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Activity and Survival of *Orrina phyllobia*: Preliminary Investigations on the Effects of Solutes[1]

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Abstract: The motility and survival of *Orrina phyllobia* fourth-stage juveniles (J4) were examined in NaCl, sucrose, and synthetic soil solutions. Synthetic soil solutions (SSSs) contained Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and NO_3^- at relative concentrations identical to those in a known agronomic soil. Nematode activity was dependent on solute composition and on water potential. In all solutions, motility ceased at a water potential of -30×10^5 Pa and nematodes partially desiccated. Activity inhibition in NaCl began at -5×10^5 Pa. At -15×10^5 Pa, a high level of activity was sustained only in SSS. Lethal effects occurred at -15 and -60×10^5 Pa in NaCl and SSS, respectively. No lethal effects were measured in sucrose solutions. Hydrogen ion concentration over the pH range 4.5–9.5 had no measurable effect on nematode activity.

Key words: osmolality, water potential, ionic solutes.

We previously examined effects of variations in temperature and dissolved oxygen concentrations on the motility and survival of fourth-stage juveniles (J4) of *Orrina phyllobia* (Thorne) Brzeski (= *Nothanguina phyllobia* Thorne). In this paper are reported effects caused by variations in water potential, solute concentrations, and the pH of ionic and nonionic aqueous solutions. The solute systems examined were NaCl, a synthetic soil solution, and sucrose. Unbalanced NaCl solutions were used by other workers examining the behavior (3), and osmo- and ionregulation of many nematode species (10,14). The synthetic soil solution (SSS) contained NaCl and four additional ions (K^+, Ca^{2+}, Mg^{2+}, and NO_3^-) at relative concentrations identical to those

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in a known soil. Sucrose provided a basis from which to predict solute-independent effects of water potential. Although sucrose is a nutrient substrate for many organisms and is not impermeant to biological membranes, it is less permeant than inorganic soil ions. It is also highly soluble, permitting the generation of water potentials ranging from those characteristic of dilute soil solutions (-0.4×10^5 Pa) to that of air at < 90% relative humidity (-200×10^5 Pa). (In order to conform to the International System of Units and simultaneously facilitate comparisons to previous literature, water potentials are expressed as multiples of 10^5 Pa = 1 bar.) Sucrose has been used in many studies on the effects of water potential on microflora (6) and in several studies on nematodes (1,2,4,5,11).

Materials and Methods

Orrina phyllobia infective J4 were obtained, stored, and hydrated as previously described (9). Their activity was then stabilized by holding them at 23 C for 2–4 hours prior to experimental use. Nematode activity was measured with an activity index (AI), defined as the number of nematode body undulations within 15 seconds, determined for 20–50 randomly selected nematodes as previously described. Confidence limits about AIs were generated from Student's *t* distribution.

The milligram equivalent (meq) concentrations of six ions were averaged for 18 soil solutions extracted 15 cm deep in situ from a loamy fine sand in a west Texas cotton field (7). The resulting meq ratios ($NA^+ : K^+ : Mg^{2+} : Ca^{2+} : Cl^- : NO_3^-$ = 8:1:6:10:17:8) were fixed to prepare a series of SSSs at water potentials of -0.25 to -240×10^5 Pa (10–8,500 meq/liter). Sucrose and NaCl solutions were prepared at concentrations isopotential to the SSSs. The responses of nematodes to test solutions were first compared by reading AIs at various intervals over a 24-hour period after the transfer of randomly drawn aliquots (ca. 1,000 nematodes/aliquot) from a stock suspension in distilled water to sucrose, NaCl, and the SSS at -15 and -30×10^5 Pa. Subsequently, activity levels were compared after nematodes had been kept for 24 hours in each solute system at various water potentials ranging from -2.5 to -240×10^5 Pa. Nematodes at -5, -15, -60, and -240×10^5 Pa were transferred from test solutions back to distilled water for an additional 24 hours to assess the reversibility of effects.

