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Freezing and Desiccation Tolerance in Entomopathogenic Nematodes: Diversity and Correlation of Traits

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Abstract: The ability of entomopathogenic nematodes to tolerate environmental stress such as desiccating or freezing conditions, can contribute significantly to biocontrol efficacy. Thus, in selecting which nematode to use in a particular biocontrol program, it is important to be able to predict which strain or species to use in target areas where environmental stress is expected. Our objectives were to (i) compare inter- and intraspecific variation in freeze and desiccation tolerance among a broad array of entomopathogenic nematodes, and (ii) determine if freeze and desiccation tolerance are correlated. In laboratory studies we compared nematodes at two levels of relative humidity (RH) (97% and 85%) and exposure periods (24 and 48 h), and nematodes were exposed to freezing temperatures (-2°C) for 6 or 24 h. To assess interspecific variation, we compared ten species including seven that are of current or recent commercial interest: *Heterorhabditis bacteriophora* (VS), *H. floridensis*, *H. georgiana*, (Kesha), *H. indica* (HOM1), *H. megidis* (UK211), *Steinernema carpocapsae* (All), *S. feltiae* (SN), *S. glaseri* (VS), *S. rarum* (17C&E), and *S. riobrave* (355). To assess intraspecific variation we compared five strains of *H. bacteriophora* (Baine, Fl1-1, Hb, Oswego, and VS) and four strains of *S. carpocapsae* (All, Cxrd, DD136, and Sal), and *S. riobrave* (355, 38b, 7-12, and TP). *S. carpocapsae* exhibited the highest level of desiccation tolerance among species followed by *S. feltiae* and *S. rarum*; the heterorhabditid species exhibited the least desiccation tolerance and *S. riobrave* and *S. glaseri* were intermediate. No intraspecific variation was observed in desiccation tolerance; *S. carpocapsae* strains showed higher tolerance than all *H. bacteriophora* or *S. riobrave* strains yet there was no difference detected within species. In interspecies comparisons, poor freeze tolerance was observed in *H. indica*, and *S. glaseri*, *S. rarum*, and *S. riobrave* whereas *H. georgiana* and *S. feltiae* exhibited the highest freeze tolerance, particularly in the 24-h exposure period. Unlike desiccation tolerance, substantial intraspecies variation in freeze tolerance was observed among *H. bacteriophora* and *S. riobrave* strains, yet within species variation was not detected among *S. carpocapsae* strains. Correlation analysis did not detect a relationship between freezing and desiccation tolerance.

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

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) (EPNs) are biological control agents that are used to control a variety of economically important insect pests (Grewal et al., 2005; Lacey and Shapiro-Ilan, 2008; Lewis and Clarke, 2012). Each species of EPN has a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (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 h, 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 after which IJs exit the cadaver to find new hosts (Lewis and Clarke, 2012). At least one dozen of the > 90 EPN species described have been commercialized for use as biological control agents (Shapiro-Ilan et al., 2014).

Successful biological control applications can be hindered by various abiotic factors including adverse environmental conditions (Shapiro-Ilan et al., 2006). Thus, biocontrol potential can be enhanced by using EPNs that can best tolerate extremes in heat, cold, or desiccating conditions or other stress factors. Hence, characterization of environmental tolerance among EPN strains and species can aid in selecting which nematode to use in a particular target system. For example, diverse classification of (nonfreezing) temperature tolerance (Grewal et al., 1994) has been highly useful in predicting the temperature range for various EPN species. In this study we focus on tolerance to two stress factors: desiccation and freezing temperatures. Comparisons of desiccation tolerance among EPN species have been reported previously (e.g., Shapiro-Ilan et al., 2009; Morton and Garcia-del-Pino, 2009; Salame et al., 2010), and several investigations of within-species variation have been reported as well (Shapiro-Ilan et al., 2003; Mukuka et al., 2010; Nimkingrat et al., 2013). Nonetheless, extended comparisons among common and commercialized EPN species and strains are warranted. Unlike desiccation tolerance, relatively few comparisons have been made among EPN species and strains for tolerance to freezing temperatures, and only one to three species have been compared at a time (Brown and Gaugler, 1996; Jagdale and Grewal, 2003; Smith et al., 2008). Therefore, our first objective was to compare inter- and intraspecific variation in freeze and desiccation tolerance among a broad array of EPN species.

