Research Article

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Imperfect match between radiation exposure times required for conidial viability loss and infective capacity reduction attenuate UV-B impact on *Beauveria bassiana*

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Abstract

BACKGROUND: UV-B radiation represents a significant challenge for the widespread use of entomopathogenic fungi in pest management. This study focused on research of the asynchronous response between virulence and conidial viability against *Ceratitis capitata* adults using specific statistical models. Moreover, it was also investigated whether the observed differences in susceptibility to UV-B radiation in *in vitro* assays among three selected isolates of *Beauveria bassiana* were reflected in the above-mentioned asynchrony.

RESULTS: While the irradiation of the three isolates of *B. bassiana* was associated with a significant loss of conidial viability, their virulence was not significantly affected compared to nonirradiated treatments when exposed to 1200 mW m^{-2} for 6 h before or after the inoculation of *C. capitata*. In fact, the irradiation time needed to reduce the mortality to 50% compared to the controls was 34.69 h for EABb 10/225-Fil, 16.36 h for EABb 09/20-Fil, and 24.59 h for EABb 09/28-Fil. Meanwhile, the irradiation time necessary to reduce conidial viability to 50% was 9.89 h for EABb 10/225-Fil, 8.74 h for EABb 09/20-Fil, and 4.71 h for EABb 09/28-Fil.

CONCLUSION: These results highlight the importance of modeling the response of entomopathogenic fungi virulence and conidial susceptibility when exposed to UV-B radiation for the selection of environmentally competent isolates, regardless of the results obtained in previous *in vitro* assays on conidial germination. This strategic approach is critical in overcoming the challenges posed by UV-B radiation and holds the key to realizing the full potential of entomopathogenic fungi in pest management.

Keywords: *Ceratitis capitata*; entomopathogenic fungi; stress; ultraviolet radiation; Mediterranean fruit fly; environmental competence

1 INTRODUCTION

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera; Tephritidae) is one of the most important tephritid pests in the Mediterranean basin, particularly in Spain, as well as one of the world's most economically important fruit pests due to both crop damage and control costs.[1,2] Its wide distribution all over the world is the result of its adaptability to international exchanges of a large variety of horticultural species over time, its great reproductive potential, and its polyphagia, where *C. capitata* can feed on a large number of fruit tree species such as citrus fruits, stone fruits, and pip fruits, among others.[3] Traditionally, the main method of medfly control consists of the use of chemical insecticides, used as cover sprays or terrestrial treatments.[4] However, medflies have developed an important resistance to multiple chemical compounds, including secondary effects such as toxic residues on food systems, environmental pollution, and negative effects on nontarget organisms, which have led to the employment of integrated pest management (IPM) strategies that include cultural, chemical, and biological tools to manage pests.[5]

Microbial control agents are one of the most used strategies within IPM, particularly the use of entomopathogenic fungi (EPF), which are increasingly being applied as a biocontrol method for a wide range of insect and mite pests.[6,7] EPF are quite widespread and can be found practically all over the world in almost all terrestrial ecosystems and habitats, as naturally infecting arthropod hosts, as saprobes in soils (their main reservoir) and even associated with plants as endophyte, epiphyte, and/or

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as part of the rhizosphere, among other functions.[8–10] In agriculture, Hypocreales EPF such as *Metarhizium* spp. Sorokīn and *Beauveria* spp. Vuill., do not usually cause large-scale epizootics because the focus of their use as biological control agents (BCAs) is as inundative applications.[7] Moreover, Hypocreales EPF are also great candidates for inundative applications because they are more target-specific than chemical insecticides, easily produced, and safe for humans and nontarget arthropods.[11] In fact, nowadays there are several commercial products in the market based on EPF such as *Beauveria bassiana* (Bals.-Criv.) Vuill., which is one of the most commercially important EPF, followed by *Metarhizium anisopliae* (Metschn.) Sorokīn, *Isaria fumosorosea* Wize, and *Beauveria brongniartii* (Sacc.) Petch, and they are used against many major arthropod pests in agricultural, forest, urban, and livestock environments.[12,13] The development of mycoinsecticides is a complex process concerning the selection of the fungal isolates, which must possess environmental competence.[14] Indeed, it has been suggested that during the initial infection process (germination and cuticle penetration), the fungus must be adapted to environmental factors such as temperature, humidity, solar radiation, microbial activity, and host physiology, which influence the persistence and efficacy of infective propagules.[7,15]

C. capitata control with EPF has been investigated by targeting two nonprotected stages: pupae in soils and adults in epigeal areas.[6,16,17] However, regarding the latter, numerous environmental stresses such as ozone depletion, increased UV radiation, biodiversity destruction, and temperature may impact EPF normal development in the field, whether the EPF have been applied as part of IPM plans or they are naturally present in these habitats.[18,19]

One of the most harmful environmental factors affecting the depletion of EPF propagules is the sunlight fraction UV-B radiation (λ of 280–315 nm), which could directly affect the presence and persistence of EPF conidia in epigeal habitats.[7,20] It is well-documented that UV-B radiation acts directly on DNA, RNA, and intracellular macromolecules (proteins, ribosomes, and biomembranes), and the development of compounds such as the reactive oxygen species (ROS) resulting in genetic modifications, cell toxicity, and signal pathway modification.[7,21–24]

