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Viability and Virulence of Entomopathogenic Nematodes Exposed to Ultraviolet Radiation

David I. Shapiro-Ilan,[1] Selcuk Hazir,[2] and Luis Lete[3]

Abstract: Entomopathogenic nematodes (EPNs) can be highly effective biocontrol agents, but their efficacy can be reduced due to exposure to environmental stress such as from ultraviolet (UV) radiation. Our objectives were to 1) compare UV tolerance among a broad array of EPN species, and 2) investigate the relationship between reduced nematode viability (after exposure to UV) and virulence. Nematodes exposed to a UV radiation (254 nm) for 10 or 20 min were assessed separately for viability (survival) and virulence to *Galleria mellonella*. We compared 9 different EPN species and 15 strains: *Heterorhabditis bacteriophora* (Baine, fl11, Oswego, and Vs strains), *H. floridensis* (332), *H. georgiana* (Kesha), *H. indica* (HOM1), *H. megidis* (UK211), *Steinernema carpocapsae* (All, Cxrd, DD136, and Sal strains), *S. feltiae* (SN), *S. rarum* (17C&E), and *S. riobrave* (355). In viability assessments, steinernematids, particularly strains of *S. carpocapsae,* generally exhibited superior UV tolerance compared with the heterorhabditids. However, some heterorhabditids tended to be more tolerant than others, e.g., *H. megidis* and *H. bacteriophora* (Baine) were most susceptible and *H. bacteriophora* (Vs) was the only heterorhabditid that did not exhibit a significant effect after 10 min of exposure. All heterorhabditids experienced reduced viability after 20 min exposure though several *S. carpocapsae* strains did not. In total, after 10 or 20 min exposure, the viability of seven nematode strains did not differ from their non-UV exposed controls. In virulence assays, steinernematids (particularly *S. carpocapsae* strains) also tended to exhibit higher UV tolerance. However, in contrast to the viability measurements, all nematodes experienced a reduction in virulence relative to their controls. Correlation analysis revealed that viability among nematode strains is not necessarily related to virulence. In conclusion, our results indicate that the impact of UV varies substantially among EPNs, and viability alone is not a sufficient measure for potential impact on biocontrol efficacy as other characters such as virulence may be severely affected even when viability remains high.

Key words: biocontrol, entomopathogenic nematode, *Heterorhabditis*, *Steinernema*, tolerance, ultraviolet.

Entomopathogenic nematodes (EPNs; genera *Steinernema* and *Heterorhabditis*) are biocontrol agents used to target a variety of economically important insect pests (Grewal et al., 2005; Lacey and Shapiro-Ilan, 2008; Shapiro-Ilan et al., 2014). EPNs have a mutualistic symbiosis with a bacterium (steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids are associated with *Photorhabdus* spp.) (Poinar, 1990; Lewis and Clarke, 2012). Infective juveniles (IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle (Lewis and Clarke, 2012). After entering the host's hemocoel, nematodes release their bacterial symbionts, which are primarily responsible for killing the host usually within 24 to 48 hr, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters, 2002; Lewis and Clarke, 2012). The nematodes molt and complete up to three generations before new IJs exit the host to find insects to infect (Hazir et al., 2003). At least one dozen of the >100 EPN species described have been commercialized for use in biological control (Shapiro-Ilan et al., 2014).

Efficacy in biocontrol can be reduced by adverse environmental conditions such as desiccation, temperature extremes, and UV radiation (Shapiro-Ilan et al., 2006, 2012). In this study, we focused on the impact of UV radiation. Prior research indicates that exposure to UV radiation can affect EPN survival, virulence, or reproduction (Gaugler and Boush, 1978; Gaugler et al., 1992; Mason and Wright, 1997; Fujiie and Yokoyama, 1998). Thus, in selecting a particular nematode species or strain for use in biocontrol programs, the relative ability to withstand exposure may be an important consideration, especially for applications that are likely to entail extensive exposure to UV radiation. However, prior to this study, only limited comparisons for UV tolerance had been made among EPNs (four species or fewer) (Gaugler and Boush, 1978; Gaugler et al., 1992; Mason and Wright, 1997; Fujiie and Yokoyama, 1998). Therefore, our first objective was to conduct a broad comparison to determine variation in UV tolerance among an array of EPN species and strains.

