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Influence of Salinity on Survival and Infectivity of Entomopathogenic Nematodes

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Abstract: Exposure to NaCl, KCl, and $CaCl_2$ affected the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema glaseri* differently. Survival, virulence, and penetration efficiency of *S. glaseri* were not affected by these salts. At high concentrations, however, all three salts inhibited its ability to move through a soil column and locate and infect a susceptible host. Calcium chloride and KCl had no effect on *H. bacteriophora* survival, penetration efficiency, or movement through a soil column, but moderate concentrations of these salts enhanced *H. bacteriophora* virulence. NaCl, however, adversely affected each of these parameters at high salinities (>16 dS/m). Salt effects on *S. glaseri* are attributed solely to interference with nematode host-finding ability, whereas the NaCl effects on *H. bacteriophora* are attributed to its toxicity and possibly to interference with host-finding behavior.

Key words: Entomopathogenic nematode, *Heterorhabditis bacteriophora,* infectivity, nematode, salinity, survival, *Steinernema glaseri.*

Salinity in soils, especially on irrigated land, is a major problem for crop production (19,26). Solutions to this problem have included breeding salt-tolerant crops, building regional drainage systems, and devising improved irrigation and management techniques (25). However, soil salinity will continue to be a problem to crops in arid and semi-arid parts of the world. In California alone, 30% of the irrigated area is considered to be saline (electroconductivity >4.0 dS/m) (1). Tolerance of soil salinity varies with crop (21). For example, carrot yields are reduced in soils with electroconductivity as low as 1.0 dS/m, whereas cotton yields are not adversely affected until soil salinity reaches 7.7 dS/m.

Only microorganisms seem to be affected by very high soil salinities (27). Wallace (30) states that "Osmotic pressure appears to exert very little influence on nematode activity in soil because salt concentrations rarely rise to inhibitory levels." However, the data from the plant-parasitic nematode literature are mixed. *Rotylenchus reniformis* occurred equally in highly saline (16.5 dS/m) and lower saline (4.0 dS/m) soils in Texas cotton fields (13). Maggenti and Hardan (22) found no difference in population densities of the root-knot nematode, *Meloidogyne javanica,* on tomato roots in soils between 4 and 16 dS/m, but the population density was twice that in nonsodic soils. In contrast, infectivity and development of the root-knot nematode, *Meloidogyne incognita,* were impaired by increasing soil solution concentrations (from 1.5 to 5.0 dS/m) of NaCl, $CaCl_2$, or combinations of both salts (6). In addition, concentrated saline solutions reduced body size of the feeding stages of the free-living nematode, *Rhabditis terrestris* (29).

Entomopathogenic nematodes have been recovered from soil in many parts of the world (16). These nematodes occur more commonly in sandy to loamy soils than in clay soils (2,10–12,14,28). Most reports, however, have not included soil salinity. In one case, Hara et al. (12) isolated a *Heterorhabditis* species (recently described as *Heterorhabditis hawaiiensis* by Gardner et al. (8)) from 22 sites and a *Steinernema* species from 2 sites in the Hawaiian Islands. Twenty-one of the 22 heterorhabditid sites were within 100 m of the ocean. In spite of their proximity to the ocean, the salinity of

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the sandy soils (15 cm deep) averaged <1 dS/m. The two steinernematid sites were located inland, with the soil salinity being 0.55 and 0.98 dS/m.

Because soil salinity is increasing in many parts of the world and we know little about its effect on entomopathogenic nematodes, we investigated the effects of saline conditions on entomopathogenic nematodes in relation to their ability to infect insects. We selected *Heterorhabditis bacteriophora* and *Steinernema glaseri* because these two species actively seek out host insects (17,20) and are therefore more likely to have their efficacy affected by soil conditions.

Materials and Methods

Insects and nematodes: Steinernema glaseri NC strain and *Heterorhabditis bacteriophora* NC1 strain were reared in larval *Galleria mellonella* (Northern Bait, Chetek, WI) according to the technique of Dutky et al. (5) and stored at 10 C until used. All nematodes were used within 2 weeks of harvest.

