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A Comparison of Entomopathogenic Nematode Longevity in Soil under Laboratory Conditions

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Abstract: We compared the longevity of 29 strains representing 11 entomopathogenic nematode species in soil over 42 to 56 d. A series of five laboratory experiments were conducted with six to eight nematode strains in each and one or more nematode strains in common, so that qualitative comparisons could be made across experiments. Nematodes included *Heterorhabditis bacteriophora* (four strains), *H. indica* (Hom1), *H. marelatus* (Point Reyes), *H. megidis* (UK211), *H. mexicana* (MX4), *Steinernema carpocapsae* (eight strains), *S. diaprepesi, S. feltiae* (SN), *S. glaseri* (NJ43), *S. rarum* (17C&E), and *S. riobrave* (nine strains). Substantial within-species variation in longevity was observed in *S. carpocapsae*, with the Sal strain exhibiting the greatest survival. The Sal strain was used as a standard in all inter-species comparisons. In contrast, little intra-species variation was observed in *S. riobrave*. Overall, we estimated *S. carpocapsae* (Sal) and *S. diaprepesi* to have the highest survival capability. A second level of longevity was observed in *H. bacteriophora* (Lewiston), *H. megidis, S. feltiae*, and *S. riobrave* (3–3 and 355). Lower levels of survivability were observed in other *H. bacteriophora* strains (Hb, HP88, and Oswego), as well as *S. glaseri* and *S. rarum*. Relative to *S. glaseri* and *S. rarum,* a lower tier of longevity was observed in *H. indica* and *H. marelatus,* and in *H. mexicana*, respectively. Although nematode persistence can vary under differing soil biotic and abiotic conditions, baseline data on longevity such as those reported herein may be helpful when choosing the best match for a particular target pest.

Key words: entomopathogenic, *Heterorhabditis,* longevity, nematode, persistence, soil, *Steinernema.*

Entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* are capable of controlling a wide variety of economically important insect pests (Klein, 1990; Grewal and Georgis, 1999; Shapiro-Ilan et al., 2002). In nature, entomopathogenic nematodes are obligate parasites of insects (Poinar, 1990; Adams and Nguyen, 2002). In order to reproduce, infective juvenile nematodes (IJ), the only free-living stage, generally must persist in soil until encountering a host, which they enter through natural openings (mouth, anus, and spiracles) or, in some cases, through the cuticle. After entering the host's hemocoel, the nematodes release their symbiotic bacteria, which are primarily responsible for killing the host (Dowds and Peters, 2002). The nematodes reproduce within the host cadaver and ultimately produce up to several hundred thousand IJ that emerge to search out new hosts (Shapiro et al., 1999a; Shapiro and Gaugler, 2002). The nematodes contribute to biological pest suppression through the actions of endemic populations (Campbell et al., 1998; Duncan et al., 2003) and through purposeful release using classical, inoculative, or inundative approaches (Parkman et al., 1993; Grewal and Georgis, 1999, Shapiro-Ilan et al., 2002). Due to sensitivity to UV light and desiccation, entomopathogenic nematodes are usually applied to protected environments, particularly soil (Kaya and Gaugler, 1993; Grewal and Georgis, 1999).

The efficacy of entomopathogenic nematodes in biological control depends on certain beneficial traits such as host-finding and virulence (Gaugler, 1987; Shapiro-Ilan et al., 2002). The nematode's ability to persist in soil can contribute to pest control efficacy (Kaya, 1990; Kaya and Gaugler, 1993; Smits, 1996). Extended persistence in soil can result in greater cumulative insect mortality and a reduced need for multiple nematode applications. Some factors that affect nematode survival in soil include environmental conditions, such as moisture and temperature (Molyneux and Bedding, 1984; Molyneux, 1985; Kung et al., 1991), antagonists (Kaya, 2002), soil type (Kung et al., 1990; Shapiro et al., 2000), cultural conditions (Shapiro et al., 1996, 1999b; Millar and Barbercheck, 2002), and nematode species or strain (Molyneux, 1985; Ferguson et al., 1995; Grewal et al., 2002b).

Laboratory assays have characterized diverse nematode species and strain differences for a number of traits, such as virulence to particular host species or among host stages (Morris and Converse, 1991; Shapiro and McCoy, 2000; Grewal et al., 2002a; Koppenhöfer and Fuzy, 2004), temperature tolerance (Grewal et al., 1994), and desiccation tolerance (Patel et al., 1997; Liu and Glazer, 2000). These studies, though conducted under controlled conditions, provide baseline data that can be useful in choosing which entomopathogenic nematode might be the best match for a particular target pest. Similar experiments addressing variation in overall nematode persistence or longevity may be considered lacking in that they have generally been limited to comparisons of only one or a few species (Molyneux, 1985; Kung et al., 1990, 1991; Hass et al., 1999; Shapiro et al., 2000; Hass et al., 2001, 2002; Grewal et al., 2002b;). Our objective was to conduct a broader comparison of intra- and inter-specific variation in entomopathogenic nematode longevity in soil.

Materials and Methods

All nematodes were cultured in the last instar of the greater wax moth *Galleria mellonella* according to proce-

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dures described by Kaya and Stock (1997). Nematodes were stored at 13 °C for up to 3 wk prior to use in experiments. All experiments were conducted at 25 °C and repeated once in entirety.

A series of five experiments was conducted to compare intra- and inter-specific variation in entomopathogenic nematode longevity. The nematode strains and species used in each experiment are listed in Table 1. Experiments I and II focused on intra-species variation in *S. carpocapsae* and *S. riobrave*, respectively. Experiments III to V focused primarily on inter-species variation, except four different strains of *H. bacteriophora* were tested and an additional *S. riobrave* strain was added. To facilitate qualitative comparisons of inter-species variation across experiments III to V, several nematode strains were included in more than one experiment. The Sal strain of *S. carpocapsae*, which exhibited the highest overall survival in experiment I, was included in all three inter-species comparisons. Additionally, three other strains that exhibited relatively high survival, i.e., *S. riobrave* strain 3–3 and 355 and the SN strain of *S. feltiae*, were each included in two of the inter-species comparison experiments.

Nematode survival in soil was measured by dilution and direct counting (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999; Perez et al., 2003). Experimental units consisted of 30-ml plastic cups (3 to 4-cm-diam., 3.5-cm-deep), each containing 5 g of loamy sand soil (84% sand, 10% silt, 6% clay, pH 6.1, and organic matter = 2.8%). Nematodes were applied in 0.5 ml tap water, and the final moisture level in each cup was brought to field capacity (14%). One d after inocula-

TABLE 1. Entomopathogenic nematode strains used in longevity experiments I to V and their extraction efficiencies in soil.