If a correlation between or among traits can be established, the relationship may assist further in predicting which strains or species of EPNs are most suitable for the environmental conditions associated with a particular target system. Furthermore, relationships

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among stress factors are of fundamental interest in understanding EPN physiology and ecology. Freeze and desiccation tolerance have been reported to be correlated in certain other organisms such as insects, e.g., the goldenrod gall fly, *Eurosta solidaginis* (Williams and Lee, 2011; Levis et al., 2012). Desiccation tolerance has been reported to correlate with other stress factors (e.g., heat tolerance) in EPNs (Chen et al., 2005). Additionally some evidence suggests that tolerance to cold temperatures and desiccation may be related in EPNs, e.g., both stress factors cause an increase in trehalose levels, which is implicated as a physiological protectant (Solomon et al., 2000; Grewal and Jagdale, 2002). Thus, our second objective was to determine if freeze and desiccation tolerance are correlated in EPNs.

Materials and Methods

Nematode cultures: Nematodes used in all experiments were cultured in parallel in commercially obtained last instar *Galleria mellonella* (L.) according to Kaya and Stock (1997); IJs were stored at 13°C for less than 2 wk before use in experiments. For each nematode strain, the number of culture passages from the time of first isolation or receiving the nematodes was kept to a minimum (e.g., less than 15 passages). To assess interspecific variation we compared 10 species: *Heterorhabditis bacteriophora* Poinar (VS strain), *H. floridensis* Nguyen, Gozel, Koppenhöfer & Adams (Stacy strain), *H. georgiana* Nguyen, Shapiro-Ilan, & Mbata (Kesha strain), *H. indica* Poinar, Karunakar & David (HOM1 strain), *H. megidis* Poinar, Jackson & Klein (UK211 strain), *Steinernema carpocapsae* (Weiser) (All strain), *S. feltiae* (Filipjev) (SN strain), *S. glaseri* (Steiner) (VS strain), *S. rarum* (Doucet) (17 C&E strain), and *S. riobrave* Cabanillas, Poinar & Raulston (355 strain). Seven of these species are currently or have recently been in commercial use (*H. bacteriophora*, *H. indica*, *H. megidis*, *S. carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. riobrave*). To assess intraspecific variation we compared five strains of *H. bacteriophora* (Baine, Fl1-1, Hb, Oswego, and Vs) and four strains of *S. carpocapsae* (All, Cxrd, DD136, and Sal), and *S. riobrave* (355, 38b, 7-12, and Tp); experiments comparing all 13 strains were run simultaneously. Experiments addressing each stress factor (freezing temperatures or desiccation), and interspecies and intraspecies variation were conducted separately.

Comparative EPN tolerance to desiccating conditions: Desiccation tolerance was compared among nematode species and strains based on procedures described by Shapiro-Ilan et al. (2009). Approximately 2,000 IJs were pipetted onto filter paper (55 mm, Whatman No. 1) in a 60-mm petri dish. Excess moisture was removed through vacuum filtration. The filter paper containing nematodes was then placed in a plastic desiccator (23-cm-maximum-diam. × 24-cm-height, Nalgene®, Rochester, NY) that was maintained at 85% or 97% RH, based on a saturated solution of KCl or K_2SO_4, respectively. After 24 and 48 h of incubation at 25°C, the filter paper was removed and placed in approximately 5-ml tap water for an additional 24 h, at which time percentage survival was determined by probing nematodes with a dissecting needle (Shapiro-Ilan et al., 2009). A minimum of 50 IJs were counted per sample. Dishes containing the various nematode strains and species were randomized within each desiccator. A single replicate of each RH level (85% and 97%) and each exposure time (24 and 48 h) was run simultaneously, and then repeated three times with a different batch of nematodes (resulting four replicates total).

A number of studies have indicated that a preacclimation period (e.g., 72 h at 97% RH) can enhance desiccation tolerance (Solomon et al., 2000). However, we chose not to implement a preacclimation phase in our study. Preacclimation may have substantial value when considering preparation of nematodes for formulation and extended shelf-life, but such controlled conditions are extremely unlikely to occur in nature. Our emphasis was on predicting relative environmental tolerance in nature and the impact on biocontrol applications. In nature, exposure to desiccating conditions can be rapid (e.g., aboveground application to a plant or tree or to dry soil). Thus, for our purposes, preacclimation for 72 h at 97% RH would not be appropriate.