To assess the impact of environmental stress, several options are available in terms of phenotypic characteristics that may be measured. Among the quickest and least labor intensive is an assessment of viability (the number or proportion of surviving nematodes). However, for assessment of UV tolerance, the relationship between viability and other important characteristics has not been studied. Certainly nematode survivability is essential to biocontrol success. However, in terms of biocontrol potential, a viable population will not be successful if virulence is lacking. Conceivably, the impact of UV radiation on viability may differ markedly from other important biocontrol traits. Therefore, our second objective was to investigate the relationship between nematode viability (after exposure to UV) and another important biocontrol trait, virulence.

Materials and Methods

Nematode cultures: Nematodes were cultured in commercially obtained last instar *Galleria mellonella* (L.)

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[1]USDA-ARS, Southeastern Fruit and Tree Nut Research Lab, Byron, GA 31008.
[2]Adnan Menderes University, Faculty of Arts and Sciences, Biology Department, Aydin, Turkey.
[3]Instituto Biologico, APTA, CP 70, Campinas, SP 13001-970, Brazil.
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E-mail: david.shapiro@ars.usda.gov.
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according to Kaya and Stock (1997). Infective juveniles were stored at 13°C for less than 2 wk prior to use in experiments. Measuring UV tolerance simultaneously in all 15 nematode strains was not feasible. Therefore, nematodes were divided into two groups. Nematodes included in the first group (hereafter referred to as (Group I) were *Heterorhabditis bacteriophora* Poinar (fl11 and Oswego strains), *Steinernema carpocapsae* (Weiser) (Cxrd, DD136, and Sal strains), *S. riobrave* Cabanillas, Poinar, and Raulston (355 strain) and *S. rarum* (Doucet) (17C&E strain). Nematodes included in the second group (hereafter referred to as Group II) were *H. bacteriophora* (Baine and Vs strains), *H. floridensis* Nguyen, Gozel, Koppenhöfer, and Adams (332 strain), *H. georgiana* Nguyen, Shapiro-Ilan, and Mbata (Kesha strain), *H. indica* Poinar, Karunakar, and David (Hom1 strain), *H. megidis* Poinar, Jackson, and Klein (UK211 strain), and *S. feltiae* (Filipjev) (SN strain). A list of the EPNs used in the UV tolerance assays is also presented in Table 1 along with associated abbreviations. In addition, one nematode, *S. carpocapsae* (All strain) was included in both groups to allow for qualitative comparisons between groups. Nematodes within each group were cultured in parallel, and the two groups were run as separate experiments.

Comparative UV tolerance: Procedures were based on previous studies that measured UV tolerance in EPNs (Gaugler and Boush, 1978; Gaugler et al., 1992; Wang and Grewal, 2002). Approximately 1,000 IJs (based on multiple dilution counts, Kaya and Stock, 1997) were suspended in 4 ml dH_2O in 60-mm petri dishes (approximately 4 mm in depth). Dishes were then placed in a Labconco Purifier Class II Biosafety Cabinet (model 36209; Labconco, Kansas City, MI). The nematodes were then exposed to UV radiation (254 nm) for 10 and 20 min using the UV lamp in the biosafety cabinet (separate dishes were used for each exposure period). An equal number of control dishes were concurrently set up without UV radiation and set on a bench next to the biosafety cabinet. Exposing the nematodes to UV (254 nm) in a thin layer of water prevents IJ desiccation and escape while allowing for UV penetration and impact (Gaugler and Boush, 1978; Gaugler et al., 1992; Wang and Grewal, 2002). There were two separate sets of dishes for each group and each time of exposure. One set was designated for viability assessment and one set for virulence. There were four replicate dishes per treatment. Thus, within each group there were 8 treatments (nematode strains) × 2 exposure periods × 4 replicates × 2 assays (viability and virulence) = 128 UV-exposed dishes, and an equal number of unexposed controls. Furthermore, each experiment was repeated in entirety once in time (two separate trials).

Table 1. List of *Heterorhabditis* spp. and *Steinernema* spp. used in bioassays to determine UV tolerance.