	Species	Strain	Source	Extraction efficiency[a]
I	*Steinernema carpocapsae* (Weiser)	Agriotos	Nguyen[b]	74.8 ± 6.3 AB
	S. carpocapsae	All	Nguyen[b]	95.0 ± 5.3 A
	S. carpocapsae	Breton	Nguyen[b]	93.5 ± 4.8 A
	S. carpocapsae	DD136	Nguyen[b]	82.8 ± 4.1 AB
	S. carpocapsae	Italian	Nguyen[b]	86.3 ± 5.4 A
	S. carpocapsae	Kapow	Nguyen[b]	64.1 ± 8.3 B
	S. carpocapsae	Mexican	Nguyen[b]	92.0 ± 6.5 A
	S. carpocapsae	Sal	Nguyen[b]	68.6 ± 6.0 AB
II	*S. riobrave* (Cabanillas, Poinar & Raulston)	3–2	S-I[c]	77.0 ± 6.8 A
	S. riobrave	3–3	S-I[c]	78.1 ± 1.9 A
	S. riobrave	3–8a	S-I[c]	84.1 ± 4.8 A
	S. riobrave	7–12	S-I[c]	71.1 ± 4.1 A
	S. riobrave	8–14	S-I[c]	84.8 ± 5.3 A
	S. riobrave	9–5	S-I[c]	73.7 ± 5.0 A
	S. riobrave	NF	S-I[c]	74.4 ± 6.7 A
	S. riobrave	TP	S-I[c]	78.7 ± 5.7 A
III	*Heterorhabditis bacteriophora* (Poinar)	Hb	VPI + SU[d]	80.6 ± 3.7 A
	H. bacteriophora	HP88	MicroBio, Ltd.[e]	81.5 ± 4.4 A
	H. bacteriophora	Lewiston	IBS[f]	81.6 ± 7.7 A
	H. bacteriophora	Oswego	Shields-Villani[g]	78.7 ± 10.1 A
	H. megidis (Poinar, Jackson & Klein)	UK211	MicroBio, Ltd.[e]	93.5 ± 7.1 A
	S. carpocapsae	Sal	Nguyen[b]	88.3 ± 6.3 A
	S. riobrave	3–3	Si-I[c]	89.8 ± 9.0 A
IV	*H. mexicana* (Nguyen, Shapiro-Ilan, Stuart, McCoy, James & Adams)	MX4	S-I[c]	59.5 ± 4.1 B
	S. carpocapsae	Sal	Nguyen[b]	62.5 ± 4.3 B
	S. diaprepesi (Nguyen and Duncan)		Duncan[h]	64.5 ± 2.3 B
	S. feltiae (Filipjev)	SN	Nguyen[b]	69.1 ± 2.7 B
	S. riobrave	355	TT[i]	86.7 ± 10.9 A
	S. rarum (Doucet)	17C&E	S-I[c]	57.2 ± 2.9 B
V	*H. indica* (Poinar, Maranukar & David)	Hom1	IBS[f]	83.6 ± 3.9 A
	H. marelatus (Liu & Berry)	Point Reyes	Stock[j]	74.2 ± 5.7 A
	S. carpocapsae	Sal	Nguyen[b]	96.4 ± 5.3 A
	S. feltiae	SN	Nguyen[b]	96.3 ± 3.0 A
	S. glaseri (Steiner)	NJ43	Nguyen[b]	74.2 ± 12.1 A
	S. riobrave	3–3	SiI[c]	71.3 ± 7.3 A
	S. riobrave	355	TT[i]	78.3 ± 2.8 A

[a] Percentage extraction efficiencies (± SE) from soil. Different letters following numerals indicate statistical differences (SNK test, $P \leq 0.05$) within experiments.
[b] K. Nguyen, University of Florida, Gainesville.
[c] Shapiro-Ilan culture collection.
[d] VPI + SU, Blacksburg, VA.
[e] MicroBio, Ltd., Cambridge, UK.
[f] Integrated BioControl Systems, Lawrenceburg, IN.
[g] E. Shields & M. Villani, Cornell University, Ithaca, NY.
[h] L. Duncan, University of Florida, CREC, Lake Alfred.
[i] Thermo Trilogy, Columbia, MD.
[j] P. Stock, University of Arizona.

tion, the initial number of extractable live nematodes was determined by rinsing the entire contents from a cup into a 1-liter beaker and adding water to a final volume of 304 ml. The suspension was stirred on a stir-plate with a 5-cm stir bar at constant speed. One ml of suspension was removed and placed on a 60-mm-diam. petri dish lid that was marked with lines 1 cm apart to facilitate counting. Additional water was added to a depth of approximately 0.5 cm, and the number of live IJ was determined using a dissecting microscope (nematodes were considered live if they were moving naturally or if they responded to probing with a needle). To avoid basing survival estimates on small sample sizes, a minimum count of 30 IJ per sample was considered acceptable. If fewer than 30 IJ were observed from a single 1-ml sample, additional samples from the beaker were added to the petri dish. Based on the dilution factor, the total number of live nematodes was then estimated for the experimental unit. The process to determine number of surviving nematodes was repeated 7, 14, 28, 42, and 56 d post-inoculation (except experiment II ended on d 42 and experiment IV had the last sample date on d 53 rather than 56). For the first two experiments, 10,000 IJ were initially added to each cup, but toward the end of these experiments numerous 1-ml samples were required to achieve a count of 30 IJ. Therefore, the number of IJ initially added to each cup increased to 15,000 for experiments III and IV, and 20,000 for experiment V. Extraction efficiency for each nematode in each experiment was calculated based on the percentage of IJ extracted 1 d post-inoculation relative to the number applied. In each trial, there were five replicate cups for each treatment (10 replicates total).

Nematode longevity over time was estimated based on the total number of live nematodes remaining in the cups. The percentage survival for each nematode was determined by dividing the number of live nematodes detected on a particular sample date by the number initially extracted 1 d post-inoculation (similar to Hass et al., 2002). Even when applying the same technique, soil extraction efficiency can vary for different nematode species or strains (Curran and Heng, 1992) (Table 1). Therefore, our approach of relating longevity to the number of nematodes initially extractable (1 d post-inoculation) was designed to minimize differences in extraction efficiency. Further, basing longevity on the number of nematodes extractable 1 d post-inoculation emphasizes longevity once the nematodes are established in the soil and eliminates mortality that might have occurred during application.

Analysis of variance (ANOVA) was used to detect treatment differences in nematode longevity for each sample date (starting 7 d post-application), as well as over the whole experimental period (i.e., for the 42-, 53-, or 56-d period) (Shapiro et al. 2000) (SAS Software, version 9.1, 2001, SAS Institute, Cary, NC). In the analyses covering the entire experimental period, a conservative error term (replicate*treatment) was used to test for treatment effects (Cochran and Cox, 1957). ANOVA was also used to compare extraction efficiencies among treatments. When ANOVA detected statistical significance ($P \leq 0.05$), treatment differences were further elucidated with the SNK test. In all analyses, percentage survival or extraction efficiency was arcsine transformed (arcsine of square root) prior to analysis (Southwood, 1978); untransformed means are presented in figures.

Results

Differences in nematode extraction efficiencies were detected among nematode strains and species (Table 1). Specifically, differences in extraction efficiencies were detected in experiments I ($F = 3.68$, df = 7,72, $P = 0.002$), IV ($F = 3.94$, df = 5,54, $P = 0.004$), and V ($F = 2.64$, df = 6,63, $P = 0.024$). Note that the F test was significant in experiment V, but the SNK test did not differentiate among treatments (Table 1). No differ-

Table 2. Statistics generated from entomopathogenic nematode experiments.

