidia were produced on a glucose-yeast extract semi-synthetic medium incubated at 25 °C for 3 weeks in the dark. Conidia were suspended in distilled water and deposited on the surface of filter membranes by filtration. Conidia were aseptically air dried for 20 min prior to irradiation	Conidia were re-suspended in water, plated onto agar medium, and incubated for 4 to 8 d in the dark at 15 °C. Colonies were enumerated and conidial survival was estimated as percent of CFUs
Temperature-controlled chambers adjusted to 27 °C provided 920 mW m^{-2} Quaite-weighted irradiance in the low-irradiance chamber, and 1200 mW m^{-2} Quaite-weighted irradiance in the high-irradiance chamber. Spectral irradiance of both chambers ranged from 290 to 380 nm (Braga et al. 2001d)	Conidia were produced on PDAY medium at 28 °C for 21 d in the dark. Conidia were suspended in distilled water and filtered on polycarbonate membrane. Suspension was stored for 48 h before exposure Effect on culturability: Inoculum was spread onto the surface of PDAY in Petri dishes, covered with cellulose diacetate film to block radiation below 290 nm, and irradiated Effect on germination: Inoculum was PDAY + 0.002 % benomyl, covered with cellulose diacetate, and irradiated	Effect on culturability: Irradiated dishes were held at 28 °C for 5 d in the dark. CFUs were counted and conidial relative percentage culturability calculated Effect on germination: Irradiated dishes were held at 28 °C for 12, 24, or 48 h in the dark. Germination was assessed to determine the relative percentage germination of conidia

Table 2 continued

Irradiance	Exposure of fungi*	Evaluation of survivals**
Solar spectral irradiance weighted from 290 to 365 nm: Full-spectrum midday sunlight, or UVA sunlight. Both at 14 °C to avoid plates being heated (Braga et al. 2001c)	Conidia were produced on PDAY medium at 28 °C for 21 d in the dark. Conidia were suspended in distilled water and filtered on polycarbonate membrane. Suspension was stored for 48 h before exposure Effect on culturability: Inoculum was spread onto the surface of PDAY in Petri dishes, and exposed to full-spectrum sunlight (plates covered with cellulose triacetate film and plastic filters) or to UV-A sunlight (plates covered with polyester film to block UV-B). Plates floated on water at 14 °C Effect on germination: Inoculum was transferred to PDAY + 0.002 % benomyl, and irradiated as described	Effect on culturability: Irradiated dishes were held at 23 °C for 4 d in the dark. CFUs were counted and conidial relative percentage culturability calculated Effect on germination: Irradiated dishes were held at 28 °C for 12, 24, or 36 h in the dark. Germination was assessed to determine the relative percentage germination of conidia
UV radiation at 2200 mW m^{-2} (Bidochka et al. 2001)	Conidia were produced on PDA slants at 25 °C. Light period and age of cultures were not mentioned. Water conidial suspension was inoculated in wells of a 96-well flat-bottomed cell culture plate filled with PDA, and irradiated	Cell culture plates were incubated at 25 °C for 6 d. Fungal growth was assessed spectrophotometrically, and an A_{630} of >0.25 was used as indicative of UV resilience
Temperature-controlled growth chamber adjusted to 27 °C provided 800 mW m^{-2} Quaite-weighted irradiance. Spectral irradiance ranged from 290 to 380 nm (Braga et al. 2002)	Conidia were produced on PDAY medium at 28 °C for 12 d in the dark. Conidia were suspended in water 0.01 % Tween, and filtered on polycarbonate membrane Effect on culturability: Inoculum was spread onto the surface of PDAY in Petri dishes, and immediately irradiated Effect on germination: Inoculum was transferred to PDAY + 0.003 % benomyl, and irradiated	Effect on culturability: Irradiated dishes were held at 24 °C for 4 d in the dark. CFUs were counted and conidial relative percentage culturability calculated Effect on germination: Irradiated dishes were held at 28 °C for 12, 24, or 36 h in the dark. Conidia were stained with a drop of Methyl Blue solution. Germination was assessed to determine the relative percentage germination of conidia Effect of visible light on culturability: Irradiated dishes were exposed to visible light (12 W m^{-2} or 150 W m^{-2}) for 2 consecutive photoperiods (18:6, light:dark) at 28 °C. Plates were covered with Llumar film to block wavelengths shorter than 400 nm
Temperature-controlled growth chamber adjusted to 27 °C provided 768 or 897 mW m^{-2} Quaite-weighted irradiance, with UV-B (peak at 313 nm) and minimal UV-A radiation (Rangel et al. 2004)	Conidia were produced on different media: Czapek Solution Agar, Emerson YpSs Agar, PDA, PDAY, enriched rice, and on insect cadavers. Cultures were incubated at 28 °C in the dark for 14 d. Larvae of *Zophobas morio* and *Galleria mellonella* were infected and incubated at 28 °C in the dark until conidiation was completed (8–14 d). Conidia were suspended in water 0.1 % Tween 80, filtered in a polycarbonate membrane, spread onto PDAY plates, and irradiated	Irradiated plates were held at 28 °C for 4 d in the dark, and CFUs were counted and conidial relative percentage culturability was calculated
Solar simulator provided radiation at 19.1 ± 0.5 mW cm^{-2} at room temperature and majority of infrared spectra was removed (Leland and Behle, 2005)	Dry conidia supplied by Emerald BioAgriculture Corp. were formulated as follows: (1) conidia suspended in water 0.04 % Silwet L77, (2) conidia suspended in corn oil, (3) lignin-coated conidia in water, (4) lignin-coated conidia in oil, (5) cross-linked lignin-coated conidia in water, (6) cross-linked lignin-coated conidia in oil. Conidia suspensions were deposited on nylon membranes by filtration and irradiated	Conidia were re-hydrated for 1 h at 100 % RH, suspended in water 0.04 % Tween 80, and soaked for 48 h at 4 °C. Germination was assessed 48 h after inoculation on MEA + 0.02 g l^{-1} benomyl and 0.2 g l^{-1} chloramphenicol. Regression time (RT) was calculated for each formulation. RT values represented estimated exposure time (h) to reduce conidial germination to 50 or 75 % (RT_{50} and RT_{75}, respectively)
Temperature-controlled growth chamber adjusted to 27 °C provided 768 mW m^{-2} Quaite-weighted irradiance, with UV-B (peak at 313 nm) and minimal UV-A radiation (Rangel et al. 2005a)	Conidia were produced on PDAY or insect cadavers. Cultures were incubated at 28 °C in the dark for 14 d. Adults of *Melanoplus sanguinipes* (Orthoptera: Acrididae) were infected and incubated at 28 °C in the dark until conidiation was completed (8–14 d). Conidia were suspended in water 0.1 % Tween 80, filtered in a polycarbonate membrane, spread onto PDAY plates, and irradiated	Irradiated plates were held at 28 °C for 4 d in the dark, and CFUs were counted, and conidial relative percentage culturability was calculated