EPF have developed several adaptation mechanisms against UV-B radiation to protect their cellular structures from damage caused, such as the production of protective pigments (i.e., melanin), antioxidant enzymes (i.e., catalases, superoxide dismutases), and resistant structures (i.e., microsclerotia).[25,26] To improve fungal persistence in the field, night-time application and/or the addition of photoprotectors to formulated EPF products have been applied, with partial success. However, whether the addition of photoprotectors to biocontrol formulations has been proven to be economically viable, the real sense of this protection in the field is still not well understood.[7,27] The high variability in UV and particularly the UV-B radiation tolerance of different species, strains, and isolates of multiple entomopathogenic fungal species has been repeatedly reported,[19,21,28–30] making the selection of strains with a higher UV-B resistance an option from which much is to be gained.[14]

Since it usually takes around 24 h from the arrival of the fungal spore on the insect cuticle to invade the host body,[7] it is likely that the insect body does not protect the fungal conidia from irradiation if radiation takes place immediately after inoculation. However, since germination is negatively affected by UV radiation,[7] inoculation efficiency might be influenced by the irradiation, making it possible that inoculation with conidia right before radiation treatment will lead to less successful inoculation than the treatment in which the inoculation takes place after irradiation (and in which the conidia might have begun recovering). It is expected that a longer UV treatment will result in lower virulence of the fungus. However, recent *in vivo* studies have shown the virulence of EPF such as the strain of *M. brunneum* (EAMa 01/58-Su), which is particularly susceptible to UV-B radiation in *in vitro* assays (germination and colony growth), is not significantly affected by the radiation even after long periods of exposure in most of the treatments performed.[30] In fact, the exposure time at 1200 mW m^{-2} to achieve a 50% reduction in *C. capitata* mortality was 47.2 h, which was longer than the 5.6 h required to reduce conidial viability at 50%.[30] However, these experiments were performed using only one isolate of *M. brunneum*, and if this behaviour can be extrapolated to other EPF isolates within or among species is still unknown. For that, the objective of this study was to evaluate the effect of UV-B radiation on the virulence of three isolates of *B. bassiana* obtained from phylloplane of plants (two of them belonging to the same genotype) against adults of *C. capitata* adults with different degrees of UV-B resistance determined in previous *in vitro* assays.[19]

2 MATERIALS AND METHODS

2.1 Fungal isolates and inoculum preparation

Isolates were obtained from phylloplane of holm oaks (*Quercus ilex* L.) in Castilblanco de los Arroyos, Sevilla (Spain) within the frame of a study about the occurrence and diversity of EPF in five Mediterranean ecosystems[9] (Table 1). The three *B. bassiana* isolates were selected based on their environmental response to UV-B radiation in a former study in *in vitro* assays (Table 1).[19] The isolates selected were EABb 09/28-Fil, which showed the lowest *in vitro* tolerance to UV-B radiation in terms of germination, followed by EABb 10/225-Fil and EABb 09/20-Fil, with a medium and high *in vitro* tolerance to UV-B radiation, respectively (Table 1).[19] Isolates EABb 10/225-Fil and EABb 10/20-Fil belonged to the same

Table 1. *Beauveria bassiana* isolates evaluated, their ecosystem, and habitat of isolation

Isolate[a,b]	Ecosystem	Habitat	Irradiated germination %[c]	Nonirradiated germination %[d]
EABb 10/225-Fil*	Holm oak dehesa	*Quercus ilex* (phylloplane)	49.6 ± 7.2	97.67
EABb 09/20-Fil*	Holm oak reforestation	*Quercus ilex* (phylloplane)	66.1 ± 3.6	98.33
EABb 09/28-Fil	Holm oak dehesa	*Quercus ilex* (phylloplane)	18.8 ± 4.6	94.67

Note: Response to UV-B radiation after 6 h at 1200 mW m^{-2} (relative germination) and fresh nonirradiated conidia germination are shown.
[a] Suffix '-Fil' indicates that isolates were obtained from phylloplane.
[b] Isolates with '*' belonged to the same genotype based on Fernandez-Bravo *et al.*[19]
[c] Relative germination was calculated as indicated in Fernandez-Bravo *et al.*[19]
[d] Based on fresh conidial germination in *in vitro* tests Fernandez-Bravo *et al.*[19]

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genotype described by Fernández-Bravo *et al.*[19] The three isolates were deposited at the culture collection of the Agricultural Entomology Research Group (PAIDI AGR 163 group) from the Department of Agronomy of the University of Córdoba (Córdoba, Spain).

To produce experimental inoculum for bioassays, the three *B. bassiana* isolates were grown on 150-mm Petri plates with malt-agar medium (MA) in darkness at 25 °C for 15 days. With the aim to make the inoculum more user-friendly and lasting, scraped conidia were frozen at −80 °C for 1 h and then lyophilized for 24 h (−50 °C, 0.02 Mb) using a lyophilizer Telstar LyoQuest (Spain). Lyophilized materials were then sieved, which resulted in a dry conidia product that was kept at 4°C until used within the next 24 h.

To produce experimental inoculum for UV-B radiation exposure time effect on the virulence bioassays, fresh conidia, scraped from 15-day colonies grown in 150-mm Petri plates, were transferred into a sterile aqueous solution of 0.1% Tween 80. Conidial suspensions of the three *B. bassiana* isolates were filtered through several layers of cheesecloth to remove the mycelium. Finally, a suspension of 1.0×10^8 conidia mL^{-1} was prepared and used within the next 6 h.