Comparative EPN tolerance to freezing temperatures: Methods used to determine IJ tolerance to freezing temperatures were based on those described previously by Lewis and Shapiro-Ilan (2002). Approximately 5,000 IJs of each nematode were pipetted into 30-ml plastic cups (4-cm-diam. × 3.5-cm-deep, Bioserv, Frenchtown, NJ) containing 10 g of sand at final moisture content of 10%. Given that gradual acclimation to lower temperatures has been shown to improve EPN survival (Brown and Gaugler, 1996; Ali and Wharton, 2013) and gradual lowering of temperatures is reflective of natural conditions in soil, the cups were incubated at 10°C for 24 h, then 4°C for 24 h, and then subjected to incubation at freezing temperatures (-2°C). Half of the cups were removed after 6 h and half after 24 h at -2°C. The nematodes were then allowed to reacclimate for 24 h at 25°C, at which time IJ survival was determined by placing the sand from each cup in 10 to 50 ml water and estimating the number living using procedures described above. There were four replicate cups per treatment (nematode species or strain), and each experiment (interspecies comparison and intraspecies comparison) was repeated once in time using a new batch of nematodes.

Data analysis: Effects of desiccating or freezing conditions on nematode survival were analyzed with analysis of variance (ANOVA). If the ANOVA detected a significant overall treatment effect ($P \leq 0.05$), then treatment differences were elucidated through the Student-Newman-Keuls' (SNK) test (SAS, 2002). For

replicated experiments that were repeated in time, e.g., freezing tolerance, data from both trials were combined, and variation among trials was accounted for as a block effect. Percentage data were arcsine transformed (arcsine of square root) before analysis (Southwood, 1978); residuals of the transformed means were plotted to indicate that equality of variance and the normality assumptions were met. Nontransformed means are presented in figures.

The potential relationship between desiccation and freezing tolerance was determined using Spearman's correlation analysis (Steel and Torrie, 1980; SAS, 2002). Correlation analysis was applied to experiments that exhibited differential nematode survival among treatments. Experiments that did not exhibit treatment differences were excluded because it was deemed that the nematodes were not substantially stressed, i.e., there was no point in comparing stress regimes when the stress level did not elicit a response. Thus, for interspecies comparisons, correlation analysis was applied to nematodes exposed to 48 h desiccation at 85% RH, and to nematodes exposed to freezing temperatures for 6 and 24 h; among intraspecies comparisons, correlation analysis was applied to nematodes exposed to 24 and 48 h desiccation at 85% RH, and to nematodes exposed to freezing temperatures for 6 and 24 h (see Results section).

Results

Comparative EPN tolerance to desiccating conditions: When analyzing interspecific variation in desiccation tolerance across all parameters, significant effects of time of exposure ($F = 32.1$; degree of freedom (df) = 1, 136; $P < 0.0001$), RH level ($F = 19.5$; df = 1, 136; $P < 0.0001$), and the interaction between treatment (nematode) effect and RH level ($F = 5.2$; df = 9, 136; $P < 0.0001$) were detected. For the intraspecific comparisons, although time of exposure did not have a significant effect ($F = 1.75$; df = 1, 181; $P = 0.187$), RH level ($F = 693.31$; df = 1, 181; $P < 0.0001$) and the interaction between RH level and treatment effects ($F = 20.36$; df = 12, 181; $P < 0.0001$) were significant. Therefore, for both inter- and intraspecies variation, we chose to analyze the treatment effects separately at each exposure time and RH level.

When exploring interspecies effects, treatment differences were not detected at 97% RH ($F = 1.98$; df = 9, 29; $P = 0.08$ for the 24-h exposure and $F = 1.14$; df = 9, 29; $P = 0.37$ for the 48-h exposure); mean survival ± SE ranged from 79.0 ± 11.36 in *S. carpocapsae* (All) to 97.75 ± 0.75 in *H. megidis* (UK211) for 24 h and 76.25 ± 11.35 in *S. rarum* (17C&E) to 97.75 ± 0.63 in *H. megidis* (UK211) for 48 h. Treatment differences were also not detected at the 24-h exposure with 85% RH ($F = 1.35$; df = 9, 29; $P = 0.25$); mean survival ± SE ranged from 12.75 ± 7.19 in *H. indica* (HOM1) to 78.25 ± 6.57 in *S. carpocapsae* (All).

Exposure to desiccating conditions at 85% RH for 48 h resulted in substantial variation in survival among EPN species ($F = 31.57$; df = 9, 30; $P < 0.0001$). *S. carpocapsae* (All) exhibited the highest level of desiccation tolerance among species followed by *S. feltiae* (SN) and then *S. rarum* (17C&E). The heterorhabditid species exhibited the lowest desiccation tolerance and *S. riobrave* (355) and *S. glaseri* (VS) were intermediate between survival of *S. rarum* (17C&E) and the heterorhabditids (*S. riobrave* 355 exhibited higher survival than three of the heterorhabditids but not the others) (Fig. 1).