Salt solutions: NaCl and KCl solutions were prepared at 0.5, 2.0, 4.1, 9.4, and 18.0 g/l (electroconductivities of 1, 4, 8, 16, and 30 dS/m, respectively). Calcium chloride solutions were prepared at 0.2, 1.0, 2.1, 4.7, and 9.0 g/l (electroconductivities of <1, 2, 4, 9, and 16 dS/m, respectively). All solutions were made with reagent-grade salts and sterile double-distilled water. The control solution was sterile double-distilled water. Salt concentrations were selected to extend well beyond the normal range of total soil salinities in the field. Solution pH values were between 5.1 and 6.2. Because pH values in this range are benign to entomopathogenic nematodes (7,18), solutions were not set to a standard pH.

Nematode survival and virulence: Infective juveniles (IJ) of *H. bacteriophora* and *S. glaseri* were placed in salt solutions in 150-ml tissue culture flasks. Flasks contained 40 ml of a nematode suspension, at the concentration of 1,000 IJ/ml for *H. bacteriophora* and 500 IJ/ml for *S. glaseri*. Each of the salt concentrations was replicated four times. Control flasks contained 1,000 or 500 IJ/ml in sterile distilled water. Initial counts of live and dead nematodes were made before exposing the nematodes to the salt solutions. At 2, 4, 8, 14, 20, 36, and 56 days after the nematodes were placed in the flasks, four counts of approximately 100 nematodes, determining number alive and number dead, were made from each flask. After 20 days of exposure to the salt solutions, some IJ were removed from the salt solutions and their virulence to *G. mellonella* determined. Ten *Galleria* larvae were exposed to 20 nematodes on moist filter paper in plastic petri dishes. A minimum of 50 *G. mellonella* larvae was used to test nematodes from each of four electroconductivities for each salt: 0, 8, 16, and 30 dS/m for NaCl and KCl, and 0, <1, 9, and 16 dS/m for $CaCl_2$. *Galleria* were exposed to the IJ for 24 hours, rinsed with sterile distilled water, and placed individually on clean, moist filter paper. Nematode-caused mortality was assessed 4 days later.

Nematode penetration efficiency: To determine if salt solutions affect the ability of *S. glaseri* or *H. bacteriophora* to penetrate into a susceptible insect host, the penetration efficiency of nematodes stored in salt solutions at room temperature for 20 days was assessed. The nematodes used in this experiment were stored in the highest concentration of each salt at nematode densities of 1,000 IJ/ml (*H. bacteriophora*) and 500 IJ/ml (*S. glaseri*). *Galleria mellonella* larvae were individually exposed to 50 IJ of either nematode on moist filter paper in 5-cm-d petri dishes. After 24 hours, larvae were rinsed to remove any external nematodes and placed on moist filter paper in clean petri dishes. After 3 days for *S. glaseri* and 4 days for *H. bacteriophora,* the cuticles were removed from dead wax moth larvae, the internal tissues digested with a pepsin-HCl solution (23), and the nematodes within each cadaver were counted.

Nematode movement through soil columns: We conducted a sand column assay to quantify the effect of salt solutions on the motility and ability of *H. bacteriophora* and

S. glaseri to find and infect hosts. Sand (150–250 μm particle size) was washed thoroughly in hot tap water, rinsed three times with distilled water, and oven dried overnight at 110 C. The sand was moistened to 10% by weight (−0.03 bars) with a salt solution. Each of the salt solutions described above was used for each nematode species; control columns contained sand moistened with double-distilled water. Plexiglass cylinders (5-cm-high × 5-cm-d; inside d = 4.4 cm) were filled with 76 cm^3 moistened sand, one *G. mellonella* larva was placed on the bottom of the column, and the ends of the column were capped with small plastic petri dish bottoms and sealed with parafilm. The columns were placed in plastic bags and incubated at 25 C. After 24 hours, exactly 5 IJ of *H. bacteriophora* or 3 IJ of *S. glaseri* were pipetted in a minimum volume of liquid (<50 μl) onto the sand at the top of the column. Columns were incubated at 25 C for 24 hours for *S. glaseri* or 48 hours for *H. bacteriophora*. Following incubation, the *Galleria* were removed, rinsed in sterile distilled water to remove any external nematodes, and placed individually on moist filter paper in small petri dishes. Four days later, nematode-caused *Galleria* mortality was assessed by dissection.