2.2 Insects

Adults of the tephritid *C. capitata* originated from the stock culture El Encin (I.N.I.A., Madrid, Spain) and maintained since 2004 in the Agricultural Entomology Research Group (PAIDI AGR 163 group) at the Department of Agronomy of the Excellence Unit Maria de Maeztu (DAUCO) of the University of Córdoba (Córdoba, Spain), were the selected insects for all bioassays. New adults from the field have been periodically introduced every year in the laboratory colony to prevent consanguinity. The colony was maintained in an environmental growth chamber at 25 ± 2 °C, 50%–60% relative humidity (RH), and a photoperiod of 16 h:8 h (light:dark). Adult flies were fed with water and a standard artificial diet consisting of hydrolyzed protein (ICN Biomedicals, Aurora, OH, USA) and sucrose (Panreac, Barcelona, Spain) in a proportion of 4:1. Larvae were reared until pupation on an artificial diet consisting of 300 g of wheat bran, 75 g of sucrose, 38 g of brewer yeast, 2 g of nipagin, 2 g of nipasol, and 2.4 g of benzoic acid dissolved in 600 mL of distilled water per kilogram of diet. All experiments were performed using 1-day-old adults.

2.3 UV-B radiation effect on virulence of *B. bassiana* isolates against *C. capitata* adults

Irradiation bioassays were conducted as described in Fernandez-Bravo *et al.*[30] in a temperature-controlled chamber (Fitoclima S600PL, ARALAB, Portugal) at 25 ± 1 °C. To describe the process briefly, the irradiated material was covered with a 0.13-mm thick cellulose diacetate film (with UV-B or irradiated treatments), which removed the radiation below 290 nm. Control treatments nonexposed to UV-B radiation were covered with aluminium foil, which physically protected the inoculum from radiation inside the UV-B chamber (no UV-B or nonirradiated treatments). The selected irradiance was 1200 mW m^{-2}, which is the mean irradiance in the south of Spain after 30% ozone depletion.[19,30] All UV-B measurements were made with a PMA2106 UV-B detector, which provides fast and accurate irradiance measurement within the UV-B region.

2.3.1 *UV-B radiation effect on the virulence of* B. bassiana *isolates against* C. capitata *adults when the conidia were exposed prior to the insect inoculation*

The procedure for the bioassays was performed as described in Fernández-Bravo *et al.*[30] Briefly, 1 g of lyophilized and sieved dry conidia was spread to create a thin layer (one conidium tall, approximately) covering the bottom of a 90-mm Petri plate for optimal UV-B irradiation. The number of plates was adapted to the requirements of each bioassay. Plates with the inoculum were covered with the appropriate filters as mentioned in Section 2.3. for 'with UV-B' (cellulose-diacetate) and 'No UV-B' (aluminium foil) treatments. Treatments were then exposed to UV-B radiation at 1200 mW m^{-2} for 6 h.

Immediately after irradiation, 1-day-old *C. capitata* adults (10 individuals per plate) were placed into each Petri plate containing the irradiated (with UV-B treatment) and nonirradiated (no UV-B treatment) inoculum and allowed to walk over the inoculum for 5 min. Then insects were transferred into clean ventilated methacrylate boxes (6 × 8 × 8 cm) as described in Fernández-Bravo *et al.*[30] Non-inoculated and nonirradiated *C. capitata* were established as controls. Water and diet for *C. capitata* adults were provided *ad libitum* during all bioassays. Three replicates were performed for each treatment (with UV-B, no UV-B, and control). Mortality was monitored every day for 8 days. Dead flies were removed immediately. The dead diagnosis was performed by surface sterilizing dead insects with 1% sodium hypochlorite followed by three rinses with sterile distilled water. Then, dead surface-sterilized flies were placed on sterile glass slices on wet filter papers, and in sterile Petri plates, sealed with Parafilm, and kept at 25 °C in darkness to promote the fungal outgrowth on the corpses.

2.3.2 *UV-B radiation effect on the virulence of* B. bassiana *isolates against* C. capitata *adults when the conidia were exposed after insect inoculation*

The procedure for the bioassays was performed as described in Fernández-Bravo *et al.*[30] Briefly, 1 g of lyophilized and sieved dry conidia was spread to create a thin layer (one conidium tall, approximately) covering the bottom of a 90-mm Petri plate for optimal UV-B irradiation. The number of plates was adapted to the requirements of each bioassay. One-day-old *C. capitata* adults (10 individuals per plate) were placed into each Petri plate containing the dry conidia and allowed to walk over the inoculum for 5 min. Then insects were transferred into clean ventilated methacrylate boxes (6 × 8 × 8 cm) as described in Fernández-Bravo *et al.*[30] The boxes containing inoculated flies were covered with the appropriate filters as mentioned above for 'with UV-B' (cellulose-diacetate) and 'no UV-B' (aluminium foil) treatments. Treatments were then exposed to UV-B radiation at 1200 mW m^{-2} for 6 h. As mentioned in Section 2.3.1, noninoculated and nonirradiated *C. capitata* were established as controls. Water and diet for *C. capitata* adults were provided *ad libitum* during all bioassays. Three replicates were performed for each treatment (with UV-B, no UV-B, and control). Mortality was monitored every day for 8 days and dead flies were extracted and processed as described in Section 2.3.1.

2.4 Effect of different UV-B radiation exposure times on the virulence of *B. bassiana* isolates against *C. capitata* adults

First, 1×10^8 conidia mL^{-1} suspensions of each of the three *B. bassiana* isolates were prepared as described in Section 2.2. Then, 1-day-old *C. capitata* adults were separately sprayed with 1 mL of each suspension in a Potter Spray Tower (Burkard Scientific, Ulbridge, UK) at a uniform working pressure of 0.7 kg cm^{-2}. Control treatments were sprayed with the same volume of a sterile aqueous solution of 0.1% Tween 80. Inoculated flies were then immediately transferred into clean ventilated methacrylate boxes (6 × 8 × 8 cm) as described in Fernández-Bravo *et al.*[30] Then boxes with inoculated flies were covered with the appropriate

filters as mentioned above for 'with UV-B' (cellulose-diacetate) and 'no UV-B' (aluminum foil) treatments. Treatments were then exposed to UV-B radiation at 1200 mW m^{-2} for 6, 12, 24, 36, and 48 h. For '0 h treatment', boxes with inoculated insects were covered with aluminum foil and irradiated at 1200 mW m^{-2} for 48 h. Water and diet for *C. capitata* adults were provided *ad libitum* during all bioassays. Three replicates were performed for each treatment dosage (with UV-B, no UV-B, and control). Mortality was monitored every day for 10 days and dead flies were extracted and processed as described above.