Table 2 continued

Irradiance	Exposure of fungi*	Evaluation of survivals**
Temperature-controlled growth chamber adjusted to 25 °C provided 978 mW m^{-2} Quaite-weighted irradiance, with UV-B (peak at 313 nm) and minimal UV-A radiation (Fernandes et al. 2007)	Conidia were produced on PDAY at 25 °C in the dark for 15 d. Conidia were suspended in water 0.1 % Tween 80, filtered in a polycarbonate membrane, spread onto plates with PDAY + 0.002 % benomyl, and irradiated	Irradiated dishes were held at 25 °C for 48 h in the dark. Conidia were stained with a drop of Methyl Blue solution and covered with a coverslip, and germination was assessed to determine the relative percentage germination of conidia
UV radiation chamber provided weighted UV-B wavelength of 312 nm (280–320 nm) at gradient doses of 0.1-1.6 J cm-2. Wavelengths were controlled by a microprocessor (Huang and Feng 2009)	Conidia were produced on slants of ¼ SDAY, at 25 °C for 7 d and the regime of Light:Dark 12:12. Conidia were suspended in water (Tween 80 0.02 %), dripped on sterilized glass slides, smeared over an area of ca. 10-mm diameter, and irradiated	Irradiated slides were incubated at 25 °C and L:D 12:12 under saturated humidity. After 24 h, the inocula were stained with cotton blue and examined for counts of germinated and non-germinated conidia. Survival index was calculated
Laminar flow chamber provided 4.8 W cm^{-2} s^{-1} of total irradiance with UV-B wavelengths (peak at 312 nm) (Posadas et al. 2012)	Conidia were produced on CMA medium. Temperature, light period, and age of cultures were not mentioned. Conidia were suspended water (Tween 80, 0.05 %), or in water plus 0.5 % sunscreen, or in 5 % oil-in-water emulsions prepared with soybean oil, sunflower oil, corn oil, or mineral oil. Aliquots were spread onto CMA plus 0.002 % benomyl in Petri plates and irradiated	Irradiated dishes were held at 25 °C for 24 h or 48 h in the dark. Conidia were stained with a drop of lactophenol cotton blue, covered with coverslip, and germination was assessed to determine the relative percentage germination
Chamber provided 2.3 W m^{-2} of total irradiance with UV-B (80 %) and UV-A wavelengths (Le Grand and Cliquet 2013)	Conidia were produced on PDA at 25 °C for 7 or 14 d. Light regime was not mentioned. Conidia were washed with sterile distilled water and filtered through cheese cloth to remove mycelium Fresh conidia: Droplets of 200 µl of each conidial suspension were placed in plastic Petri plates and irradiated. The plastic lid of each Petri plate was not removed and used as a filter to cut off wavelengths <280 nm Dried conidia: Suspensions were filtered on cellulose filters and placed at room temperature at 75 % RH for 12 h prior to irradiation	Fresh conidia: Aliquots from irradiated droplets were transferred to 2-cm square pieces of cellophane on the surface of agar water plates. Plates were incubated at 25 °C for 10 and 12 h. Cellophane pieces were stained with lactophenol cotton blue and germination was assessed to determine the relative percentage germination Dried conidia: Irradiated filters were gently washed with sterile water and germination assessed as described above