When exploring intraspecies variation in desiccation tolerance, treatment differences were not detected at 97% RH ($F = 0.49$; df = 12, 39; $P = 0.906$ for 24 h, and $F = 0.55$; df = 12, 39; $P = 0.868$ for 48 h). Treatment differences were detected at 85% RH ($F = 8.72$; df = 12, 39; $P < 0.0001$ for 24 h, and $F = 24.56$; df = 12, 39; $P < 0.0001$ for 48 h), yet variation among strains within species was not detected after 24 or 48 h exposure (Figs. 2,3). All *S. carpocapsae* strains had higher survival than *S. riobrave* or *H. bacteriophora* strains (Figs. 2,3).

Comparative EPN tolerance to freezing temperatures: When analyzing interspecific variation in tolerance to freezing

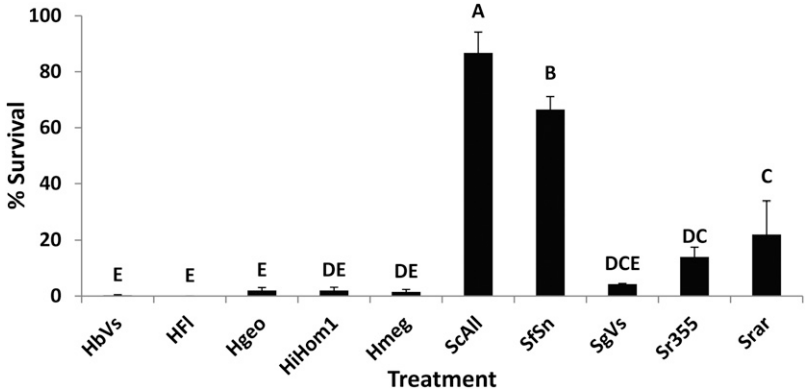

Fig. 1. Mean percentage survival (± SEM) of entomopathogenic nematodes in an interspecies comparison following exposure to desiccating conditions (48 h at 85% RH). Nematodes included *Heterorhabditis bacteriophora* (VS), *H. floridensis* (Stacy), *H. georgiana* (Kesha), *H. megidis* (UK211), *Steinernema carpocapsae* (All), *S. feltiae* (SN), *S. glaseri* (VS), *S. riobrave* (355), and *S. rarum* (17C&E). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

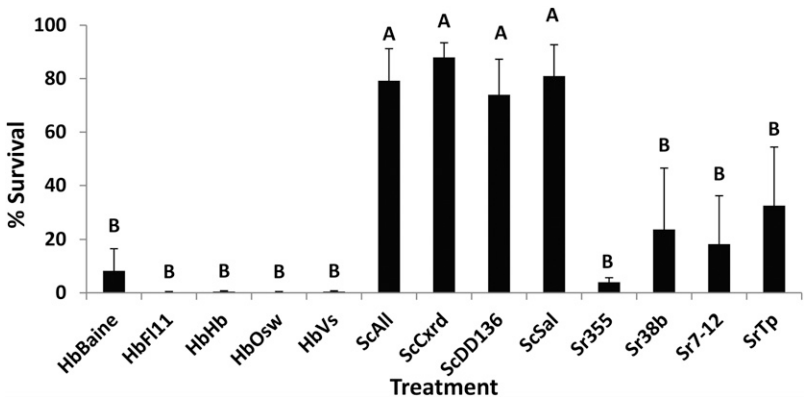

FIG. 2. Mean percentage survival (± SEM) of entomopathogenic nematodes in an intraspecies comparison following exposure to desiccating conditions (24 h at 85% RH). Nematodes included five strains of *H. bacteriophora* (Baine, Fl1-1, Hb, Oswego, and VS) and four strains of *S. carpocapsae* (All, Cxrd, DD136, and Sal), and *S. riobrave* (355, 38b, 7-12, and TP). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

temperatures significant effects of time of exposure (F= 48.75; df = 1, 139; $P < 0.0001$) were detected and the interaction between treatment effect and exposure time was also significant (F = 6.76; df = 9, 139; $P <$ 0.0001) were detected. Similarly, for the intraspecific comparisons, significant effects of time of exposure (F= 317.32; df = 1, 181; $P < 0.0001$) and the interaction between treatment effect and exposure time (F = 2.82; df = 9, 181; P = 0.0014) were detected. Therefore, for inter and intraspecies variation, we chose to analyze the treatment effects at each exposure time individually.