2.5 Conidial viability

Additional insects per treatment ('with UV-B' and 'no UV-B' treatments) were included in each bioassay of Sections 2.3 and 2.4, with the aim of determining the viability of the inoculum before or after the irradiation. For that, 10 inoculated insects, irradiated and nonirradiated, were transferred into a 2-mL Eppendorf tube containing 1 mL of sterile distilled water plus 0.1% Tween 80. Tubes containing inoculated insects were vortexed for 2–3 min to dislodge conidia from the fly bodies. Then, 500 µL of this suspension was added to an Erlenmeyer flask with 25 mL of Sabouraud broth plus cycloheximide 250 µg mL^{-1}, chloramphenicol 500 µg mL^{-1}, ampicillin 500 µg mL^{-1}, and streptomycin 500 µg mL^{-1} to prevent bacterial growth. Flasks were incubated for 24 h at 25 °C in darkness. Finally, the percentage of germinated conidia of each treatment was determined. For that, 20 µL from each flask was observed at 400× magnification. Then, germinated conidia, among 100 conidia randomly selected, were counted. The procedure was carried out three times per flask/treatment.

2.6 Data analysis

Mortality in all bioassays was analysed using a one-way ANOVA and the Tukey-HSD test was used to determine the pairwise comparison among the treatments. Mortality percentages were transformed using the arcsin transformation to meet the ANOVA assumptions.[31] The percentage of total mortality caused by the three isolates of *B. bassiana* in experiments described in Section 2.3 was corrected for mortality in the controls using Abbott's formula.[32] The cumulative mortality response across the assessment period was analysed with Kaplan–Meier survival analysis.

The relative germination percentage after each treatment was calculated by the following equation:

$$\text{relative germination}\,(\%) = \frac{\text{Wt}}{\text{Wc}} \times 100$$

where Wt is the number of germlings for the UV-B treatments and Wc is the number of germlings for the no UV-B treatments.

Insect mortality and conidial viability were evaluated to assess the effect of exposure time on the virulence of the three *B. bassiana* isolates against *C. capitata* adults. The effect on insect mortality was modeled by a three-parameter logistic equation with binomial distribution for the response:

$$Y = \frac{a}{1 + \left(\frac{x}{c}\right)^b}$$

where *x* is the exposure time, *a* is the mortality rate, *b* is the reduction of mortality (slope of the curve), and *c* is the time required for the reduction of mortality by 50% for the treatments.[33]

The effect of the exposure time on conidial viability along the time (count data) was evaluated using the exponential function:

$$Y = e^{(a\prime + b\prime X)}$$

where *x* is the exposure time, *a* is the viability rate, *b* is the reduction of viability (slope), and the response is Poisson distributed.

3 RESULTS

3.1 UV-B radiation effect on the virulence of *B. bassiana* isolates against *C. capitata* adults when the conidia were exposed prior to the insect inoculation

Conidia irradiated at 1200 mW m^{-2} for 6 h showed a reduction of the virulence of the three isolates compared to the nonirradiated treatments. All isolates presented a higher mortality when the isolates were not exposed to UV-B radiation ('no UV-B' treatments) compared to the ones that were exposed ('with UV-B' treatment) (Table 2). EABb 09/20-Fil showed the highest mortality, followed by EABb 09/28-Fil and then the isolate EABb 10/225-Fil (Table 2). However, only two of the three isolates showed a significant reduction of mortality among the treatments and the controls: EABb 10/225-Fil ($F_{2,6} = 4.45$, $P = 0.065$), EABb 09/20-Fil ($F_{2,6} = 47.09$, $P = 0.002$), and EABb 09/28-Fil ($F_{2,6} = 30.04$, $P = 0.007$), but not between the treatments for any of the three isolates (Table 2).

In line with the mortality, average survival time (AST) was shorter for the EABb 09/20-Fil isolates, followed by EABb 09/28-Fil and longer for the EABb 10/225-Fil isolate, where no significant differences were found among the treatment.

Conidial viability was reduced when the fungi were irradiated ('with UV-B' treatment) compared to the non-irradiated ones ('no UV-B' treatments) prior to insect inoculation for the three isolates, where the relative germination was 46.29 ± 3.21% for EABb 10/225-Fil, 36.78 ± 0.75% for EABb 09/20-Fil, and 45.54 ± 24.57% EABb 09/28-Fil, respectively.

3.2 UV-B radiation effect on the virulence of *B. bassiana* isolates against *C. capitata* adults when the conidia were exposed after the insect inoculation

C. capitata mortality was significantly different between the treatments ('with UV-B' and 'no UV-B' treatments) and the controls but not among the treatments when the fungi were exposed to UV-B radiation after the insect inoculations ($F_{2,6} = 48.19$, $P = 0.002$, $F_{2,6} = 101.47$, $P < 0.000$, and $F_{2,6} = 32.36$, $P = 0.006$ for EABb 10/225-Fil, EABb 09/20-Fil and EABb 09/28-Fil, respectively) (Table 2). All isolates presented a higher mortality when the isolates were not exposed to UV-B radiation ('no UV-B' treatments) compared to the ones that were exposed ('with UV-B' treatment) (Table 2). Significant differences were found among the AST of treatments and the controls but not between the treatments for the three *B. bassiana* isolates (Table 2).