Fresh conidia were used to prepare the suspensions, unless specified differently

* *SMA* sabouraud maltose agar, *MA* maltose agar, *SDA* sabouraud dextrose agar, *PDAY* potato dextrose agar supplemented with 1g l^{-1} yeast extract, *PDA* potato dextrose agar, *MEA* malt extract agar, *CMA* complete medium agar;

** *CFU* colony-forming units, *SMAY* sabouraud maltose agar + yeast; percentage reduction was calculated as $((CFU_{Treated} - CFU_{Treated})/CFU_{Control})100$; Conidial relative percentage culturability was calculated as $(CFU_{Treated}/CFU_{Control}) \times 100$

wavelengths (as shown in Table 2). Although it may not reflect a realistic condition, incubating the fungi in darkness avoids the addition of an extra variable to the tests. Moreover, if an isolate restricted from photoreactivation presents high tolerance to UV, it may be expected that the isolate will achieve a higher performance in natural conditions if long wavelengths are available. Efficient photoreactivation was reported for conidia of *Hirsutella thompsonii* previously exposed to UV (200–300 nm) (Tuveson and McCoy 1982), and for conidia of *Trichoderma* sp. exposed to UV-B and short wavelength UV-A (800 mW m^{-2} Quaite-weighted irradiance, at 5.5 and 8.8 kJ m^{-2}) (Braga et al. 2002). In contrast, no significant difference was detected in relative culturability between irradiated conidia of *S. lanosoniveum* or *L. aphanocladii* incubated in visible light (12 W m^{-2}) in comparison to those incubated in the dark after UV-B exposure (Braga et al. 2002). The efficiency in photoreactivation may be related to the high-intensity sunlight habitat where the fungus normally grows (Tuveson and McCoy 1982). In addition, the exposure to visible light (400–700 nm, 150 W m^{-2}, for 2 consecutive photoperiods: 18:6, light:dark) was also detrimental to *S. lanosoniveum* conidia previously exposed to UV-B or non-irradiated controls (held in the dark for 48 h after inoculation). The exposure of *M. robertsii* ARSEF 2575 to visible light during growth, however, induced significantly increased tolerance to UV-B radiation (978 mW m^{-2} Quaite-waited irradiance for 2 h, 7.04 kJ m^{-2}) (Rangel and Roberts 2007; Rangel et al. 2011).

A variety of tests are needed to select isolates with both natural ability to remain viable for long periods in insolated fields and with marked capacity for controlling arthropod pests. This review does not suggest that one method is more appropriate than another for selecting isolates with outstanding natural tolerance to UV, but it emphasizes simple laboratory conditions, that may previously have been believed to be insignificant, that may seriously affect the susceptibility of isolates.

Formulation to protect entomopathogenic fungi against UV radiation

Most attempts to increase the persistence of microbial agents on the field have relied heavily on the use of protectants as additives in biological products as reviewed by Ignoffo and Garcia (1992). In laboratory tests with *M. acridum* (=*M. flavoviride*), conidia formulated in groundnut or mineral oil were protected from UV compared to conidia suspended in water due to the radiation absorption of the oil; however, higher levels of UV protection would be advantageous for field applications (Moore et al. 1993). Conidia of *M. anisopliae* s.l. formulated in peanut oil or refined paraffinic oil also were significantly protected against the deleterious effects of UV radiation in a simulated solar radiation chamber (Alves et al. 1998). In addition, survival of *B. bassiana* conidia exposed to UV (260–400 nm, with irradiance of 675 μW cm^{-2}) was enhanced in paraffinic oil applied on wheatgrass leaves in comparison to water formulation (Inglis et al. 1995). The benefits of oil have led to oil dispersions being one of the most common formulation types available on the market for application of entomopathogenic fungi worldwide (Faria and Wraight 2007).