Group	Species	Strain(s)	Abbreviation
I	*H. bacteriophora*	fl11	Hbfl11
		Oswego	HbOsw
	S. carpocapsae	All	ScAll
		Cxrd	ScCxrd
		DD136	ScDD136
		Sal	ScSal
	S. riobrave	355	Sr355
	S. rarum	17C&E	Srar17C&E
II	*H. bacteriophora*	Baine	HbBai
		Vs	HbVs
	H. floridensis	332	HFL332
	H. georgiana	Kesha	HgKesha
	H. indica	Hom1	HiHom1
	H. megidis	UK211	Hmeg
	S. carpocapsae	All	ScAll
	S. feltiae	SN	SfSN

Viability and virulence were assessed after 24 hr of incubation at 25°C. In half of the dishes, viability was determined by measuring the percentage of IJs that died following UV exposure relative to mortality in the strain's corresponding control (no UV exposure). Viability was assessed based on percentage nematode mortality. Abbott's formula (Abbott, 1925) was applied to each replicate to correct for control mortality. The formula was ([survival in the control − survival in the treatment]/survival in the control) × 100. In this manner, any potential differences in control mortality among the nematode treatments could be accounted for. Nematode mortality was determined based on lack of movement response when probed with a dissecting needle (Shapiro-Ilan et al., 2009) (a minimum of 50 IJs were counted per dish). Average IJ survival in controls was high ranging from 87.5% to 100%.

For determination of virulence, the suspensions from the other half of the dishes were poured into 100-mm petri dishes containing three filter papers (Whatman No. 1), and 10 last instar *G. mellonella* larvae were added to each dish. The number of live and dead larvae was determined after 48 hr of incubation at 25°C. Potential differences in innate nematode virulence among the strains was corrected by applying a modified Abbott's formula to larval mortality in the exposed treatments compared with their respective controls (no UV exposure). The formula used was (100 − [(mortality in the control − mortality in the treatment)/mortality in the control]) × 100. Average mortality of *G. mellonella* was high in the controls ranging from 95% to 100%.

Data analysis: For each strain and each assessment variable (viability and virulence), the effect of UV radiation was assessed by comparing UV-exposed and nonexposed nematodes using a t test (SAS, 2002; $P \leq 0.05$). In addition, the comparative effects of UV tolerance among different strains in terms of corrected IJ mortality (to assess impact on viability) and corrected *G. mellonella* mortality (to assess virulence) were analyzed with analysis of variance (SAS, 2002). Data from experiments that were repeated in time were combined, and variation among trials was accounted for as

a block effect. Percentage data were arcsine transformed (arcsine of square root) prior to analysis (Southwood, 1978). Nontransformed means are presented in figures.

The potential relationship between viability and virulence was determined using Spearman's correlation analysis (Steel and Torrie, 1980; SAS, 2002). Correlation analysis was applied separately to corrected viability and mortality levels in each nematode group and exposure time.

Results

Viability: The viability of EPNs was differentially affected following exposure to UV radiation. Overall, the steinernematids (especially *S. carpocapsae* strains) tended to withstand UV radiation better than most heterorhabditid strains though some heterorhabditids also exhibited tolerance, e.g., *H. bacteriophora* (Vs) (Fig. 1). For Group I strains, after 10 min exposure, a comparison of individual EPN strains to their respective controls (using *t* tests) indicated that *S. carpocapsae* (Cxrd, DD136, and Sal strains), *S. riobrave*, and *S. rarum* were not affected by UV radiation (df = 14, $P > 0.05$ for all strains), whereas the other strains were affected (df = 14, $P \leq 0.05$ for all strains) (Fig. 1). In comparing corrected IJ mortality among strains because of UV radiation, steinernematids, especially *S. carpocapsae* strains, tended to have lower mortality ($F = 11.25$; df = 7, 55; $P < 0.0001$) (Fig. 1).

For Group I after 20 min of UV exposure, three *S. carpocapsae* strains (All, Cxrd, and DD136) were not affected compared with their respective controls (df = 14, $P > 0.05$ for all strains), whereas the other strains were affected (df = 14, $P \leq 0.05$ for all strains) (Fig. 1). In comparing corrected IJ mortality among strains, results paralleled those observed in the 10 min exposure with *H. bacteriophora* (Hbfl11 and Oswego strains) exhibiting notably higher mortalities than the other strains ($F = 96.78$; df = 7, 55; $P < 0.0001$) (Fig. 1).