Conidial viability was reduced when the fungi were irradiated ('with UV-B' treatment) compared to the nonirradiated ones ('no UV-B' treatments) prior to insect inoculation for the three isolates, where the relative germination was 56.84 ± 2.16% for EABb 10/225-Fil, 51.47 ± 4.25% for EABb 09/20-Fil, and 34.37 ± 9.96% EABb 09/28-Fil, respectively.

3.3 UV-B radiation exposure time effect on the virulence of *B. bassiana* isolates against *C. capitata* adults

The effect of UV-B radiation exposure time at 1200 m W m^{-2} was calculated by spraying 1-day-old *C. capitata* adults with 1.0×10^8

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Table 2. Effect of UV-B radiation on pathogenicity of the three *Beauveria bassiana* isolates EABb 10/225-Fil, EABb 09/20-Fil and EABb 09/28-Fil irradiated prior or after *Ceratitis capitata* inoculation

	Prior to inoculation[a]			After inoculation[b]		
	Kaplan–Meier survival analysis			Kaplan–Meier survival analysis		
Treatment[c]	AST[d]	95% FL	Total mortality[e,f]	AST[e]	95% FL	Total mortality[e,f]
EABb 10/225 with UV-B	4.0 ± 0.4a	3.1–4.9	12.1 ± 9.9a	3.2 ± 0.2a	2.8–3.6	76.6 ± 7.2a
EABb 10/225 no UV-B	4.8 ± 0.6a	3.6–6.0	36.4 ± 18.2a	3.3 ± 0.2a	2.9–3.8	88.5 ± 6.7a
Control	6.0 ± 0.5a	4.9–7.0	0.0 ± 0.0a	8.2 ± 0.6b	6.9–9.5	0.0 ± 0.0b
EABb 09/20 with UV-B	2.9 ± 0.2A	2.4–3.4	77.2 ± 4.5A	3.4 ± 0.2A	2.9–3.8	80.8 ± 3.8A
EABb 09/20 no UV-B	2.6 ± 0.2A	2.1–3.1	86.4 ± 7.9A	3.3 ± 0.2A	2.7–3.8	80.8 ± 3.8A
Control	6.0 ± 0.5B	4.9–7.0	0.0 ± 0.0B	8.2 ± 0.6B	6.9–9.5	0.0 ± 0.0B
EABb 09/28 with UV-B	3.9 ± 0.1a′	3.5–4.3	63.6 ± 16.4ab′	3.3 ± 0.2a′	2.8–3.8	69.2 ± 3.8a′
EABb 09/28 no UV-B	3.3 ± 0.2a′	2.8–3.8	77.3 ± 4.5a′	3.1 ± 0.2a′	2.6–3.5	88.5 ± 11.5a′
Control	6.0 ± 0.5b′	4.9–7.0	0.0 ± 0.0b′	8.2 ± 0.6b′	6.9–9.5	0.0 ± 0.0b′

Note: The treatments were conducted at 1200 mW m^{-2} for 6 h.
[a] Conidia irradiated prior to fly inoculation. Petri plates with pure, dry conidia were covered with cellulose-diacetate film ('with UV-B' treatment) or aluminum foil ('no UV-B' treatment) and then irradiated at 1200 mW m^{-2} for 6 h. Immediately after irradiation, the adult flies were placed inside the Petri plates containing the irradiated conidia and allowed to walk around for 5 min.
[b] Conidia irradiated after flies inoculation. *C. capitata* adults were placed in Petri plates containing nonirradiated pure, dry conidia, and flies were allowed to walk on the inoculum for 5 min, then the flies were transferred to clean ventilated methacrylate boxes. The boxes were covered with cellulose-diacetate film ('with UV-B' treatment) or aluminum foil ('no UV-B' treatment) and then irradiated at 1200 mW m^{-2} for 6 h.
[c] Control: noninoculated and nonirradiated insects placed outside the chamber.
[d] AST (mean ± SE) limited to 8 days. Data in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the log-rank test.
[e] Data (mean ± SE) in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to Tukey-HSD test.
[f] Abbott-corrected mortality.

conidia mL^{-1} suspension for the three isolates studied. Immediately after the insect inoculation, these were exposed to the radiation from 0 ('no UV-B' 48-h treatments), 6, 12, 24, 36, and 48 h ('with UV-B' treatments). Mortalities produced by the three *B. bassiana* isolates were highest for treated and nonirradiated insects (0 h treatments), followed by treated and exposed to 6, 12, 24, 36, and 48 h treatments (Table 3). Mortality percentages were inversely correlated with the exposure time for the three *B. bassiana* isolates evaluated, showing a graduate reduction in their mortalities. All isolates induced high ratios of mortalities which ranged between 46.67 and 76.67% for EABb 10/225-Fil, 66.67% and 90.00% for EABb 09/20-Fil, and 72.56% and 86.67% for EABb 09/28-Fil (Table 3). AST was also directly correlated with the time of exposure for the three *B. bassiana* isolates, which ranged between 6.36 and 7.42 h for EABb 10/225-Fil, 4.63 and 6.75 h for EABb 09/20-Fil, and 5.11 and 7.00 h for EABb 09/28-Fil (Table 3). Conidial viability significantly decreased with the exposure time for the three *B. bassiana* isolates ($F_{6,14} = 8.13$, $P = 0.0001$, $F_{6,14} = 25.22$, $P = 0.0001$, and $F_{6,14} = 15.50$; $P = 0.003$, for EABb 09/28-Fil, EABb 09/20-Fil and EABb 10/225-Fil, respectively) (Fig. 1).