Spray-dried conidia of the commercialized isolate *B. bassiana* GHA (provided as technical grade spore powder in Mycotrol®; Emerald BioAgriculture Corp., Butte, MT, USA) coated with lignin provided protection against simulated solar radiation. The formulation that provided the greatest UV protection was the cross-linked lignin-coated conidia suspended in water, in which lignin-coated conidia were complemented with $CaCl_2$ as a cross-linking agent of lignin to reduce the water solubility. The weakest formulation against UV used non-coated conidia suspended in water. The loss of UV protection of cross-linked lignin-coated conidia or lignin-coated in oil formulations may be related to the formation of crusts of lignin in oil (Leland and Behle 2005).

Variation in conidial protection by sunscreens has also been reported. Many water-soluble adjuvants used in aqueous formulations of *B. bassiana* s.l. enhanced survival of conidia exposed to UV radiation in comparison to water formulation alone (Inglis et al. 1995). In agreement, Tinopal (Calcofluor white, 1 to 10 g L^{-1}) protected *B. bassiana* conidia sprayed onto leaves and exposed to UV radiation (mostly UV-B, 260-400 nm, with a peak at 313 nm); and Tinopal had no inhibitory effect on growth of *B. bassiana* (Reddy et al. 2008). Also, oxybenzone added at 0.5 % (w/v) to water suspension (Tween 80, 0.05 %) of *B. bassiana* s.l. significantly increased the tolerance of conidia to UV in comparison to the controls (without the sunscreen) for four of six isolates investigated; however, higher protection was reached when conidia were suspended in 5 % oil-in-water emulsions prepared with soybean oil, sunflower oil, corn oil, or mineral oil prior to UV exposures (Posadas et al. 2012). Soyscreen in sunflower oil reduced transmittance of light energy, with UV transmission reduced to 99 % at 10 % concentration of sunscreen in sunflower oil; "soyscreen is a modified- soybean oil that offers UV protection as a result of biocatalytic esterification with a vegetable antioxidant (ferulic acid) that absorbs UV radiation" as described by Behle et al. (2009). In agreement, sunflower oil at 10 % concentration of soyscreen provided significant protection of *B. bassiana* conidia from simulated UV radiation; the protection, however, was similar to the protection provided by 0.15 % of oxyl methoxycinnimate (OMC), that is

a known sunscreen commonly found in skin-care products to provide UV protection (Behle et al. 2009). An evaluation of conidial protection by three different chemical groups of sunscreen revealed that the addition of oxybenzone to vegetable oil provided significantly more protection to the conidia of *M. acridum* than ethyl cinnamate or Graessorb S (Moore et al. 1993). In general, the addition of sunscreens expanded the protection of conidia and increased absorption over the 208-320 nm range. In contrast, possible reactions and polarity shifts may occur in combination with vegetable oils and sunscreens, and this may be a risk when using vegetable oils in final fungal formulations. Moreover, "the high UV absorption of the vegetable oils can have an effect on the perceived UV absorption maxima of the sunscreens if these are near the absorption range of the oil" (Moore et al. 1993).

The addition of certain sunscreens gave only limited protection to *M. acridum* conidia formulated in distilled mineral oil. The most beneficial effects of sunscreens were evident only within the first 24 h of incubation of irradiated conidia, whereas incubation for 48 h allowed unprotected conidia (formulated in kerosene alone) to germinate at a similar level to the protected ones in kerosene plus sunscreens; after 48 h of incubation, only conidia formulated in kerosene plus Eusolex 8021 had higher levels of germination than conidia formulated with no sunscreen (Hunt et al. 1994). In agreement, nine adjuvants tested in oil-compatible formulations did not present greater survival of *B. bassiana* conidia than that of conidia exposed to radiation in paraffinic oil alone; in longer periods of exposure, however, conidial survival in oil formulated with Parsol MCX and 2,2-hydroxy-4-octoxybenzophenone was significantly superior to the survival of conidia in only oil (Inglis et al. 1995).