Columns were established in blocks of 15, replicated three times, and the entire experiment was repeated three to five times. The number of repetitions was determined by the variability of the response, *H. bacteriophora* generally producing more variable results. To confirm that nematodes exposed to salt solutions were still virulent to *G. mellonella*, filter paper assays, in which conditions for infection by the nematodes were optimal, were conducted using nematodes stored in salt solutions for 2 days. The technique used was the same as described (nematode survival).

Results and Discussion

Exposure to high concentrations of NaCl, KCl, and $CaCl_2$ affected survival of the entomopathogenic nematodes *S. glaseri* and *H. bacteriophora* differently (Fig. 1). Neither nematode showed a decline in survival when stored for 56 days in KCl or $CaCl_2$ solutions, but NaCl reduced survival of *H. bacteriophora* from 87% alive (distilled water) to 59% (30 dS/m) while not affecting the survival of *S. glaseri* (Fig. 1).

After 20 days storage at room temperature, survival of *H. bacteriophora* IJ was adversely affected at 30 dS/m NaCl. Moreover, the virulence of the IJ surviving at this time was reduced (Table 1). Survival of *H. bacteriophora* was unaffected by the other salts, whereas virulence of the survivors was enhanced by moderate levels of both KCl and $CaCl_2$ (Table 1). No such effects were noted on *S. glaseri* (data not shown). The reasons for enhanced virulence of *H. bacteriophora* IJ following exposure to KCl and $CaCl_2$ solutions are unknown but could be due to moderate levels of salts stimulating nematode activity or causing less osmotic stress to the nematodes. Stimulation of nematode activity has been reported for *S. carpocapsae* treated with the insecticide-nematicide oxamyl (15), but the increased activity did not result in increased infectivity of the nematodes (9). Enhancing the virulence of stored entomopathogenic nematodes by the addition of specific salts to the storage solution is an intriguing possibility that should be investigated further.

Sodium chloride at 30 dS/m also reduced the penetration efficiency of *H. bacteriophora* while not affecting that of *S. glaseri* (Table 2). Of the *H. bacteriophora* IJ exposed to 30 dS/m NaCl for 20 days, only 12% infected *G. mellonella* larvae, compared with 38% of those IJ exposed to distilled water for 20 days (Table 2).

The effect of salts on nematode movement in sand columns also varied with nematode species (Fig. 2). *Steinernema glaseri* was less able to find and (or) infect *G. mellonella* larvae when higher concentrations of all three salts were present. *Heterorhabditis bacteriophora*, on the other hand, was not adversely affected by increasing concentrations of KCl or $CaCl_2$. Sodium chloride at high concentrations, however,