Experiment	Day[a]	*F*	df	*P*
I	7	8.86	7, 72	<0.0001
	14	12.95	7, 72	<0.0001
	21	6.40	7, 72	<0.0001
	28	14.11	7, 72	<0.0001
	42	15.43	7, 72	<0.0001
	56	18.91	7, 71	<0.0001
	7–56[a]	193.79	7, 28	<0.0001
II	7	2.57	7, 71	0.0202
	14	3037	7, 72	0.0036
	21	1.85	7, 72	0.0903
	28	1.31	7, 71	0.2569
	42	2.39	7, 71	0.0300
	7–42[a]	2.61	7, 28	0.0329
III	7	3.92	6, 63	0.0022
	14	6.81	6, 63	<0.0001
	21	5.03	6, 63	0.0003
	28	7.51	6, 61	<0.0001
	42	8.71	6, 63	<0.0001
	56	4.24	6, 62	0.0012
	7–56[a]	25.63	6, 24	<0.0001
IV	7	1.48	5, 54	0.2124
	14	5.25	5, 54	0.0005
	21	4.67	5, 54	0.0013
	28	5.20	5, 54	0.0006
	42	12.59	5, 53	<0.0001
	53	7.60	5, 53	<0.0001
	7–53[a]	94.80	5, 20	<0.0001
V	7	4.08	6, 63	0.0016
	14	9.56	6, 63	<0.0001
	21	15.22	6, 63	<0.0001
	28	8.80	6, 63	<0.0001
	42	12.55	6, 63	<0.0001
	56	16.20	6, 58	<0.0001
	7–56[a]	67.96	6, 24	<0.0001

[a] Days after nematode inoculation; the last row in each experiment (e.g., 7–56) represents an analysis averaging longevity over the entire experimental period. See Table 1 for a list of nematodes that were included in each experiment.

ences in extraction efficiency were detected among treatments in experiments II ($F = 0.88$, df = 7,72, $P = 0.527$) and III ($F = 0.60$, df = 6,63, $P = 0.728$). Extraction efficiencies throughout the study ranged from 57% to 96% (Table 1).

Intra- and inter-specific differences in longevity were detected in all five experiments (Figs. 1–6) (Table 2). In experiment I, which focused on intra-specific variation among *S. carpocapsae* strains, Agriotos and Sal strains tended to exhibit the highest survival on sample dates 7 to 28, and Sal strain had the highest survival in the latter part of the experiment (i.e., on days 42 and 56) (Fig. 1). When longevity was analyzed over the entire experimental period, survival was greatest in the Sal strain followed by Agriotos, and among the lowest in Kapow, Breton, and All strains (Fig. 6A).

Little intra-specific variation in longevity was observed among *S. riobrave* strains compared with variation among *S. carpocapsae* strains (compare Figs. 2,6B to Figs. 1,6A). On sample dates 7, 14, and 42, strains 9–5, 3–3, and 3–8a exhibited greater survival than four, five, and one other strain, respectively (Fig. 2). No other differences were detected on individual sample dates. Additionally, no differences were detected among strains when longevity was analyzed over the entire experimental period (Fig. 6B).

In experiment III, *S. carpocapsae* Sal strain exhibited the greatest longevity relative to other nematodes; the Sal strain's survival was greater than *S. riobrave* (3–3), *H. megidis*, and the four *H. bacteriophora* strains tested at 14 d post-inoculation, and when compared over the whole experimental period (Figs. 3,6C). *Steinernema riobrave* 3–3 had greater survival than all heterorhabditids on day 28 and all heterorhabditids except *H. megidis* on day 21. When analyzed over the entire period, survival of the 3–3 strain was not different from *H. megidis* or

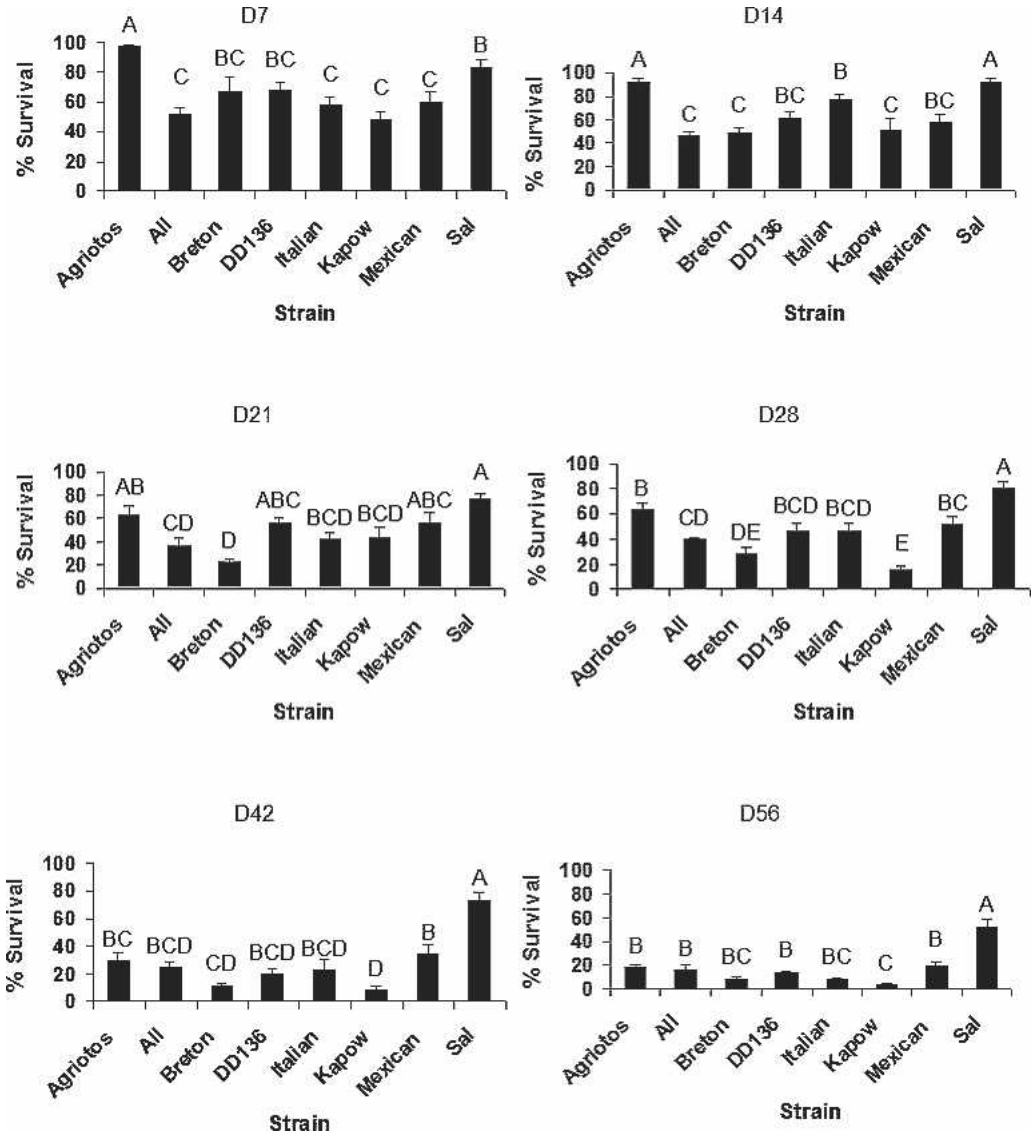

FIG. 1. Average longevity of *Steinernema carpocapsae* strains in soil 7, 14, 21, 28, 42, and 56 d after inoculation (D7-D56). Different letters above bars within each day of evaluation indicate statistical differences (SNK test, $P \leq 0.05$).