In Group II following 10 min of UV exposure, only *H. bacteriophora* (Vs) and *S. carpocapsae* were unaffected relative to their respective controls (df = 14, $P > 0.05$ for both strains), whereas the other strains were affected (df = 14, $P \leq 0.05$ for all strains) (Fig. 1). UV radiation caused higher IJ mortality in *H. bacteriophora* (Baine) and *H. megidis* than the other strains, and the other strains were for the most part similar to each other in response ($F = 53.87$; df = 7, 55; $P < 0.0001$) (Fig. 1). After 20 min exposure to Group II nematodes, only *S. carpocapsae* (All) remained unaffected by UV radiation compared with its nonexposed control (df = 14; $P > 0.05$). *S. carpocapsae* also exhibited the greatest tolerance compared with all other strains ($F = 56.18$; df = 7, 55; $P < 0.0001$) (Fig. 1).

Virulence: Relative virulence after UV exposure, as indicated by corrected *G. mellonella* mortality, varied among EPNs (Fig. 2). Unlike the results observed for viability (Fig. 1) based on *t* tests, all of individual

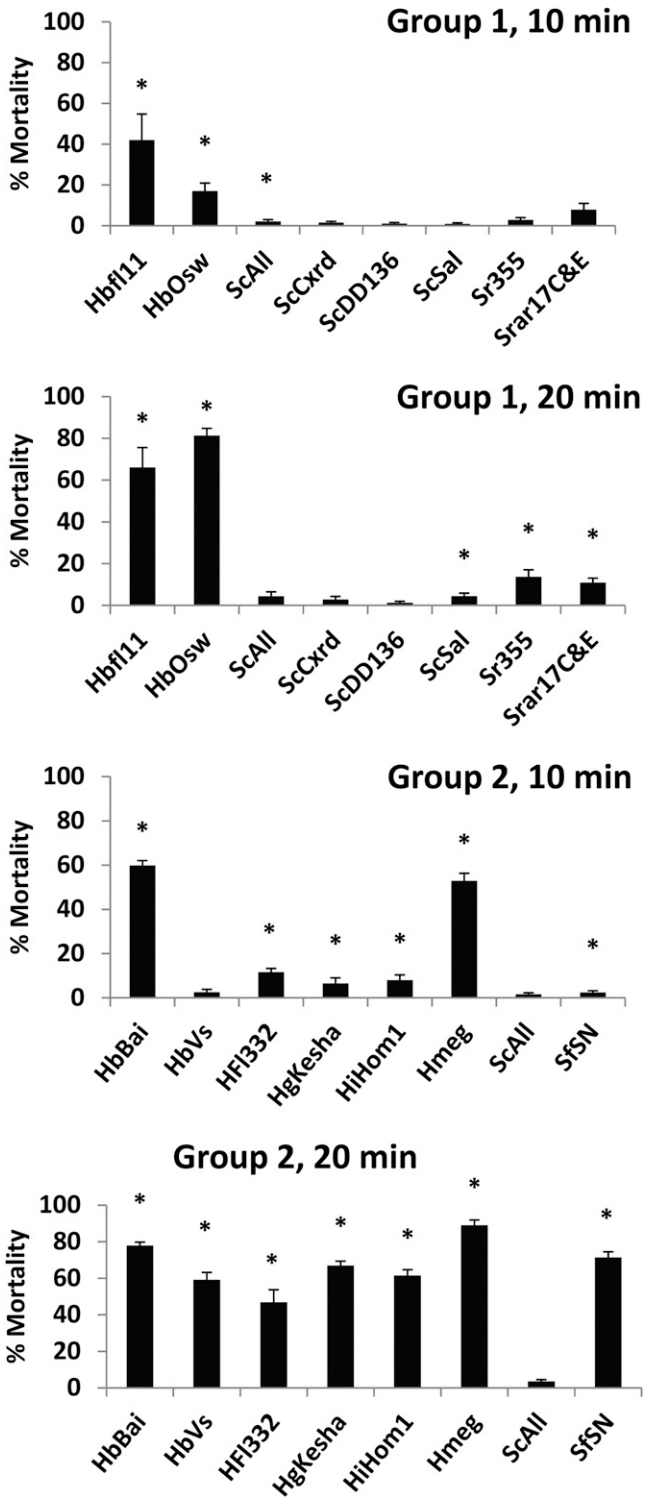

Fig. 1. Corrected percentage mortality in two groups of entomopathogenic nematode infective juveniles after exposure to UV radiation for 10 or 20 min. Corrected nematode mortality was calculated using Abbott's formula relative to mortality of nematodes that were not exposed to UV. Nematodes included *Heterorhabditis bacteriophora* (Baine, fl11, Oswego strains, and Vs strains), *H. floridensis* (332), *H. georgiana* (Kesha), *H. indica* (Hom1), *H. megidis* (UK211), *S. carpocapsae* (All, Cxrd, DD136, and Sal), *S. feltiae* (SN), *S. riobrave* (355), and *S. rarum* (17C&E). See Table 1 for abbreviations associated with each species and strain. For each nematode strain, * indicates a significant difference between the UV-treated nematodes and nematodes not exposed to UV (*t* test, $\alpha = 0.05$).