The three-parameter logistic model showed that the irradiation time needed to reduce the morality to 50% of *C. capitata* adults treated with 1.0×10^8 conidia mL^{-1} compared to the controls were 34.69 h for EABb 10/225-Fil, 16.36 h for EABb 09/20-Fil, and 24.59 h for EABb 09/28-Fil (Fig. 1). The exponential model showed that the irradiation time necessary to reduce the conidial viability to 50% during the same treatments mentioned above were 9.89 h for EABb 10/225-Fil, 8.74 h for EABb 09/20-Fil, and 4.71 h for EABb 09/28-Fil.

4 DISCUSSION

Entomopathogenic fungi (EPF) have shown great potential to control pests by reducing the use of chemical products in recent decades.[8] However, the sensitivity of these fungi to environmental conditions, particularly UV-B radiation, may hamper their efficiency as BCAs.[34] The effect of UV-B radiation on conidial fitness has been well-documented *in vitro* in recent years, and inter- and intra-specific variations in tolerance have been reported, showing that UV-B radiation does not influence each fungal genus or species, or even isolate within species, equally.[15,19,28,30,35] *Metarhizium* spp. has been discovered to be more susceptible, in terms of germination and colony growth, than *B. bassiana* at the same level of UV-B irradiation and time of exposure in previous studies,[19,30] where the presence of melanin or melanin-like pigments does not necessary increase UV-B resistance, as some authors have hypothesized.[25]

Even when the effect of UV-B radiation on EPF conidia fitness (conidia germination and colony growth) has been well documented *in vitro*, if this effect is significant on EPF virulence has been poorly addressed.[30] In general, the parameter used as an index to reveal the susceptibility of EPF to UV-B radiation for further field applications has been the mentioned variation of conidia UV-B resistance in *in vitro* tests.[24] The reality, however, is that the *in vitro* conidia susceptibility to UV-B radiation does not necessarily translate into a significant loss of virulence for certain EPF such as the strain EAMa 01/58-Su of *M. brunneum*.[30] However, if this tolerance to UV-B radiation is a general behaviour of all EPF is still unknown. For that, in this study, we explored the impact of UV-B radiation on the virulence and conidial viability after insect inoculations of three *B. bassiana* isolates whose conidia presented different degrees of susceptibilities to UV-B radiation in previous *in vitro* assays.

Overall, we demonstrated that the virulence, whether the conidia of three *B. bassiana* isolates were irradiated before or after insect inoculations, did not show significant differences compared to the nonirradiated treatments. However, a significant

Table 3. Effect of UV-B radiation (1200 mW m^{-2}) exposure time on the virulence of the three *Beauveria bassiana* isolates EABb 10/225-Fil, EABb 09/20-Fil and EABb 09/28-Fil against *C. capitata* adults

	Kaplan–Meier survival analysis			
Treatment[a]	AST[b]	Lower	Upper	Total mortality
EABb 10/225-Fil 0 h	6.3 ± 1.1a	4.0	8.6	76.6 ± 3.3
EABb 10/225-Fil 6 h	6.5 ± 1.2a	3.9	9.0	66.6 ± 3.3
EABb 10/225-Fil 12 h	6.7 ± 1.1ab	4.5	8.9	66.6 ± 6.6
EABb 10/225-Fil 24 h	7.1 ± 1.5ab	4.1	10.0	63.3 ± 8.8
EABb 10/225-Fil 36 h	7.2 ± 1.9ab	3.4	10.9	56.6 ± 12.0
EABb 10/225-Fil 48 h	7.4 ± 1.0ab	5.3	9.4	46.6 ± 6.6
Control	7.6 ± 2.1b	2.9	11.2	23.3 ± 3.3
EABb 09/20-Fil 0 h	4.6 ± 0.8A	2.8	6.3	90.0 ± 5.7
EABb 09/20-Fil 6 h	5.2 ± 0.9A	3.2	7.1	83.3 ± 8.8
EABb 09/20-Fil 12 h	5.8 ± 1.3AB	3.3	8.4	80.0 ± 5.8
EABb 09/20-Fil 24 h	6.5 ± 1.2AB	4.2	8.8	76.6 ± 3.3
EABb 09/20-Fil 36 h	6.7 ± 1.5AB	3.6	9.8	73.3 ± 8.8
EABb 09/20-Fil 48 h	6.7 ± 1.0AB	4.6	8.9	66.6 ± 8.8
Control	7.3 ± 2.8B	1.7	12.9	23.3 ± 8.8
EABb 09/28-Fil 0 h	5.1 ± 0.9a′	3.3	6.9	86.6 ± 3.3
EABb 09/28-Fil 6 h	5.6 ± 0.9a′	3.7	7.4	83.3 ± 3.3
EABb 09/28-Fil 12 h	6.0 ± 1.3a′	3.2	8.7	76.6 ± 3.3
EABb 09/28-Fil 24 h	6.6 ± 1.1ab′	4.3	8.9	73.3 ± 6.6
EABb 09/28-Fil 36 h	6.9 ± 1.6ab′	3.8	10.3	75.6 ± 3.3
EABb 09/28-Fil 48 h	7.0 ± 1.2ab′	4.6	9.4	72.5 ± 5.7
Control	7.3 ± 3.3b′	0.7	13.9	23.3 ± 3.3

[a] *C. capitata* adults were sprayed with 1.0×10^8 conidia ml^{-1} suspension and placed in methacrylate boxes, which were covered with cellulose-diacetate film and exposed to 6, 12, 24, 36, and 48 h. 0 h treatments were covered with aluminum foil and exposed for 48 h. Control: noninoculated and nonirradiated insects placed outside the chamber.
[b] AST (mean ± SE) limited to 7 days. Data in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the log-rank test.

reduction of conidia viability was previously detected for all isolates, which could reach more than 80% compared to fresh and nonirradiated conidia viability (Table 1). Fernández-Bravo *et al.*[30] found a significant reduction in the virulence of the *M. brunneum* strain EAMa 01/58-Su, but only when the conidia were exposed to UV-B before insect inoculation, not after insect inoculation.