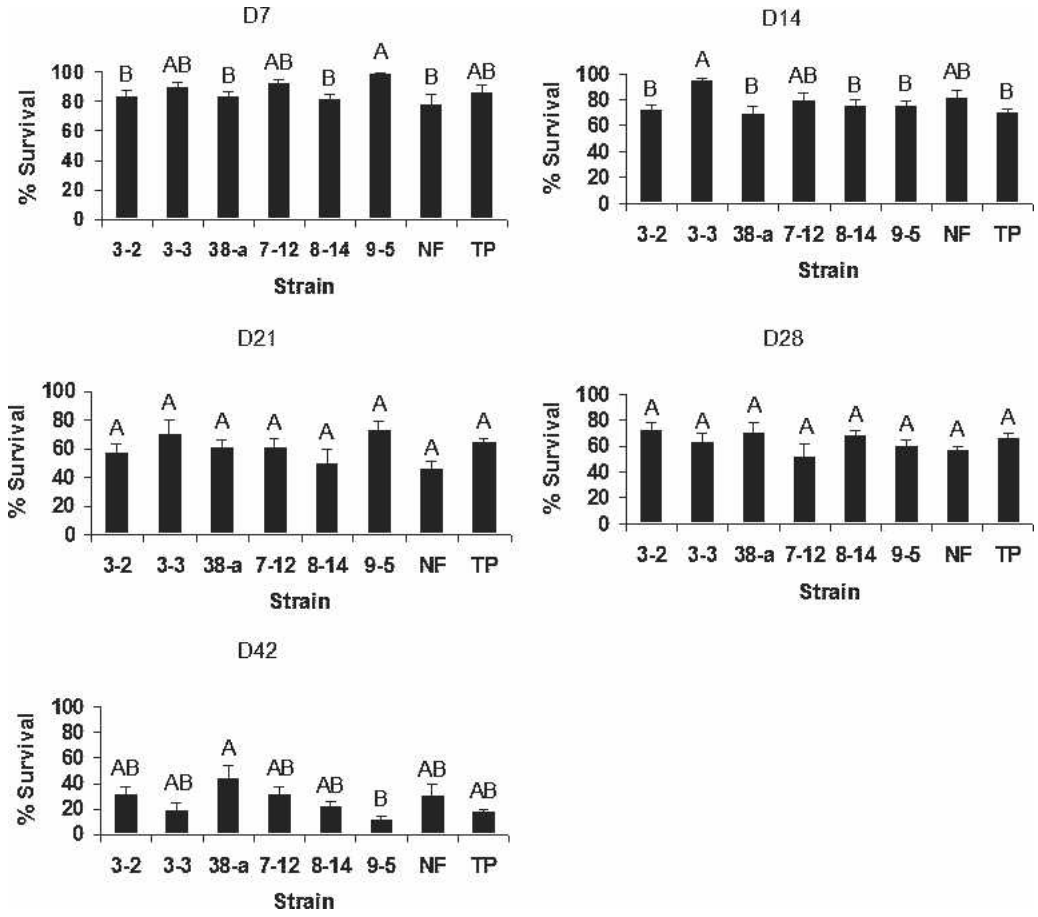

FIG. 2. Average longevity of *Steinernema riobrave* strains in soil 7, 14, 21, 28, and 42, d after inoculation (D7–D42). Different letters above bars within each day of evaluation indicate statistical differences (SNK test, $P \leq 0.05$).

H. bacteriophora (Lewiston); *H. megidis* exhibited greater survival than all of the *H. bacteriophora* strains except Lewiston (Fig. 6C).

In Experiment IV, inter-species comparisons revealed no significant differences 7 d post-inoculation, yet differences were detected subsequently (Fig. 4). On day 14 and thereafter, *S. carpocapsae* (Sal) remained in the highest-ranked group for survival throughout the experimental period. *Steinernema diaprepesi* survival was lower than *S. carpocapsae* (Sal) on day 21, but exhibited similar longevity thereafter. Over the whole experimental period, survival of *S. carpocapsae* (Sal) and *S. diaprepesi* was the highest, followed by *S. feltiae*, which was greater than *S. rarum* but not different from *S. riobrave* (355) (Fig. 6D). Survival of *H. mexicana* was the lowest among the nematodes tested in experiment IV 14 d post-inoculation and thereafter as well as when averaged over the whole experimental period (Figs. 4,6D).

In experiment V, the two heterorhabditids, *H. marelatus* and *H. indica*, exhibited lower survival than all steinernematids within the first 4 wk except *S. glaseri*, and survival of these heterorhabditids was lower than all steinernematids in the latter part of the experiment (Fig. 5). Similarly, over the entire experimental period *H. marelatus* and *H. indica* had the lowest survival, followed by *S. glaseri* (Fig. 6E). Also, when averaged over the experimental periods, no difference in survival was detected among *S. carpocapsae* Sal, *S. feltiae* (SN), or *S. riobrave* (strains 3–3 and 355) (Fig. 6E).

As an illustrative summary of the inter-species comparisons, a schematic diagram was generated based on experiments III to V (Fig. 7). The relative rankings in the diagram were derived from nematode survival when averaged over the duration of the experiments, i.e., the entire 53 or 56 d (Fig. 6C–E). In general, to construct the diagram nematode species or strains estimated to have greater survival abilities were placed above those estimated to have lower survival. Specifically, if a nematode strain was found to have greater survival than another strain in at least one experiment, then that relationship of superior survival was represented in the diagram by a solid vertical line. Strains on the same level connected through horizontal lines were estimated to have approximately equivalent survival based on direct comparison or by inference from comparisons made among strains common to more than one experiment. For example, *H. megidis* and *S. feltiae* were not directly compared but were placed at the same level because (in separate experiments) their survival capabilities were both similar to *S. riobrave* (3–3). In direct comparisons

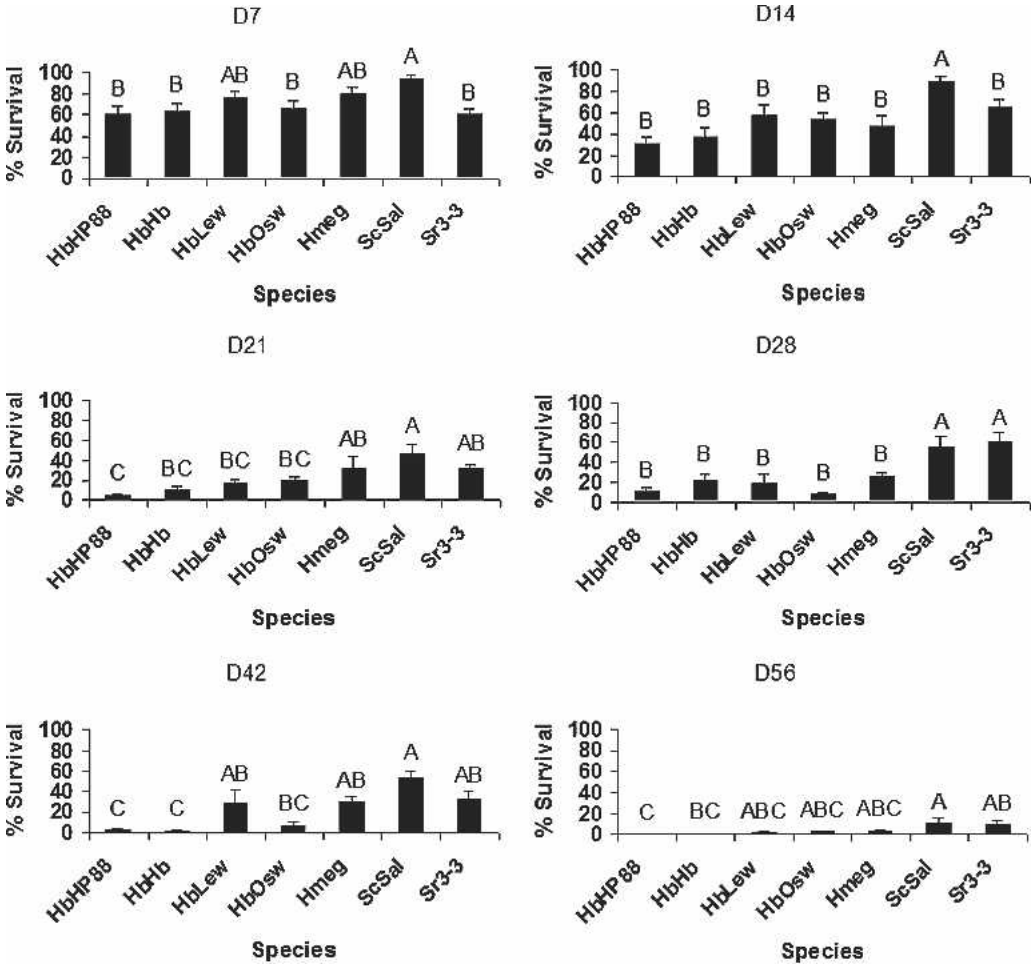

FIG 3. Average longevity of entomopathogenic nematodes in soil 7, 14, 21, 28, 42, and 56 d after inoculation (D7–D56). Hb = *Heterorhabditis bacteriophora* (HP88, Hb, Lewiston and Oswego strains), Hmeg = *H. megidis* (UK211), ScSal = *Steinernema carpocapsae* (Sal), Sr3–3 = *S. riobrave* (3–3). Different letters above bars within each day of evaluation indicate statistical differences (SNK test, $P \leq 0.05$).