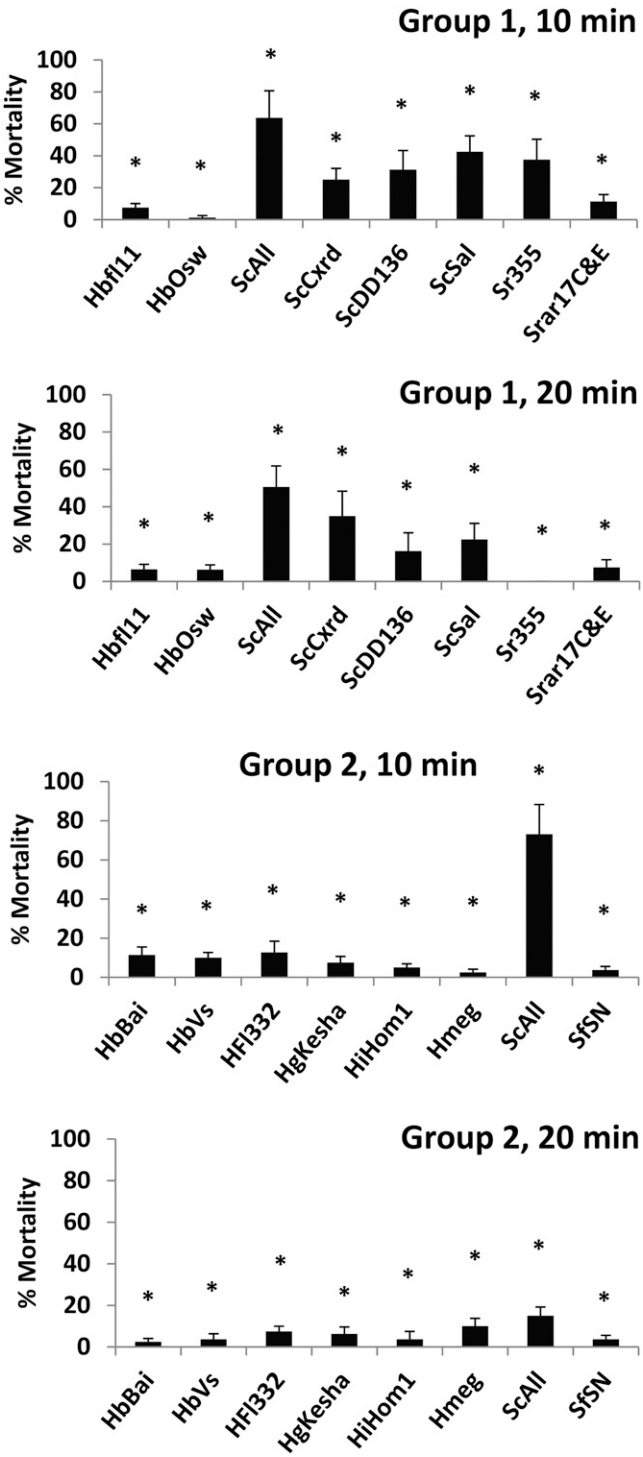

FIG. 2. Corrected percentage mortality of *Galleria mellonella* caused by entomopathogenic nematodes (two different groups tested separately) after exposure to UV radiation for 10 or 20 min. Corrected insect mortality for each nematode treatment was calculated using Abbott's formula relative to mortality in the corresponding control (nematodes not exposed to UV). Nematodes included *Heterorhabditis bacteriophora* (Baine, fl11, Oswego strains, and Vs strains), *H. floridensis* (332), *H. georgiana* (Kesha), *H. indica* (Hom1), *H. megidis* (UK211), *S. carpocapsae* (All, Cxrd, DD136, and Sal), *S. feltiae* (SN), *S. riobrave* (355), and *S. rarum* (17C&E). See Table 1 for abbreviations associated with each species and strain. For each nematode strain, * indicates a significant difference between the UV-treated nematodes and nematodes not exposed to UV (*t* test, $\alpha = 0.05$).