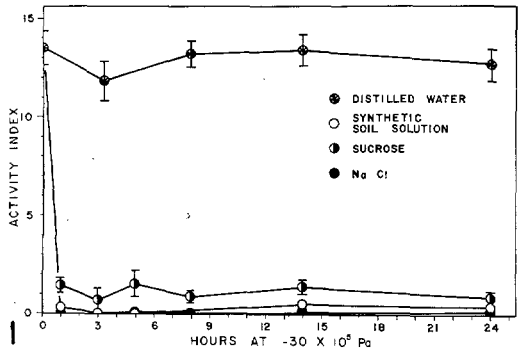
Effects of pH were compared by reading AIs 24 hours after transferring nematodes from distilled water to SSS at -5, -10, -20, and -40×10^5 Pa with pHs adjusted to 4.5, 7.0, and 9.5 with HCl/NaOH for each SSS tested.

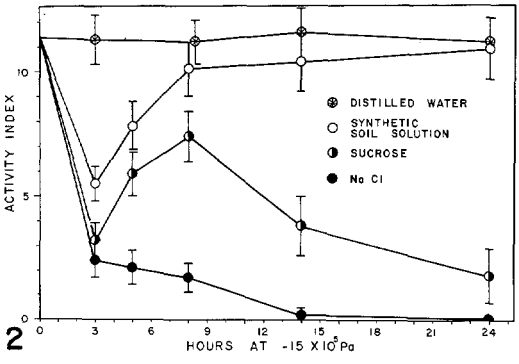
Since *O. phyllobia* J4 are known to die after 5–10 days in distilled water at 23 C (9), nematode longevity was compared in distilled water and in SSS at two water potentials, -0.25 and -2.5×10^5 Pa, by reading AIs 0, 3, and 5 days after nematode transfer from distilled water. The total concentrations of ions in SSS solutions at the two water potentials examined were 10 and 100 meq/liter. Activity and survival were also compared in several modifications of the SSS at -2.5×10^5 Pa by measuring AIs 1 and 6 days after nematode introduction. These solutions included all combinations resulting from deleting one, or including only one, of the four cations of the SSS.

The water potentials of all solutions tested were verified (± 1%) by freezing point depression with an osmometer calibrated against solutions of oven-dried NaCl. Temperature was controlled in all experiments at 23 ± 0.5 C. Microscope stage temperature during AI readings was controlled with a water jacket as previously described (8).

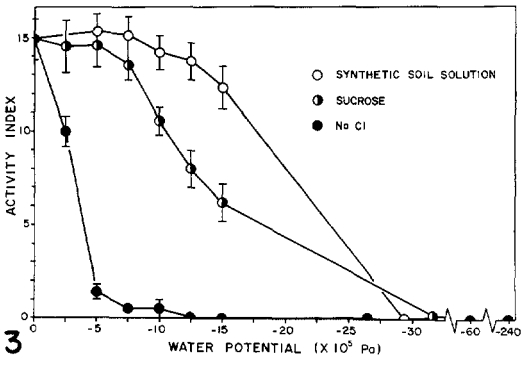
Results

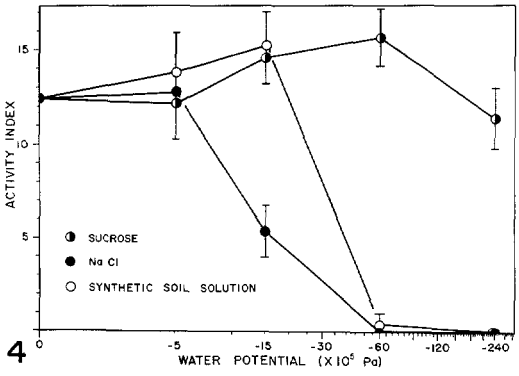
When nematodes were transferred from distilled water to NaCl, sucrose, or the SSS at -30×10^5 Pa, they became inactive within 3 hours, shrunk lengthwise by ca. 50%, and remained inactive for 24 hours (Fig. 1). At -15×10^5 Pa, a similar initial decrease in activity (60–80%) during the first 3 hours was followed by solute-dependent responses (Fig. 2). In the SSS, the initial drop in activity was followed by 90% recovery within 8 hours, with the AI at 24 hours asymptotically approaching the AI of nematodes in distilled water. In sucrose, a partial recovery of activity from 3 to 8 hours was followed by a reversal of this effect, with activity degenerating toward ca. 25% of the control at 24 hours. In NaCl, no recovery of activity occurred, and the