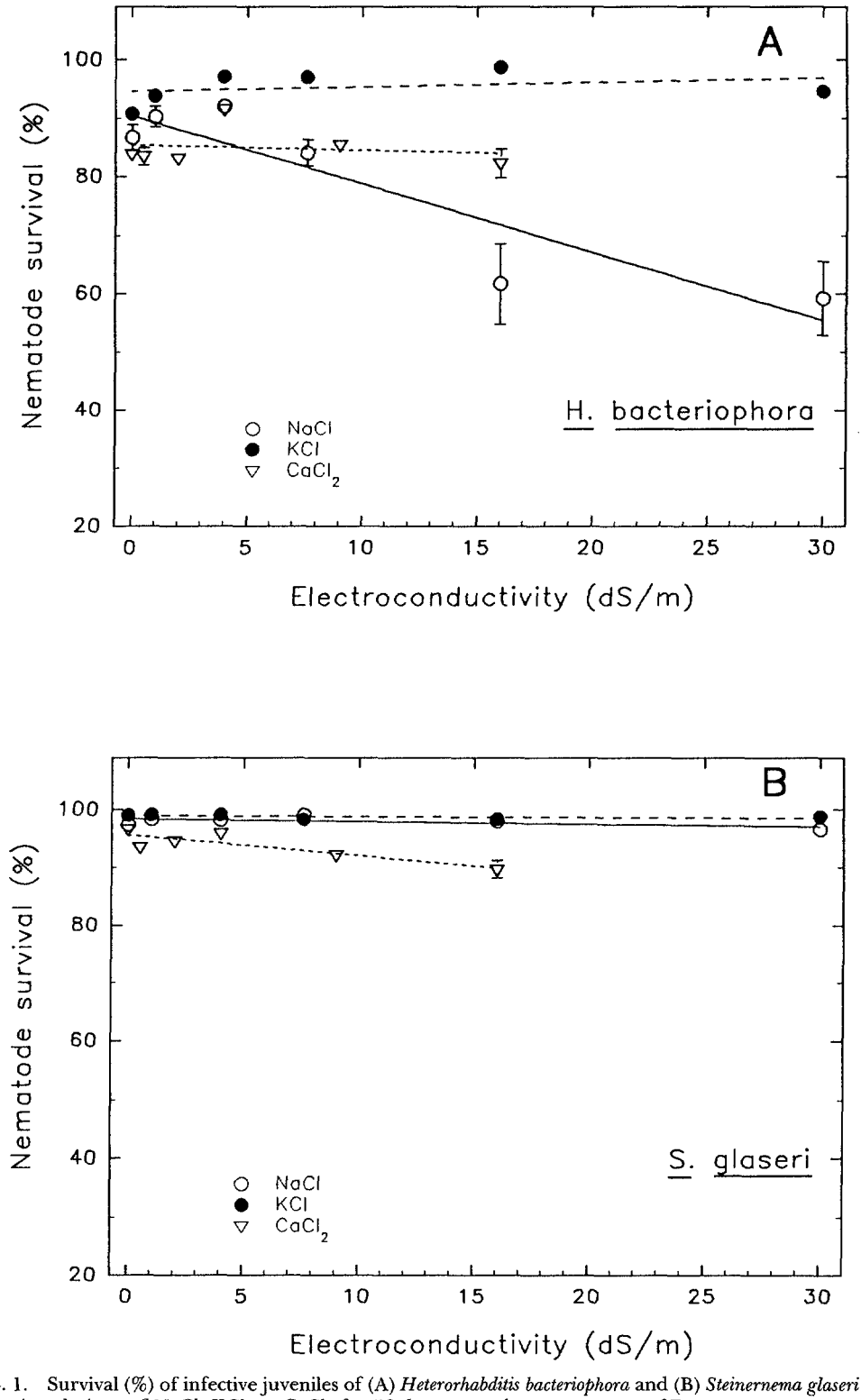

FIG. 1. Survival (%) of infective juveniles of (A) *Heterorhabditis bacteriophora* and (B) *Steinernema glaseri* after storage in solutions of NaCl, KCl, or $CaCl_2$ for 56 days; error bars represent ± SE.

TABLE 1. Survival† and virulence‡ of *Heterorhabditis bacteriophora* infective juveniles after storage in salt solutions at 24 C for 20 days.

	NaCl		KCl			$CaCl_2$		
EC§	Survival†	Virulence‡	EC	Survival	Virulence	EC	Survival	Virulence
0	97.8 ± 1.3	82.0 ± 5.8	0	97.8 ± 1.3	56.7 ± 3.3	0	97.8 ± 1.3	42.2 ± 2.7
8	97.5 ± 0.7	82.0 ± 3.7	8	98.3 ± 0.2	61.7 ± 7.0	4	96.8 ± 1.2	66.3 ± 4.8**
16	95.2 ± 1.5	74.0 ± 5.1	16	97.7 ± 0.4	73.3 ± 4.9*	9	96.7 ± 0.7	83.4 ± 5.8**
30	62.5 ± 3.8**	62.0 ± 5.8*	30	93.9 ± 2.4	55.0 ± 5.6	16	98.7 ± 1.0	50.9 ± 5.9