nematode strains exposed to UV exhibited reduced virulence compared with their respective nonexposed controls (all groups and exposure times, df = 14; $P < 0.05$). In Group I after 10 min exposure, the comparison of virulence among treatments indicated that *S. carpocapsae* (All) exhibited notably higher tolerance than *H. bacteriophora* (fl11 and Oswego strains) whereas the other strains exhibited intermediate levels of tolerance ($F = 4.67$; df = 7, 55; $P = 0.0004$) (Fig. 2). Similarly, for Group I after 20 min exposure, *S. carpocapsae* (All) exhibited higher tolerance than *H. bacteriophora* (fl11 and Oswego strains) as well as *S. riobrave* and *S. rarum* ($F = 5.8$; df = 7, 55; $P < 0.0001$) (Fig. 2). In Group II after 10 min, *S. carpocapsae* (All) exhibited the highest tolerance ($F = 11.48$; df = 7, 54; $P < 0.0001$) and after 20 min no differences were detected ($F = 1.86$; df = 7, 55; $P = 0.095$) (Fig. 2).

Overview of virulence and viability and correlation analysis between traits: Overall, looking at both viability and virulence, the *S. carpocapsae* strains (All, Cxrd, DD136, and Sal) may be deemed to have exhibited the highest level of UV tolerance among the EPNs tested. When considering the *t* tests comparing the UV-exposed nematodes versus their nonexposed controls, EPNs exhibited higher tolerance in viability compared with virulence. In total, after 10 or 20 min exposure, the viability of seven nematode strains did not differ from their non-UV-exposed controls. However, in contrast to the viability measurements, all nematodes experienced a reduction in virulence compared with their controls.

There was a significant correlation between viability and virulence in the Group I experiments but not in the Group II experiments. In Group I, a significant negative correlation was detected between corrected nematode mortality following UV exposure and corrected *G. mellonella* mortality (i.e., virulence increased as nematode mortality due to exposure decreased) ($r = -0.714$, $P = 0.0465$ for 10 min and $r = -7.62$, $P = 0.028$ for 20 min). In contrast, there was no correlation detected between viability and virulence in Group II experiments ($r = -0.167$, $P = 0.6932$ for 10 min and $r = -0.366$, $P = 0.373$ for 20 min).

DISCUSSION

Our results indicated significant variation in UV tolerance among EPN strains and species. Overall, several steinernematids exhibited higher levels of UV tolerance than other strains or species with *S. carpocapsae* strains generally exhibiting the highest level. Previous comparisons among EPNs for UV tolerance only included one to four species or strains (Gaugler and Boush, 1978; Gaugler et al., 1992; Mason and Wright, 1997; Fujiie and Yokoyama, 1998; Grewal et al., 2002; Wang and Grewal, 2002; Jagdale and Grewal, 2007). In one previous comparison, Gaugler et al. (1992) compared the two nematodes *H. bacteriophora* (NC1) and

S. carpocapsae (All), and concluded that the lower UV tolerance observed in the *H. bacteriophora* strain was indicative of a broader lack of environmental tolerance inherent in heterorhabditids compared with steinernematids. In comparison with the previous study, the results of our investigation emphasize the need for diverse comparisons that include a wide array of species and strains; although we generally did observe greater tolerance in some steinernematids than a number of the heterorhabditids, certain heterorhabditids such as *H. bacteriophora* (Vs) and *H. floridensis* (332) exhibited relatively high tolerance.

The impact of UV radiation in EPNs depends on wavelength. Gaugler and Boush (1978) reported that longer UV wavelengths, such as 366 nm, did not impact virulence of *S. carpocapsae*, whereas shorter wavelengths such as 254 nm negatively affected the nematode. Confirming this finding, Fujiie and Yokoyama (1998) reported sunlight and UV wavelengths of 310 and 254 nm affected *Steinernema kushidai* Mamiya virulence whereas 350 nm had no effect. In contrast to our study, Jagdale and Grewal (2007) reported that UV had no impact on the virulence of *S. feltiae* (SN), but did affect *S. carpocapsae* (All) and *S. riobrave* (TX-355 strain). We observed higher UV tolerance in *S. carpocapsae* (All) than *S. feltiae* (SN). However, Jagdale and Grewal (2007) applied a wavelength of 340 nm whereas we applied 254 nm. Thus, the reduced impact and differential effects were likely due to the longer wavelength used in the previous study.