Interspecies comparisons of tolerance to freezing temperatures indicated variation among treatments at both exposure times (F = 21.69; df = 9, 69; $P < 0.0001$ at 6 h and F = 23.73; df = 9, 69; $P < 0.0001$ at 24 h). After 6 h of exposure, survival in *H. indica* (HOM1), *S. rarum* (17C&E), and *S. riobrave* (355) was lower than in all the other species, which were not different from each other (with the exception of *H. megidis* UK211 having higher survival than *S. glaseri* VS) (Fig. 4). After 24 h of exposure, survival of *H. georgiana* (Kesha) was higher than all other nematode species except *S. feltiae* (SN), and *S. feltiae* (SN) survival was higher than the other species except *H. bacteriophora* (VS); survival of *H. floridensis* (Stacy), *S. carpocapsae* (All), and *H. megidis* (UK211) was intermediate whereas survival of *H. indica* (HOM1), *S. rarum* (17C&E), *S. glaseri* (VS) and *S. riobrave* (355) was lower (with *S. rarum* survival being least) (Fig. 5).

For both exposure periods, unlike desiccation tolerance, substantial intraspecies variation in freeze tolerance was observed among *H. bacteriophora* and *S. riobrave* nematode strains, yet no differences were observed among *S. carpocapsae* strains (F = 7.60; df = 12, 90; $P <$ 0.0001 at 6 h and F = 9.57; df = 12, 90; $P < 0.0001$ at 24 h). After 6 h of exposure to freezing temperatures, survival in *H. bacteriophora* (Oswego) was higher than two other *H. bacteriophora* strains (Fl1-1 and Hb) though similar to the Baine strain; *S. riobrave* (355) had lower survival at 6 h relative to the other three *S. riobrave* strains tested (Fig. 6). Following 24 h of exposure, the Baine and Oswego strains of *H. bacteriophora* exhibited higher survival than the Hb and Fl1-1 strains, and higher survival was observed in *S. riobrave* (TP) compared with the other three *S. riobrave* strains tested (Fig. 7).

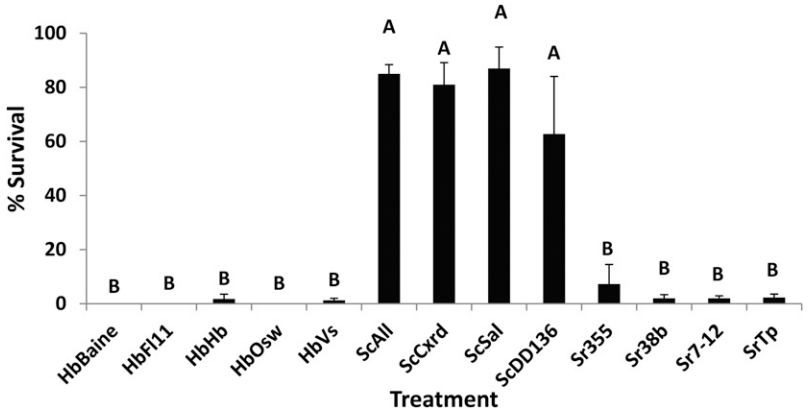

FIG. 3. Mean percentage survival (± SEM) of entomopathogenic nematodes in an intraspecies comparison following exposure to desiccating conditions (48 h at 85% RH). Nematodes included five strains of *H. bacteriophora* (Baine, Fl1-1, Hb, Oswego, and VS) and four strains of *S. carpocapsae* (All, Cxrd, Sal, and DD136), and *S. riobrave* (355, 38b, 7-12, and TP). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