Adjuvants screened in laboratory tests and selected as protectants of conidia had their efficacy investigated in experiments conducted in the field (Inglis et al. 1995). Conidia of *B. bassiana* formulated in water with each of five water-compatible adjuvants indicated that conidial survival under solar radiation declined logarithmically over time, but comparisons of the formulations with the control treatment (water formulation) indicated that the stilbene brightener Tinopal LPW-OB and clay significantly enhanced the survival of conidia, and that neither formulation tested was superior to the other (Inglis et al. 1995). The adjuvants differed in function, Tinopal LPW absorbed the UV, whereas clay blocked the UV radiation (Inglis et al. 1995). Accordingly, laboratory tests also reported that formulation of *L. lecanii* (=*Verticillium lecanii*) with montmorillonite (1 %), SCPX-1374, which is a clay mineral and a wetting agent [EM-APW#2 (1 %)], protected conidia from UV-B, allowing conidial germination to reach 93 % after 6 h exposure, whereas no survival was seen in irradiated suspensions without montmorillonite and wetting agent; similar results were reported after a 30-min UV-C (100–280 nm) exposure (Lee et al. 2006). Sunlight blockers are reported as very effective in protecting organisms from UV-B radiation, and the use of clay seems to be advantageous because it is harmless to the environment (Inglis et al. 1995). The ability to protect entomopathogenic fungi from UV with clay associated with biodegradable biopolymers and anionic dyes with suitable spectra has been investigated in laboratory tests (Cohen and Joseph 2009; Cohen et al. 2003). Clay-chitosan-dye matrices were powerful photostabilizers that protected conidia from UV radiation at 5 % clay matrices based on montmorillonite, attapulgite, and kaolinite (Cohen et al. 2003). The photoprotection of fungal conidia is mostly involved with absorption of UV radiation by the anionic dyes and attenuation of UV radiation by the clay particles (Cohen et al. 2003). *B. bassiana* conidia mixed with clay-chitosan-dye matrices (kaolinite or bentonite) presented germination rates close to 80 % when irradiated under artificial conditions that simulated sunlight (at 108 J m^{-2}) (Cohen and Joseph 2009).

Estimating field efficacy of formulated fungi is still a challenging task. Poor efficacy of formulated fungi against arthropod pests has been attributed to the influence of environmental conditions, especially temperature and UV radiation from sunlight (Inglis et al. 1997b, a). Dried conidia of *B. bassiana* GHA (Mycotech, Butte, MT) formulated in 1.5 % (w/v) oil emulsion amended with 4 % clay applied in a warm and sunny field did not reduce grasshopper populations, but some disease was observed in grasshoppers collected within 5 d of conidial application and maintained in cages adjacent to the field plots. Furthermore, in areas of intense sunlight exposure, the phenomenon known as behavioral fever or basking in sunlight to elevate grasshopper body temperature may negatively influence fungal development in the arthropod host in the field. Also, conidial survival on plant leaves can be low and decline logarithmically over time. Poor efficacy of fungi also was attributed to the fungal exposure to sunlight when field-treated grasshoppers were held in cages shaded from sunlight presented more rapid disease development and higher prevalence of final mycosis than grasshoppers held in cages receiving full-spectrum sunlight (Inglis et al. 1997a). Also, *B. bassiana* (GHA) formulated with 10 % soyscreen oil in sunflower oil did not extend persistence of conidia applied to field-grown cabbage and bean plants, in comparison with conidia suspended in water. In this case, the lower rate of oil combined with the ability of leaves to absorb or spread the oil is suspected to have resulted in conidia unprotected from sunlight (Behle et al. 2009).

In addition to the protection of fungi against UV radiation, fungal formulations should also consider many abiotic and biotic factors that may influence their efficacy in the field. In agricultural crops, entomopathogenic fungi applied to foliage

may be affected not only by sunlight exposure but also by rain, temperature variation, humidity, leaf surface chemistry, and phylloplane microbiota (see Jaronski 2010). On parasite-infested livestock, in addition to the environmental factors noted above, the efficacy of topically applied fungal products may challenge the host–skin environment, with special consideration to the skin temperature, pH, secretions, sweat (with many components such as a wide range of ions), and skin microflora (Polar et al. 2008; Fernandes et al. 2012). Therefore, screening for promising fungal strains and formulating them for their tolerance to UV is just one of the basic important factors to be considered before evaluating the efficacy of the formulation in complex field experiments for pest control.

Conclusions

The search for promising biological control agents is a complex mission. The exceptional UV tolerance of a promising candidate may be associated with its significant virulence against the arthropod target, and tolerance to several other adverse conditions imposed by the environment, such as temperature and humidity variations. Fungal formulations, on the other hand, may be connected with practical application methods and other control strategies as part of an integrated pest management system, and the efficacies of these formulations need to be monitored regularly. Much is known, but there is much more to be discovered regarding the circumstances that affect the UV tolerance of entomopathogenic fungi. We expect that since tolerance of fungi to UV may be physiologically manipulated in the laboratory, such laboratory manipulations potentially may be used to enhance the UV tolerance of promising fungal candidates during their mass production for field applications.

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