where intermediate survival relationships were indicated, dotted lines were used to show where a lack of significance occurred. For example, survival of *H. megidis* was greater than *H. bacteriophora* (Oswego) but similar to the Lewiston strain, and Lewiston was not found to be different from the Oswego strain (Fig. 6C); thus, dotted lines were used between *H. bacteriophora* Oswego and Lewiston strains (Fig. 7). Nematode strains not connected through one or more series of vertical or horizontal lines were not considered to be subject to comparison, e.g., relative survival between *S. glaseri* vs. *S. rarum* or *H. indica* vs. *H. mexicana* was not subject to ranking (even qualitatively).

Based on the ranking approach described above, a summary of experiments III to V indicates the highest survival capability in *S. carpocapsae* (Sal) strain and *S. diaprepesi* (Fig. 7). A second level of survivability was observed in *H. bacteriophora* (Lewiston), *H. megidis, S. feltiae*, and *S. riobrave* (3–3 and 355 strains). Lower levels of survivability were observed in *H. bacteriophora* (Hb, HP88, and Oswego strains), *S. glaseri* and *S. rarum*. Relative to *S. glaseri* and *S. rarum*, a lower tier of survival was observed in *H. indica* and *H. marelatus,* and *H. mexicana*, respectively (Fig. 7).

DISCUSSION

In general, treatment effects of inundatively applied entomopathogenic nematodes have been observed to persist for a relatively short term, e.g., over 2 to 6 wk (Duncan et al., 1996; Smits, 1996, McCoy et al., 2000). In some instances, however, longer-term persistence and insect control have been reported to occur over several seasons or years (Klein and Georgis, 1992; Parkman et al., 1993; Shields et al., 1999). Endemic nematode populations also can contribute to long-term suppression of insect populations (Duncan et al., 2003). Long-term persistence of entomopathogenic nematodes depends to a large extent on their ability to find and reproduce in suitable hosts (Kaya, 1990; Smits, 1996), yet the probability of recycling and potential for prolonged insect control depends on how long the nematodes can survive without a host. We observed substantial difference among various nematode species

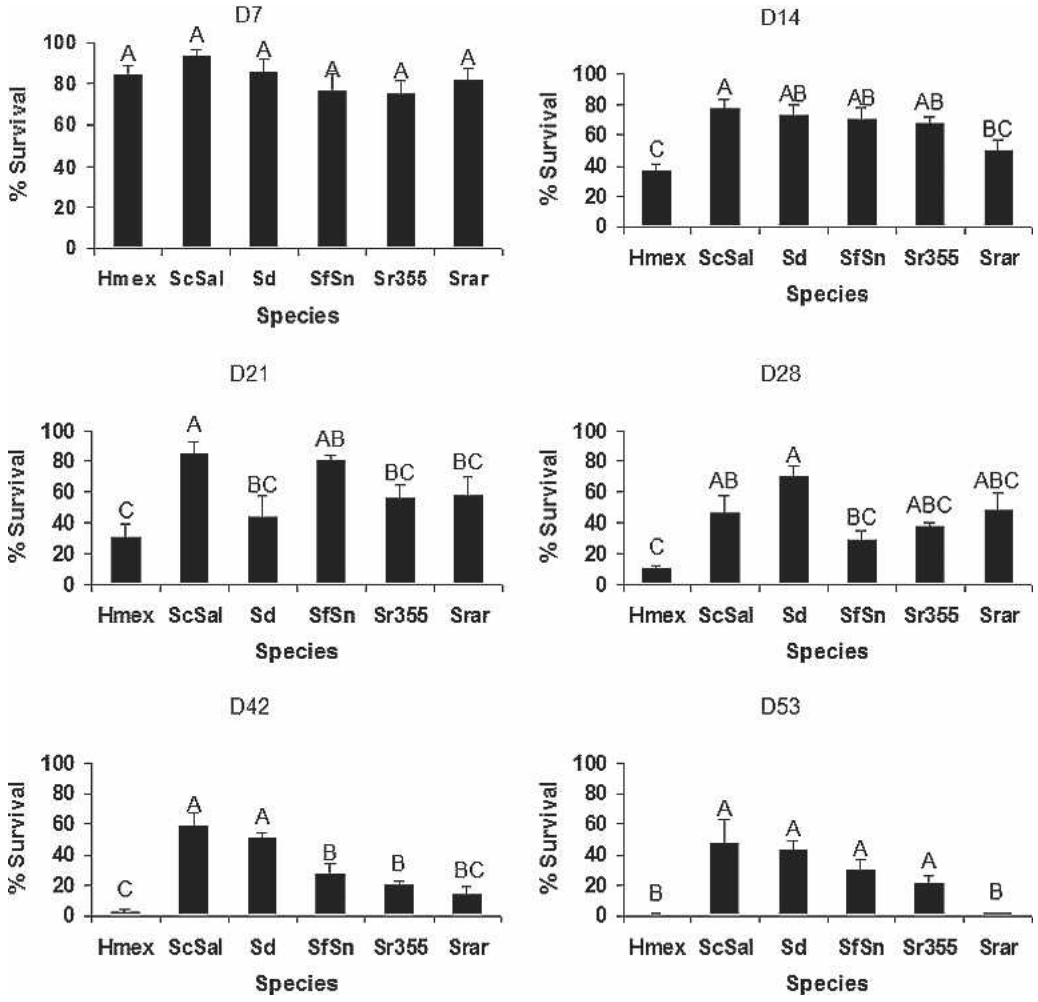

FIG. 4. Average longevity of entomopathogenic nematodes in soil 7, 14, 21, 28, 42, and 53 d after inoculation (D7-D53). Hmex = *Heterorhabditis mexicana* (MX4 strain), ScSal = *S. carpocapsae* (Sal), Sd = *Steinernema diaprepesi*, SfSn = *S. feltiae* (SN), Sr355 = *S. riobrave* (355), Srar = *S. rarum* (17C&E). Different letters above bars within each day of evaluation indicate statistical differences (SNK test, $P \leq 0.05$).

and strains in their innate abilities to survive in soil. Some nematodes, such as *S. diaprepesi* and *S. carpocapsae* (Sal), displayed superior potential for persistence, whereas others, such as *H. mexicana* and *H. indica*, exhibited relatively poor longevity. Laboratory comparisons such as those reported herein may be helpful when considering which nematode strain or species to use in field applications.