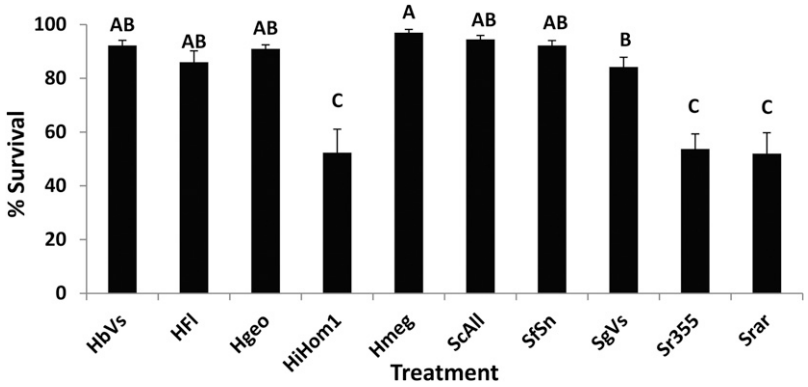

Fig. 4. Mean percentage survival (± SEM) of entomopathogenic nematodes in an interspecies comparison following exposure to freezing temperatures (6 h at -2°C). Nematodes included *Heterorhabditis bacteriophora* (VS), *H. floridensis* (Stacy), *H. georgiana* (Kesha), *H. megidis* (UK211), *Steinernema carpocapsae* (All), *S. feltiae* (SN), *S. glaseri* (VS), *S. riobrave* (355), and *S. rarum* (17C&E). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

Furthermore, survival in the Baine and Oswego strains of *H. bacteriophora* and *S. riobrave* (TP) was similar to all four strains of *S. carpocapsae* (Fig. 7).

Relationship between desiccation and freeze tolerance: No correlations between desiccation tolerance and tolerance to freezing temperatures were detected in either the interspecies comparisons (Table 1) or intraspecies comparisons (Table 2). As one would expect, the tolerance to freezing temperatures after 6 h was correlated to tolerance at 24 h (Tables 1 and 2), and similarly desiccation tolerance after 24 h was correlated to tolerance after 48 h (Table 2).

Discussion

Our findings indicate that desiccation tolerance varies greatly across EPN species. The relative levels of tolerance among species observed in our study are similar to some previous comparisons involving fewer species. For example, similar to this study, Shapiro-Ilan et al. (2009) observed the highest level of desiccation tolerance in *S. carpocapsae* followed by *S. feltiae* and with heterorhabditids being least tolerant and *S. riobrave* intermediate. Also similar to our results, Morton and Garcia-del-Pino (2009) observed greater desiccation tolerance in *S. carpocapsae* and *S. feltiae* compared with *H. bacteriophora*.

In contrast to interspecies variation in desiccation tolerance, strain variation within species was not detected for the three EPN species investigated. Conceivably, we would have observed intraspecies variation under different conditions (e.g., more stressful) or if the selection of strains or species tested was expanded. For example, within species differences have been observed in *S. feltiae* (Solomon et al., 1999; Nimkingrat et al., 2013). Additionally, Shapiro-Ilan et al. (2003) observed variation in desiccation tolerance among *S. carpocapsae* strains when they were exposed to more extreme conditions than were implemented in this study. Nonetheless, based on this study as well as earlier literature, it appears clear that variation in desiccation tolerance across species is more pronounced than strain level differences, and that certain generalizations regarding relative tolerance can be made such as the basic hierarchy observed among the 10 species we tested.

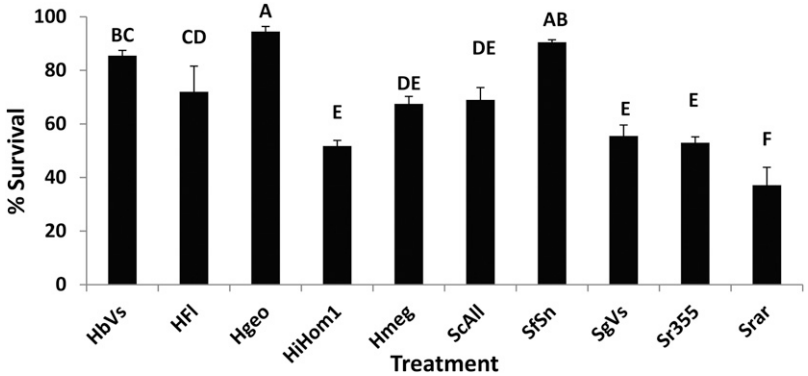

Fig. 5. Mean percentage survival (± SEM) of entomopathogenic nematodes in an interspecies comparison following exposure to freezing temperatures (24 h at -2°C). Nematodes included *Heterorhabditis bacteriophora* (VS), *H. floridensis* (Stacy), *H. georgiana* (Kesha), *H. megidis* (UK211), *Steinernema carpocapsae* (All), *S. feltiae* (SN), *S. glaseri* (VS), *S. riobrave* (355), and *S. rarum* (17 C&E). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