The choice of host species can be important when assessing the impact of UV or other stress factors on EPNs. For example, using simulated sunlight, Mason and Wright (1997) did not observe any effects of UV radiation on the virulence of four EPN strains (*Steinernema* spp. [SSL85 and M87], *Heterorhabditis* sp., and *H. indica*) to diamondback moth, *Plutella xylostella* (L.). The lack of effects observed by Mason and Wright (1997) may have been due to the use of *P. xylostella* as a target host. Conceivably *P. xylostella* is not sensitive to the level of phenotypic changes that may have occurred. The choice of target host depends on the goal of the study. For Mason and Wright (1997), it was logical to use *P. xylostella* because their objective was to develop an aboveground EPN application program for this specific pest, and hence determination of potential UV effects for that particular target was necessary. Similarly, Fujiie and Yokoyama (1998) chose *Anomala cuprea* Hope as subject insect because they were testing UV effects on *S. kushidai* Mamiya, which has a host range that is particularly adapted to scarabs (Mamiya, 1989) (and thus a lepidopteran such as *G. mellonella* would be less suitable). In our study, in which the goal was to measure relative impact of UV among diverse EPNs, the use of *G. mellonella* as the subject host was clearly justified because it is susceptible to a broad array of EPNs (Shapiro-Ilan et al., 2014), displays a positive dose response (Grewal et al., 1993; Kalia et al., 2014; Kumar et al., 2015), and has been used successfully to distinguish differential UV effects on EPN virulence in several previous studies (Gaugler and Boush, 1978; Gaugler et al., 1992; Grewal et al. 2002; Jagdale and Grewal, 2007).

For inundative biocontrol applications using EPNs, success depends on a variety of factors. In addition to having a viable product, successful biocontrol requires an EPN population that can penetrate and kill the target host, i.e., a high level of virulence (Shapiro-Ilan et al., 2002). Thus, when assessing the impact of stress levels, examination of only one phenotypic trait may lead to false conclusions for predicting tolerances among EPNs and their biocontrol potential. Similar to our study, Fujiie and Yokoyama (1998) included both viability and virulence in their investigation, but in contrast the authors did not find a discrepancy in the effects. However, the previous study (Fujiie and Yokoyama, 1998) only involved one nematode species, and so conclusions across species were not possible. On the other hand, Jagdale and Grewal (2007) observed differential effects in the two traits as they did not detect any changes in viability following UV exposure, but reported reduced virulence in *S. carpocapsae*, *S. feltiae*, and *S. riobrave*. Our study is the first to examine the effects of UV on both viability and virulence using a broad array of EPNs. We determined that a lack of impact on viability does not necessarily translate into a lack of effects in virulence. Therefore, after exposure to UV radiation, one cannot necessarily predict successful virulence or biocontrol efficacy based on viability alone. This lack of predictability may extend to effects of other stresses (e.g., heat or desiccation), and thus, for quality control purposes, virulence assessments may need to be coupled with viability.

In our study, the same populations of nematodes that were exposed to UV for viability assessment were also used in virulence assays. The goal was to determine if a given nematode population would remain viable following exposure to UV and also retain the potential to kill a target pest. Thus, our virulence assays included both live and dead IJs. It may have been interesting to also assess virulence using only live IJs. Nonetheless, the discrepancy between viability and virulence observed in our study is clear. Perhaps the strongest evidence is indicated by the seven nematode strains that did not exhibit any reduction in viability, yet virulence was significantly reduced.

In summary, we found a high level of variation in relative UV tolerance among a diverse array of EPN species and strains. Hence, differential UV tolerance may need to be considered when choosing a nematode for a biocontrol program, particularly if significant UV exposure is expected. Although our results elucidated tolerances among EPNs under laboratory conditions, additional research is needed to see how these tolerances may

compare in under field conditions. Future research may also include further testing of relationships among traits such as viability, virulence, reproductive capacity, and environmental tolerance when exposed to UV or to other stress factors. In addition, it would be interesting to investigate the cause for the discrepancy between viability and virulence assessments. Fujiie and Yokoyama (1998) reported that UV exposure harmed the nematode's symbiotic bacteria as well as the nematodes themselves. Possibly, the observation by Fujiie and Yokoyama (1998) may explain the differential impact of UV on EPN viability and virulence; the EPNs may remain alive while the bacteria (the primary virulence carrier) are killed or damaged.

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