The irradiation time needed to reduce the mortality to 50% for *C. capitata* adults treated with 1.0×10^8 conidia mL^{-1} was 34.69 h for the isolate EABb 10/225-Fil, 16.36 h for EABb 09/20-Fil, and 24.59 h for EABb 09/28-Fil. In addition, the time needed to reduce the conidial viability to 50% was 9.89 h for the isolate EABb 10/225-Fil, 8.74 h for EABb 09/20-Fil, and 4.71 h for EABb 09/28-Fil. That is, the time of exposure needed to reduce conidial viability to 50% was 3.5, 1.8, and 5.2 times less than the time needed to reduce the virulence to 50% for the EABb 10/225-Fil, EABb 09/20-Fil, and EABb 09/28-Fil isolates, respectively. Fernández-Bravo *et al.*[30] discovered that the irradiation time necessary to reduce the virulence to 50% under the same conditions as described in this paper, that is, the treatment of *C. capitata* adults with a suspension of 1.0×10^8 conidia mL^{-1} of the *M. brunneum* strain EAMa 01/58-Su, and irradiated after the insect inoculation, was 47.2 h. In addition, the time needed to reduce conidial viability was 5.6 h. In that case, the time of exposure needed to reduce conidial viability of the strain EAMa 01/58-Su of *M. brunneum* to 50% was 8.4 times less than the time needed to reduce its virulence to 50%. The comparison of both results confirmed the differences in UV-B tolerance between the two EPF species, where *B. bassiana* showed a lower tolerance than *M. brunneum* in terms of virulence, but not in conidial viability. Moreover, these results also demonstrate that even when the conidia are affected by UV-B radiation, which reduces their germination, growth, and viability, the remaining propagules, still active, have the capacity to induce high mortality rates in *C. capitata* in a similar range as the non-irradiated treatments. However, whereas it is possible to establish a relationship between the UV-B radiation tolerance and the EPF species, the responses of different isolates of *B. bassiana* were not related to the previous UV-B radiation tolerance measured in *in vitro* assays, nor the genotype.

To explain this phenomenon of low tolerance of UV-B radiation in terms of conidial viability, which does not affect the infective capacity, many studies have mentioned the ability of *B. bassiana* to recover after UV-B irradiation exposure.[19,36] After an initial delay in conidia germination (the duration of the delay varies among species or isolates within species), the conidia can recover their activity even after more extended irradiation periods with high irradiation strength (6 h at 1200 mW m^{-2}).[19,28,30,36–38] The low distinction between the infective capacity in adult flies treated with irradiated conidia and those treated with nonirradiated conidia, even after showing an important reduction in conidial viability, could be attributed to a combination of two main reasons. The first is the time of exposure to UV-B radiation at 1200 mW m^{-2} needed to reduce the infective capacity compared to the time of exposure needed to reduce the viability. This viability is much longer than the 6 h shown in *in vitro* assays, which has been documented in previous studies for several species of EPF, ranging between 12 and 24 h.[20,39] In addition, *C. capitata* adults were placed, after the UV-B treatments, in an environmental growth chamber with a photoperiod of 16 h:8 h (light:dark) for 8 days, which could have facilitated the photoreactivation of the conidia with no differences between both scenarios.[40] The second is the diet influence. Paula *et al.*[41] found that the time lag between the first contact of *Aedes aegypti* (Diptera: Culicidae) cuticle with the fungus *M. anisopliae* and the mortality of the insects was closely related to the blood-feeding since it increased the immune activity of the mosquito. *C. capitata* survival is greatly dependent on the full diet,[42] and for that, the lack of nutritional stress in our flies could preserve an effective immune response against the irradiated or nonirradiated inoculum and delay death in both scenarios.

A negative correlation between the irradiation time of conidia of each *B. bassiana* isolate and the fly mortality rate was detected. It could be observed that the mortality rate of EABb 09/20-Fil decreases faster at the first hours of irradiation and drops later in time (Fig. 1). On the other hand, EABb 09/28-Fil and especially EABb 10/225-Fil decrease more gradually at the beginning of the irradiation process and continue to decrease slowly throughout the radiation time (Fig. 1). At the longest irradiation times (36 and 48 h), none of the isolates showed much-added efficacy in comparison with noninoculated controls, hence the activity of the fungus against the flies has been reduced greatly after such long irradiation times and does not or hardly recover anymore. This can also be seen in the viability test, where the percentage of conidia that still germinated after 48 h of irradiation could be detected, but lower than 5%. The substantial reduction of conidia viability in all bioassays, independent of whether the propagules