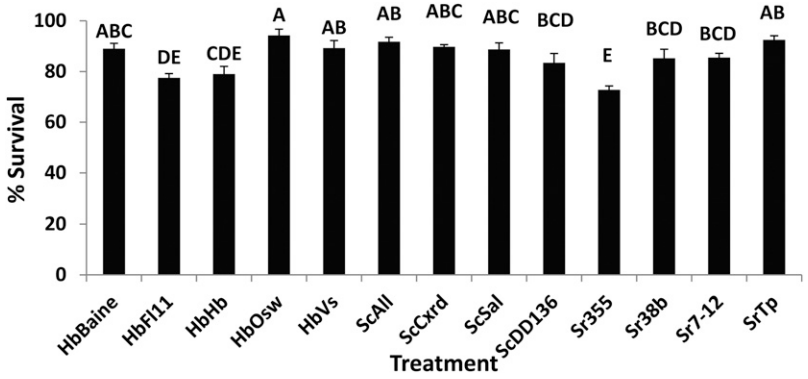

FIG. 6. Mean percentage survival (± SEM) of entomopathogenic nematodes in an intraspecies comparison following exposure to freezing temperatures (6 h at -2°C). Nematodes included five strains of *H. bacteriophora* (Baine, Fl1-1, Hb, Oswego, and VS) and four strains of *S. carpocapsae* (All, Cxrd, Sal, and DD136), and *S. riobrave* (355, 38b, 7-12, and TP). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

Survival of exposure to freezing temperatures varied both among and within species of EPNs. In terms of interspecies variation, some earlier studies reported trends similar to our findings suggesting that some generalizations can be made, e.g., Jagdale and Grewal (2003) observed lower freeze tolerance in *S. riobrave* relative to other steinernematids (*S. carpocapsae* and *S. feltiae*), and Brown and Gaugler (1996) reported greater freezing tolerance in *S. feltiae* relative to *H. bacteriophora*. These results generally reflect previous knowledge and reports in reference to temperature tolerance, e.g., *S. feltiae* is known to be relatively cold tolerant (such as in optimum infection and reproduction temperatures) (Grewal et al., 1994) and, therefore, it is not surprising that the nematode was also relatively freeze tolerant. However, our results indicate that despite some exceptions (e.g., that *S. carpocapsae* tends to be relatively freeze tolerant), predictions made at the species level could be difficult given the degree of strain variation observed.

Nematodes can survive subzero temperatures, the mechanism is determined by the individual nematode's environment, in addition to their physiology (Wharton and Brown, 1991). Different species use either a freeze-avoiding (Wharton and Surrey, 1994; Pickup 1990), freezing-tolerant strategy (Brown and Gaugler, 1996) or cryoprotective dehydration (Wharton et al., 2003). *Heterorhabditis zealandica* was shown to be freeze-avoiding (Wharton and Surrey, 1994). Several EPN species were found to be freezing tolerant, i.e., *Steinernema anomali* (Kozodoi), *S. feltiae*, and *Heterorhabditis bacteriophora* (Brown and Gaugler, 1996). Recently Ali and Wharton (2013) exposed *H. bacteriophora* and *S. feltiae* to gentle cooling regimes and discovered that both species can also survive by cryoprotective dehydration. In this study, we did not determine whether the nematodes used freeze tolerant or freeze avoiding strategies. In an effort to mimic occasional conditions in nature, we simply exposed nematodes to freezing temperatures for varying amounts of time; our results provide a broad comparison of relative ability among EPNs to withstand short periods of freezing in soil.

Enhanced environmental tolerance in EPN strains can be related to geographic origin because EPNs are expected to adapt to native conditions (Glazer, 2002; Stock et al., 2008). For example, The IS-6 strain of

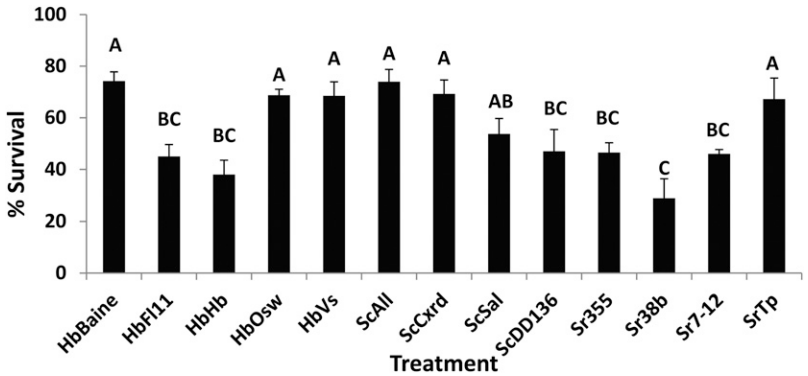

FIG. 7. Mean percentage survival (± SEM) of entomopathogenic nematodes in an intraspecies comparison following exposure to freezing temperatures (24 h at -2°C). Nematodes included five strains of *H. bacteriophora* (Baine, Fl1-1, Hb, Oswego, and VS) and four strains of *S. carpocapsae* (All, Cxrd, Sal, and DD136), and *S. riobrave* (355, 38b, 7-12, and TP). Different letters above bars indicate statistical differences (SNK test, $\alpha = 0.05$).