Our findings on relative entomopathogenic nematode longevity are consistent with some previous laboratory studies but not with others. For example, our findings of superior survival of *S. carpocapsae* relative to *S. glaseri* are similar to those of Kung et al. (1990). Also consistent with our study, Molyneux (1985) reported greater longevity in *S. carpocapsae* and *S. glaseri* relative to two heterorhabditids but, unlike our study and that of Kung et al. (1990), survival of *S. glaseri* in Molyneux's study was greater than that of *S. carpocapsae*. One possible cause for these discrepancies is strain variation. We observed considerable variation in longevity among *S. carpocapsae* strains. Similarly, Grewal et al. (2002b) reported significant variation in longevity among 15 populations of *H. bacteriophora*. However, we observed little difference among *S. riobrave* strains, indicating that within-species variation in longevity is greater for some nematodes than others. Possibly, the different degrees of intra-species variation observed is due to the substantially wider geographical range in which the *S. carpocapsae* strains originated compared with the relatively narrow range of the *S. riobrave* strains (Poinar, 1990; Stuart et al., 2004).

Our rankings of nematode longevity in the laboratory are consistent with observations on nematode persistence in certain field studies. For example, our observations of superior longevity in *S. diaprepesi* are consistent with prior evidence of this nematode's persistence capabilities under field conditions, e.g., relative to *S. riobrave* (Duncan et al., 2003). Also analogous to our study, persistence of *H. bacteriophora* under field conditions was reported to be lower than that of *S. riobrave* and *S. carpocapsae* (Duncan et al., 1996). Jansson et al. (1993), however, reported greater field per-

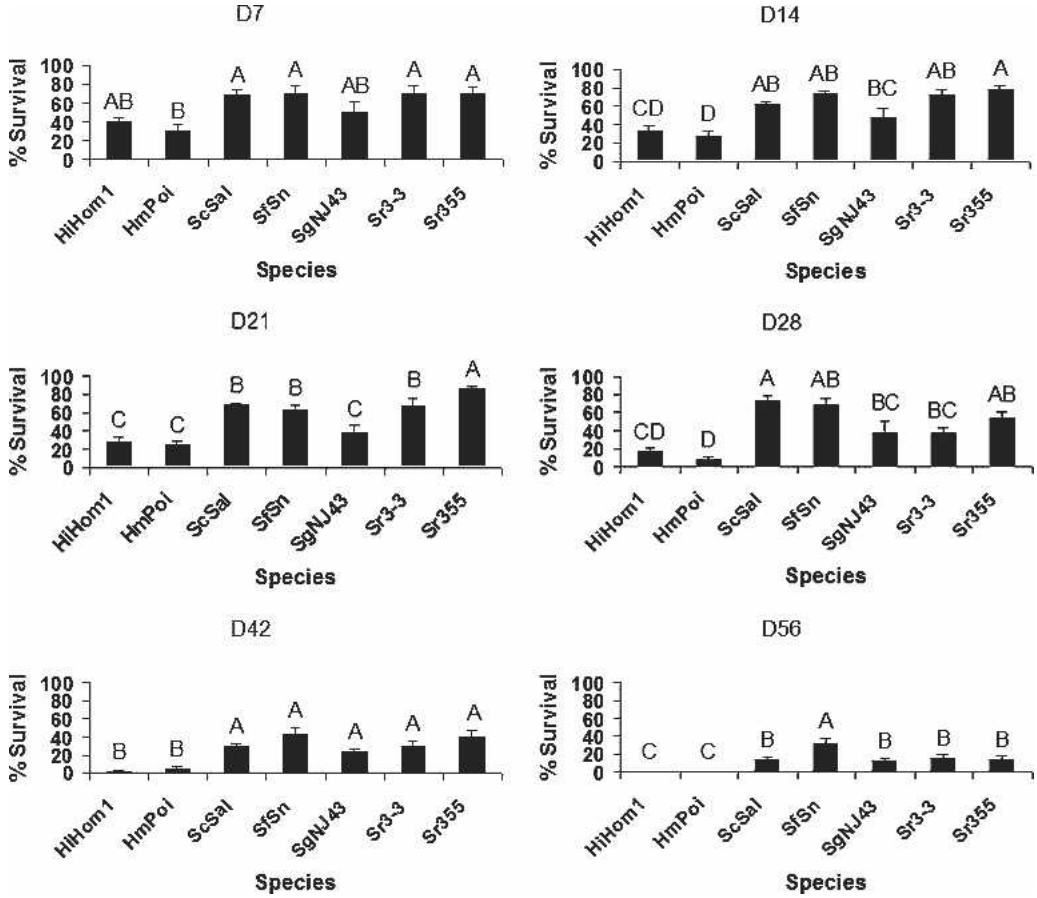

FIG. 5. Average longevity of entomopathogenic nematodes in soil 7, 14, 21, 28, 42, and 56 d after inoculation (D7-D56). HiHom1 = *Heterorhabditis indica* (Hom1 strain), Hmpoi = *H. marelatus* (Point Reyes), ScSal = *S. carpocapsae* (Sal), SfSn = *S. feltiae* (SN), SgNJ43 = *S. glaseri* (NJ43), Sr = *S. riobrave* (3–3 and 355 strains). Different letters above bars within each day of evaluation indicate statistical differences (SNK test, $P \leq 0.05$).

sistence with *S. feltiae* than with *S. carpocapsae* strains (a trend we did not observe). Additionally, we observed relatively poor longevity in *H. bacteriophora* (Oswego), yet this strain was reported to exhibit substantial persistence and infectivity for 700 d or more in the field (Ferguson et al., 1995; Shields et al., 1999). Thus, the Oswego strain's persistence under field conditions was likely due to its ability to efficiently find and reproduce in insect hosts rather than its innate longevity. Indeed, a variety of factors can contribute to variations in nematode longevity and persistence under field conditions (Kaya, 1990; Smits, 1996; Kaya, 2002).

A physiological basis for differences in longevity observed in this study is not clear. Grewal et al. (2002b) observed a strong positive correlation between *H. bacteriophora* longevity in sterile water at 25 °C and tolerance to heat, UV, and hypoxia, indicating a possible genetic link between these stress factors and longevity. Lipid content or energy reserves have been related to entomopathogenic nematode longevity (Selvan et al., 1993; Abu Hatab and Gaugler, 2001). In that case, one might have expected the longevity of *S. glaseri* to have been relatively higher in our study due to its greater energy content (Selvan et al., 1993). On the other hand, Hass et al. (2002) also reported a correlation between entomopathogenic nematode persistence and energy reserves but concluded that the effect was not directly responsible for reductions in nematode survival. The authors suggested differences in longevity were more likely attributable to other factors, such as activity of antagonists in nonsterile soil (as was used in this study as well).

Our laboratory comparison of entomopathogenic nematode longevity included 29 strains representing 11 species. At least eight of the species tested are or have been commercially available (*H. bacteriophora*, *H. indica*, *H. megidis*, *H. marelatus*, *S. carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. riobrave*). Although a number of species and numerous strains were not included (Poinar, 1990; Adams and Nguyen, 2002), our study was the broadest comparison to date. Due to limitations in space and labor requirements, it was not feasible to compare all nematodes simultaneously. However, incorporation of strains that were common among the experiments (which were conducted under essentially identical conditions) allows for substantially more valid qualitative comparisons than when comparing across studies conducted under varying conditions. It is likely that the relative