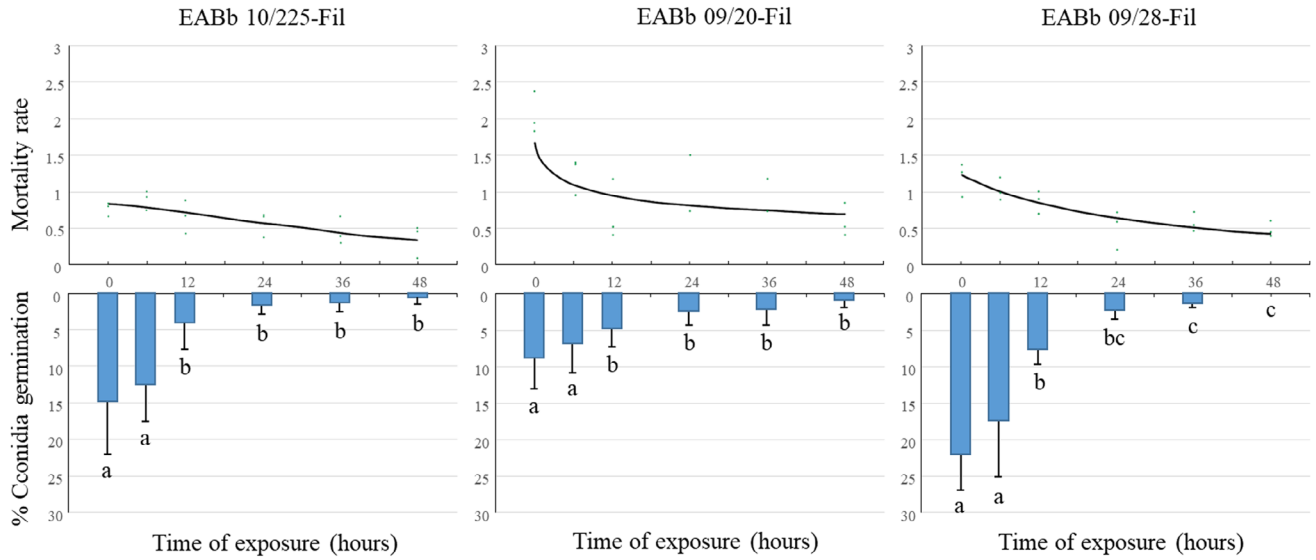

Figure 1. Effect of exposing adults of *Ceratitis capitata* sprayed with 1.0×10^8 conidia mL^{-1} of *Beauveria bassiana* isolates EABb 10/225-Fil, EABb 09/20-Fil, and EABb 09/28-Fil, to UV-B for 0 h ('no UV-B' 48 h), 6, 12, 24, 36 and 48 h ('with UV-B' treatments) on morality and conidia viability. Figures showing mortality rate (top figures): The model represents a logistic three-parameter function according to Zwietering *et al.*[33] for the effect of exposure time on fly mortality (mortality rate). Figures showing % of conidia germination (bottom figures): Percentage of conidia germination after 24 h of incubation in Saboureau broth of the three *Beauveria bassiana* isolates EABb 10/225-Fil, EABb 09/20-Fil, and EABb 09/28-Fil when the inoculum was irradiated on *C. capitata* after the times of exposure mentioned above. According to the Tukey HSD test, the same letter within each isolate means that treatments are not significantly different ($\alpha = 0.05$).

were irradiated or not, compared to fresh nonirradiated conidia suggests that the propagules suffer intense stress during the first hours of the infection process, which inactivates a high percentage of conidia. We hypothesize two scenarios: (i) the infection process must rapidly begin during the first hours after insect inoculation when the conidia have started to penetrate the insect cuticles, and for that only the remaining propagules still not attached to the insect body are counted in viability assays; and (ii) the low proportion of propagules protected from the UV-B radiation within the folds of the body insect are enough to induce the high rates of insect mortalities observed. To support these hypotheses, Bechara *et al.*[43] demonstrated that the penetration structures of *M. anisopliae* (Metschn.) Sorokīn form between 12 and 24 h after the tephritid *Anastrepha fracterculus* Wiedemann inoculation. In addition, the infection process is possible due to the action of several virulence factors not only in the adhesion and penetration stages but also in the invasion stage (Ortiz-Urquiza y Keyhani, 2016). This can lead to up-regulation of the expression of oxidative stress response genes or secretion of toxins and enzymes significantly affected by UV-B stress.[44,45]

5 CONCLUSION

In conclusion, it was observed that not all *B. bassiana* isolates showed the same response to UV-B radiation stress in terms of virulence even when they sowed high tolerance in *in vitro* assays, such as the *B. bassiana* isolate EABb 09/20-Fil. However, all three isolates were still significantly virulent after 6 h of irradiation or even longer periods of exposure to UV-B radiation. Indeed, the observed reduction in virulence of irradiated conidia after 6 h of exposure was not significant in any case, which may indicate their ability to induce high mortality rates even with a lower number of viable propagules. However, this tolerance was lost after long periods of irradiation (more than 16 h), after which the isolates lost at least half of their virulence against *C. capitata*. Nonetheless, the continuous periods of exposure at a constant high UV-B irradiation are not natural in the environment. For that, these types of scenarios with more than 6 h of high UV-B radiation have been addressed for scientific resources of information. In this paper, we demonstrated that the rates in UV-B tolerance of the different *B. bassiana* isolates differed, independently of whether the isolates showed a high UV-B tolerance in *in vitro* assays or if they share the same genotype or not. The time lag between *B. bassiana* conidia germination and *C. capitata* death is in part influenced by UV-B radiation. These results reinforce the importance of the environmental competence behaviour of each isolate for further selections to be used as BCA within IPM. For that, the selection of environmentally competent EPF isolates with a natural tolerance to UV-B radiation, in terms of virulence, must be mandatory to minimize the necessity and cost of formulated BCA products.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest associated with this publication.

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