Table 1. Correlation coefficients (*P*-values) for desiccation and freeze tolerance in an interspecies comparison among entomopathogenic nematodes.

	Desiccation	Freeze (6 h)	Freeze (24 h)
Desiccation	1	-0.07317	-0.20061
		(-0.8408)	-0.5784
Freeze (6 h)	-0.07317	1	0.65654
	-0.8408		-0.0392
Freeze (24 h)	-0.20061	0.65654	1
	-0.5784	-0.0392	

The correlation between tolerance to freezing temperatures (-2°C for 6 or 24 h) and desiccating conditions (85% RH for 48 h) (*P*-values) was tested based on entomopathogenic nematode survival following exposure to these conditions; see text for details on nematode species. *P*-values are in parentheses below the correlation coefficients ($\alpha = 0.05$).

S. feltiae isolated from the Negev desert region of Israel exhibited superior desiccation tolerance relative to other strains (Solomon et al., 1999). In our study, some variation among EPNs might also be attributed to geographic origin. For example, the superior freeze tolerance observed in the Baine and Oswego strains of *H. bacteriophora* may be related to their original isolation from South Dakota and New York, respectively. On the other hand, results for other EPN strains do not support the hypothesis, e.g., *S. riobrave* (TP) isolated from the Rio Grande valley (Texas) and *H. georgiana* (Kesha) isolated in Georgia exhibited high levels of freeze tolerance despite the warm climates where they were isolated. Thus, variation in stress tolerance among EPNs cannot be explained solely by environmental conditions at the source of isolation. One caveat to this interpretation stems from the fact that all strains used in this study were cultured numerous times since their initial isolation. Repeated subculturing can lead to alteration of beneficial traits (such as environmental tolerance) (Shapiro et al., 1996; Bai et al., 2005). Therefore, conceivably (even though we attempted to minimize the number of culture passages) stress tolerance characteristics observed in some strains may not fully reflect the state of tolerance among the original populations.

Table 2. Correlation coefficients (*P*-values) for desiccation and freeze tolerance in an intraspecies comparison of entomopathogenic nematodes.

	Desiccation (24 h)	Desiccation (48 h)	Freeze (6 h)	Freeze (24 h)
Desiccation (24 h)	1	0.84328 (0.0003)	0.25069 (0.4087)	0.28099 (0.3524)
Desiccation (48 h)	0.84328 (0.0003)	1	0.00277 0.9928	0.08576 0.7806
Freeze (6 h)	0.25069 0.4087	0.00277 0.9928	1	0.74176 0.0037
Freeze (24 h)	0.28099 0.3524	0.08576 0.7806	0.74176 0.0037	1

The correlation between tolerance to freezing temperatures (-2 C for 6 or 24 h) and desiccating conditions (85% RH for 24 or 48 h) (*P*-values) was tested based on entomopathogenic nematode survival following exposure to these conditions; see text for details on nematode species. *P*-values are in parentheses below the correlation coefficients ($\alpha = 0.05$).

For success in biocontrol applications, knowing the distribution of beneficial traits (such as environmental tolerance) among EPN species and strains can be beneficial in selecting the optimum candidate for use in a particular geographic area or even microclimate. Thus, developing predictive power on beneficial traits based on laboratory data can be helpful in decision making. Furthermore, knowledge of relative beneficial trait qualities among EPNs can assist in developing strain improvement programs, e.g., through selective breeding and hybridization (Shapiro et al., 1997; Shapiro-Ilan et al., 2003, 2005; Mukuka et al., 2010). In this study, we did not detect any correlation between desiccation and freeze tolerance among EPNs; hence results obtained on newly discovered or untested species cannot be used to predict across these stress factors. Nonetheless, the extensive screening of variation among EPN strains and species reported in this study may aid in selecting the appropriate nematode when freezing or desiccation tolerance is required, and furthermore the data can be used as a baseline for additional comparisons.

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