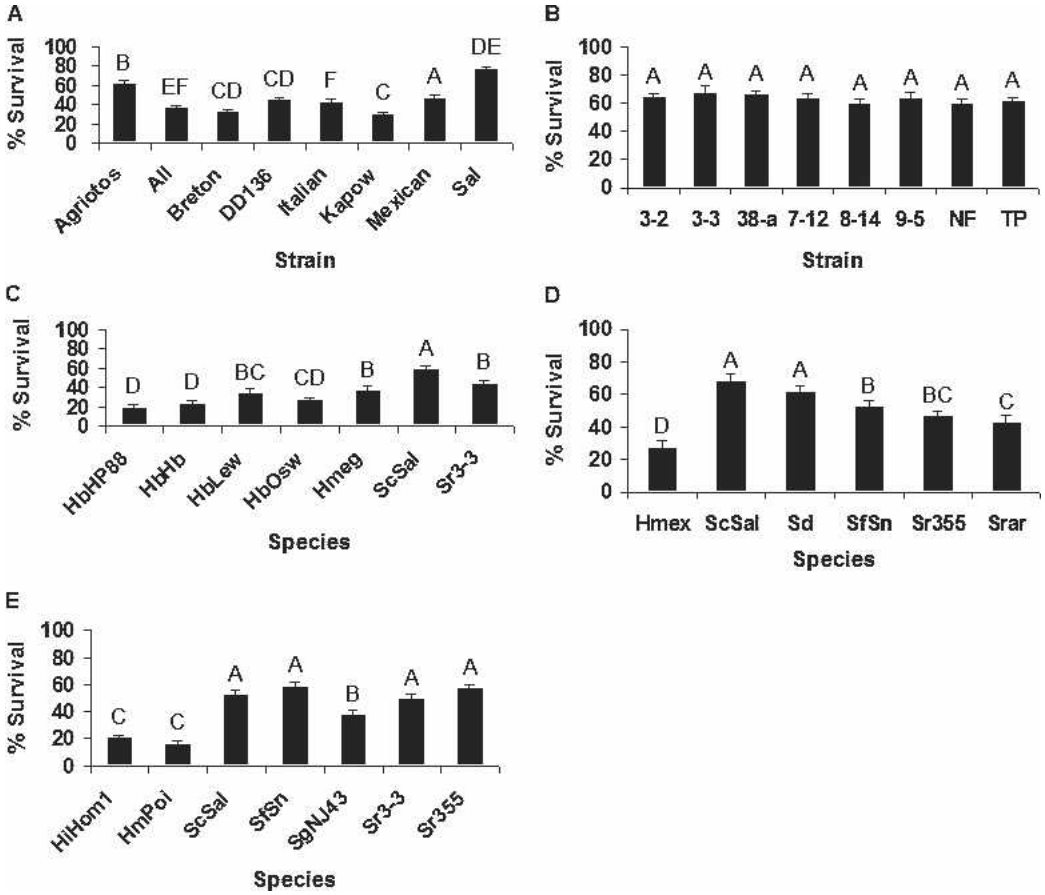

FIG. 6. Longevity of entomopathogenic nematodes in soil averaged over a 42- to 56-d experimental period. Each graph represents a separate experiment. A) *Steinernema carpocapsae* strains. B) *S. riobrave* strains. C-E) Various strains and species: Hb = *Heterorhabditis bacteriophora* (HP88, Hb, Lewiston and Oswego strains), Hmeg = *H. megidis* (UK211), ScSal = *S. carpocapsae* (Sal), Sr = *S. riobrave* (3–3 and 355 strains), Hmex = *H. mexicana* (MX4 strain), Sd = *S. diaprepesi*, SfSn = *S. feltiae* (SN), Srar = *S. rarum* (17C&E), HiHom1 = *H. indica* (Hom1 strain), Hmpoi = *H. marelatus* (Point Reyes), SgNJ43 = *S. glaseri* (NJ43). Different letters above bars within each experiment indicate statistical differences (SNK test, $P \leq 0.05$).

longevities of nematodes we tested will differ to some (or a large) extent when tested under varying temperatures, soil types, etc., or under the complexities of diverse field conditions. Additionally, it must be noted that a nematode's ability to survive for a certain period does not necessarily mean it will remain infective during that time; our focus was on longevity, not persistence of infective abilities. Clearly, further research is needed to address entomopathogenic nematode longevity and long-term efficacy in various environments.

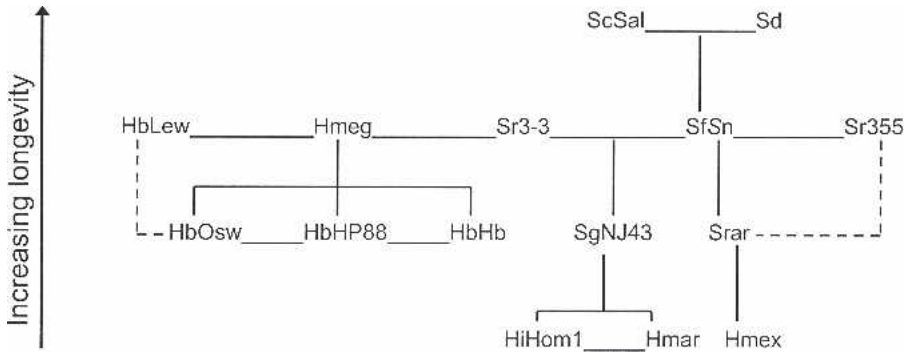

FIG. 7. A schematic summarizing a series of laboratory experiments comparing survival among entomopathogenic nematodes in soil. The illustrative summary is based on average survival over a 53- or 56-d period (see Fig. 6C-E). In general, nematode species or strains higher up on the diagram (indicated by solid vertical lines) exhibited greater survival than those below. Strains on the same level connected by horizontal lines had approximately equivalent survival (based on direct comparison or inference from comparisons made among strains common to more than one experiment). Dotted lines between two strains indicate that a statistical difference was not detected.

Nonetheless, our study provides an initial assessment and baseline data from which expanded studies can be made.

Literature Cited

Abu Hatab, M., and Gaugler, R. 2001. Diet composition and lipids of in vitro-produced *Heterorhabditis bacteriophora*. Biological Control 20:1–7.

Adams, B. J., and Nguyen, K. B. 2002. Taxonomy and systematics. Pp. 1–34 *in* R. Gaugler, ed. Entomopathogenic nematology. New York: CABI.

Campbell, J. F., Orza, G., Yoder, F., Lewis, E., and Gaugler, R. 1998. Spatial and temporal distribution of endemic and released entomopathogenic nematode populations in turfgrass. Entomologia Experimentalis et Applicata 86:1–11.

Cochran, W. G., and Cox, G. M. 1957. Experimental designs. New York: John Wiley & Sons.

Curran, J., and Heng, J. 1992. Comparison of three methods for estimating the number of entomopathogenic nematodes present in soil samples. Journal of Nematology 24:170–176.

Dowds, B. C. A., and Peters, A. 2002. Virulence mechanisms. Pp. 79–98 *in* R. Gaugler, ed. Entomopathogenic nematology. New York: CABI.

Duncan, L. W., Graham, J. H., Dunn, D. C., Zellers, J., McCoy, C. W., and Nguyen, K. 2003. Incidence of endemic entomopathogenic nematodes following application of *Steinernema riobrave* for control of *Diaprepes abbreviatus*. Journal of Nematology 35:178–186.

Duncan, L. W., McCoy, C. W., and Terranova, A. C., 1996. Estimating sample size and persistence of entomogenous nematodes in sandy soils and their efficacy against the larvae of *Diaprepes abbreviatus* in Florida. Journal of Nematology 28:56–67.

Ferguson, C. S., Schroeder, P. C., and Shields, E. J. 1995. Vertical distribution, persistence, and activity of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) in alfalfa snout beetle (Coleoptera: Curculionidae) infested fields. Environmental Entomology 24:149–158.

Gaugler, R. 1987. Entomogenous nematodes and their prospects for genetic improvement. Pp. 457–484 *in* K. Maramorosch, ed. Biotechnology in invertebrate pathology and cell culture. San Diego: Academic Press.