† Mean percentage survival ± SE of IJ stored in salt solutions for 20 days.
‡ Mean percentage mortality ± SE of *Galleria mellonella* larvae exposed to IJ of *H. bacteriophora* in standardized petri dish bioassays.
§ Electroconductivity (dS/m) of salt solution.
* and ** significantly different from control (no salt), *t*-test, $P = 0.05$ and 0.01, respectively.

suppressed the ability of *H. bacteriophora* to locate and infect the host insect (Fig. 2). The poor performance of *H. bacteriophora* in the sand column assays at high concentrations of NaCl is attributed to toxic effects of NaCl and interference with host finding ability. Survival, virulence, and penetration efficiency of *H. bacteriophora* were all adversely affected by NaCl, suggesting toxic effects of this salt. Because the sand column assay requires nematodes to move 5 cm through sand to locate and infect a host, virulence and host finding are both important components of the assay. Virulence of *H. bacteriophora* in filter paper assays was affected after 20 days exposure to high concentrations of NaCl, but was not affected by exposure to 30 dS/m NaCl for 2 days (the duration of the exposure in the sand column assays). Thus some of the problems with host-finding by IJ of *H. bacteriophora* in the sand column assay may be explained by NaCl interference.

TABLE 2. Penetration efficiency§ of infective juveniles of *Heterorhabditis bacteriophora* and *Steinernema glaseri* after storage in salt solutions at 24 C for 20 days.

		Penetration efficiency (%)§	
Salt	EC†	*H. bacteriophora*	*S. glaseri*
None	0	37.8 ± 4.6	30.7 ± 2.9
NaCl	30	12.0 ± 1.8**	39.0 ± 3.8
KCl	30	34.2 ± 2.5	28.2 ± 3.5
$CaCl_2$	16	35.2 ± 3.2	26.1 ± 3.8

§ Mean percentage ± SE of applied IJ penetrating into individual host *Galleria mellonella* larvae within 24 hours.
† Electroconductivity (dS/m) of salt solution.
** Significantly different from control (no salt), *t*-test, $P < 0.01$.

Although movement of *S. glaseri* through sand was affected by high concentrations of each salt (Fig. 2), survival (Fig. 1) and virulence of *S. glaseri* IJ surviving 20 days storage in all salts (data not shown) were unaffected. Because nematode virulence was unaffected by salt exposure, the reduced response of *S. glaseri* in the sand column assays was probably due to interference with chemotaxis; there was no evidence of toxic effects associated with the salts used.

Our data show that *H. bacteriophora* and *S. glaseri* tolerate exposure to salt solutions at concentrations found in soils with high salinity. Although salt solutions affected nematode survival and ability to infect a susceptible host in a soil column, the effects were only noticeable at high salinities. Salinities at or below the plant tolerance thresholds did not affect the nematode species used. However, the relationship of our one-salt exposure to the effects of solutions found in natural soils is unknown. The interactions of salts with entomopathogenic nematodes are poorly understood and may be varied, depending on taxon. Entomopathogenic nematodes apparently tolerate a wide range of salinities. Oetting and Latimer (24) determined that the infectivity of *S. carpocapsae* to *G. mellonella* was unaffected by NaCl concentration up to 16 dS/m. Similarly, Das (4) showed that *S. carpocapsae* could survive salinity levels up to 20 dS/m. This is in con-