Grewal, P., and Georgis, R. 1999. Entomopathogenic nematodes. Pp. 271–299 *in* F. R. Hall and J. J. Menn, eds. Methods in biotechnology, vol. 5. Biopesticides: Use and delivery. Totowa, NJ: Humana Press.

Grewal, P. S., Grewal, S. K., Malik, V. S., and Klein, M. G. 2002a. Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. Biological Control 24:230–237.

Grewal, P. S., Selvan, S., and Gaugler, R. 1994. Thermal adaptation of entomopathogenic nematodes—niche breadth for infection, establishment, and reproduction. Journal of Thermal Biology 19:245–253.

Grewal, P. S., Wang, X., and Taylor, R. A. J. 2002b. Dauer juvenile longevity and stress tolerance in natural populations of entomopathogenic nematodes: Is there a relationship? International Journal for Parasitology 32:717–725.

Hass, B., Downes, M. J., and Griffin, C. T. 1999. Persistence of *Heterorhabditis* infective juveniles in soil: Comparison of extraction and infectivity measurements. Journal of Nematology 31:508–516.

Hass, B., Downes, M. J., and Griffin, C. T. 2001. Correlation between survival in water and persistence of infectivity in soil of *Heterorhabditis* spp. isolates. Nematology 3:573–579.

Hass, B., Downes, M. J., and Griffin, C. T. 2002. Persistence of four *Heterorhabditis* spp. isolates in soil: Role of lipid reserves. Journal of Nematology 34:151–158.

Jansson, R. K., Lecrone, S. H., and Gaugler, R. 1993. Field efficacy and persistence of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) for control of sweetpotato weevil (Coleoptera: Apionidae) in southern Florida. Journal of Economic Entomology 86:1055–1063.

Kaya, H. K. 1990. Soil ecology. Pp. 93–116 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Kaya, H. K. 2002. Natural enemies and other antagonists. Pp. 189–204, *in* R. Gaugler, ed. Entomopathogenic nematology. New York: CABI.

Kaya, H. K., and Gaugler, R. 1993. Entomopathogenic nematodes. Annual Review of Entomology. 38:181–206.

Kaya, H. K., and Stock, S. P. 1997. Techniques in insect nematology. Pp. 281–324 *in* L. A. Lacey, ed. Manual of techniques in insect pathology. San Diego: Academic Press.

Klein, M. G. 1990. Efficacy against soil-inhabiting insect pests. Pp. 195–214 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Klein, M. G., and Georgis, R. 1992. Persistence of control of Japanese beetle (Coleoptera: Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. Journal of Economic Entomology 85:727–730.

Koppenhöfer, A. M., and Fuzy, E. U. 2004. Effect of white grub developmental stage on susceptibility to entomopathogenic nematodes. Journal of Economic Entomology 97:1842–1849.

Kung, S., Gaugler, R., and Kaya, H. K. 1990. Soil type and entomopathogenic nematode persistence. Journal of Invertebrate Pathology 55:401–406.

Kung, S., Gaugler, R., and Kaya, H. K. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. Journal of Invertebrate Pathology 57:242–249.

Liu, Q. Z, and Glazer, I. 2000. Desiccation survival of entomopathogenic nematodes of the genus *Heterorhabditis*. Phytoparasitica 28:331–340.

McCoy, C. W., Shapiro, D. I., Duncan, L. W., Nguyen, K. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biological Control 19:182–190.

Millar, L. C., and Barbercheck, M. E. 2002. Effects of tillage practices on entomopathogenic nematodes in a corn agroecosystem. Biological Control 25:1–11.

Molyneux, A. S. 1985. Survival of infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Revue de Nématologie 8:165–170.

Molyneux, A. S., and Bedding, R. A. 1984. Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the sheep blowfly *Lucilia cuprina*. Nematologica 30:358–365.

Morris, O. N., and Converse, V. 1991. Effectiveness of steinernematid and heterorhabditid nematodes against noctuid, pyralid, and geometrid species in soil. Canadian Entomologist 123:55–61.

Parkman, J. P., Hudson, W. G., Frank, J. H., Nguyen, K. B., and Smart, G. C., Jr. 1993. Establishment and persistence of *Steinernema scapterisci* (Rhabditida: Steinernematidae) in field populations of *Scapteriscus* spp. mole crickets (Orthoptera: Gryllotalpidae). Journal of Entomological Science 28:182–190.

Patel, M. N., Perry, R. N., and Wright, D. J. 1997. Desiccation survival and water content of entomopathogenic nematodes, *Steinernema* spp. (Rhabditida: Steinernematidae). International Journal of Parasitology 27:61–70.

Perez, E. E., Lewis, E. E., and Shapiro-Ilan, D. I. 2003. Impact of the host cadaver on survival and infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under desiccating conditions. Journal of Invertebrate Pathology 82:111–118.

Poinar, G. O., Jr. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. Pp. 23–62 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Selvan, S., Gaugler, R., and Lewis, E. E. 1993. Biochemical energy reserves of entomopathogenic nematodes. Journal of Parasitology 79: 167–172.

Shapiro, D. I., Cate, J. R., Pena, J., Hunsberger, A., McCoy, C. W. 1999a. Effects of temperature and host range on suppression of *Dia-*

prepes abbreviatus (Coleoptera: Curculionidae) by entomopathogenic nematodes. Journal of Economic Entomology 92:1086–1092.

Shapiro, D. I., and Glazer, I. 1996. Comparison of entomopathogenic nematode dispersal from infected hosts versus aqueous suspension. Biological Control 25:1455–1461.

Shapiro, D. I., and Lewis, E. E. 1999. Comparison of entomopathogenic nematode infectivity from infected hosts versus aqueous suspension. Biological Control 28:907–911.

Shapiro, D. I., and McCoy, C. W. 2000. Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. Journal of Economic Entomology 93:1090–1095.

Shapiro, D. I., McCoy, C. W, Fares, A., Obreza, T., and Dou, H. 2000. Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviatus*. Environmental Entomology 29:1083–1087.

Shapiro, D. I., Obrycki, J. J., Lewis, L. C., and Jackson, J. J. 1999b. Effects of crop residue on the persistence of *Steinernema carpocapsae*. Journal of Nematology 31:517–519.

Shapiro, D. I., Tylka, G. L., and Lewis, L. C. 1996. Effects of fertilizers on virulence of *Steinernema carpocapsae*. Applied Soil Ecology 3:27–34.

Shapiro-Ilan, D. I., and Gaugler, R. 2002. Production technology for entomopathogenic nematodes and their bacterial symbionts. Journal of Industrial Microbiology and Biotechnology 28:137–146.

Shapiro-Ilan, D. I., Gouge, D. H., and Koppenhöfer, A. M. 2002. Factors affecting commercial success: Case studies in cotton, turf, and citrus. Pp. 333–356 *in* R. Gaugler, ed. Entomopathogenic nematology. New York: CABI.

Shields, E. J., Testa, A., Miller, J. M., and Flanders, K. L. 1999. Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* 'Oswego' and *H. bacteriophora* 'NC' on Alfalfa snout beetle larvae (Coleoptera: Curculionidae). Environmental Entomology 28:128–136.

Smits, P. 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Science and Technology 6:379–387.

Southwood, T. R. E. 1978. Ecological methods. London: Chapman and Hall.

Stuart, R. J., Shapiro-Ilan, D. I., James, R. R., Nguyen, K. B., and McCoy, C. W. 2004. Virulence of new and mixed strains of the entomopathogenic nematode *Steinernema riobrave* to larvae of the citrus root weevil *Diaprepes abbreviatus*. Biological Control 30:439–445.