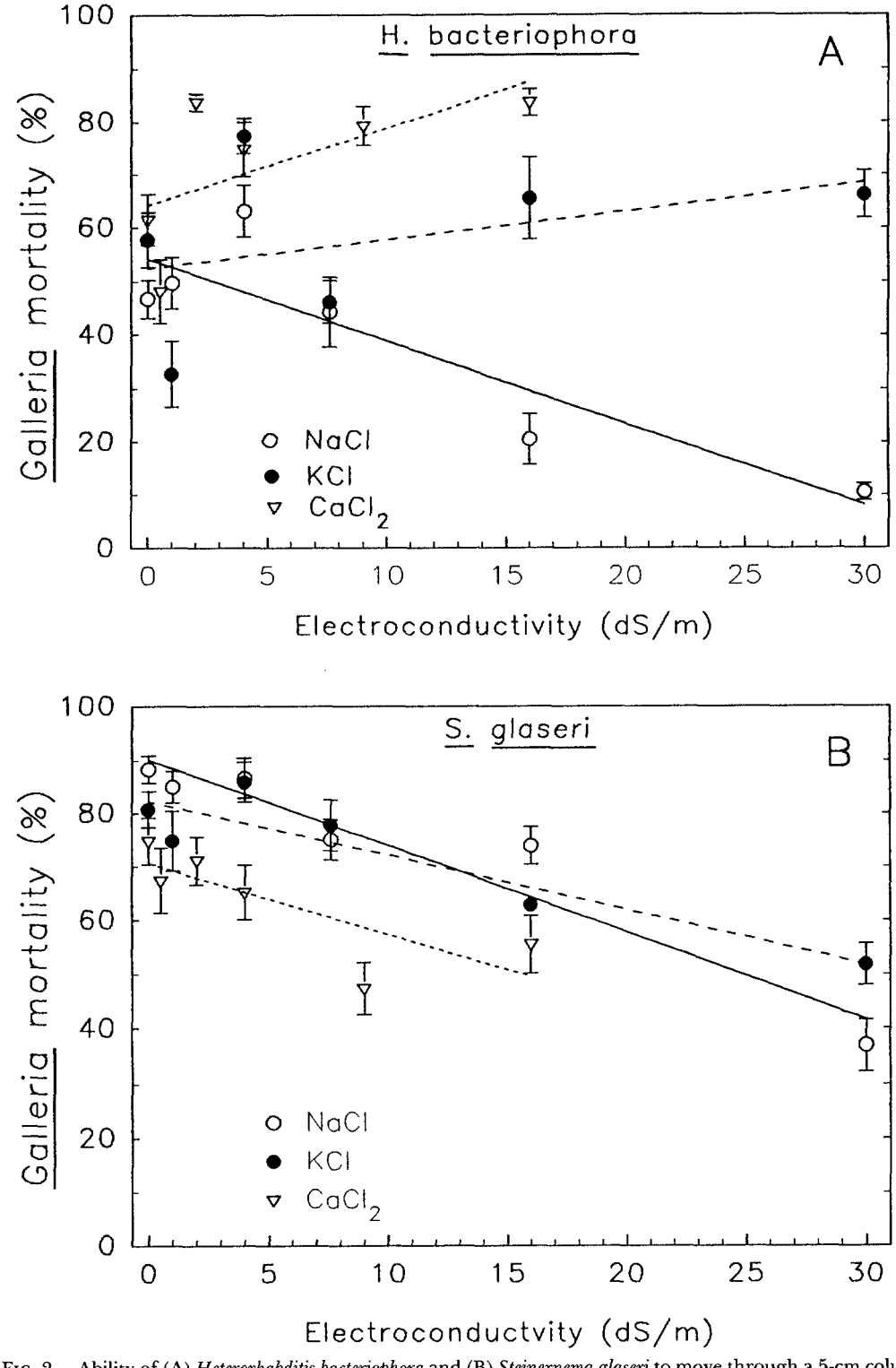

FIG. 2. Ability of (A) *Heterorhabditis bacteriophora* and (B) *Steinernema glaseri* to move through a 5-cm column of soil moistened with solutions of NaCl, KCl, or $CaCl_2$ and infect a *Galleria mellonella* larva used as bait on the bottom of the column, expressed as percentage mortality of bait *Galleria;* error bars represent ± SE.

trast to another, unrelated, entomopathogenic mermithid nematode, *Romanomermis culicovorax,* which has poor survival and infectivity at 4.8 dS/m NaCl (3). Nevertheless, it appears that the entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* may be suitable for use in saline agricultural soils, although more work with other members of these genera and in situations more representative of salinity in the field may be required to confirm this general conclusion.

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