FIGS. 1, 2. Activity index of *Orrina phyllobia* (undulations/15 seconds, 50 infective juveniles/datum) at 23 C in distilled water and after transfer from distilled water to NaCl, sucrose, and a synthetic soil solution. 1) At -30×10^5 Pa. 2) At -15×10^5 Pa. Brackets indicate confidence limits at $P = 0.05$.





FIGS. 3, 4. Activity index of *Orrina phyllobia* (undulations/15 seconds, 23 C). 3) Twenty-four hours after transfer from distilled water to NaCl, sucrose, and synthetic soil solutions at various water potentials (50 infective juveniles/datum). 4) Recovery in distilled water 24 hours after transfer from NaCl, sucrose, and synthetic soil solutions (20 infective juveniles/datum). Brackets indicate confidence limits at $P = 0.05$.

nematodes were immobilized within 14 hours. These nematodes were straight, optically refractive, and were not noticeably shrunken. Inactive nematodes in sucrose at -15×10^5 Pa were partially coiled; nematodes in the SSS did not differ in appearance from nematodes in distilled water.

After nematodes were maintained at various water potentials from 0 to -240×10^5 Pa for 24 hours, the following results were observed (Fig. 3). In sucrose and in the SSS, activity began to decrease at -5 and -10×10^5 Pa, respectively, and ceased in both solutions at -30×10^5 Pa. Twenty-four hours after the transfer of nematodes from -5, -15, -60, and -240×10^5 Pa to distilled water, irreversible effects from NaCl were measurable only in solutions with water potentials $< -15 \times 10^5$ Pa ($\geq$ 720 meq/liter); the total loss of activity that occurred after 24 hours in NaCl at -5×10^5 Pa was completely reversed (Fig. 4). Irreversible effects from exposure to the SSS occurred only in solutions at or below -60×10^5 Pa (ca. 2,800 meq/liter). No significant irreversible loss of activity in sucrose was measured.

Hydrogen ion concentration had no appreciable effect on activity in any of the SSSs examined (Table 1).

When nematodes were maintained for 6 days in distilled water, two concentrations of the SSS (10 and 100 meq/liter), and eight modifications of the SSS at 100 meq/liter), only the single cation sodium solution (NaSSS) affected activity appreciably. After 24 hours in NaSSS, nematodes were essentially immobilized (AI < 0.04); AIs for other modifications of the SSS were between 12.4 and 14.2. According to AI measurements at 6 days, survival was neither shortened nor prolonged substantially by any of the other solutions.

TABLE 1. Activity indices of *Orrina phyllobia* J4 after 24 hours in a synthetic soil solution at various pHs and water potentials.

Water potential ($\times 10^5$ Pa)	pH			
	4.5	7.0	9.5	Mean
−5	12.4 ± 1.4*	12.4 ± 1.4	13.9 ± 1.4	12.9
−10	9.8 ± 1.2	10.6 ± 1.1	10.6 ± 1.1	10.3
−20	4.9 ± 1.0	6.5 ± 1.0	4.3 ± 1.0	5.2
Mean	9.0	9.8	9.6	

* Confidence intervals about activity at $P = 0.05$ based on Student's t distribution.

DISCUSSION

Of the various solutions to which *O. phyllobia* J4 were exposed, only dilute concentrations of the SSS approximated conditions to which nematodes might be exposed in soil. The responses observed are primarily useful for comparing *O. phyllobia* to other species examined under similar conditions.

Experimental populations of *O. phyllobia* J4 incurred 50% irreversible loss of activity after 24 hours in NaCl at -10×10^5 Pa. Viglierchio et al. (12) similarly exposed six nematode species to various NaCl-induced water potentials from -0 to -80×10^5 Pa for 24 hours. Fifty percent irreversible activity reduction for *Rhabditis* sp., *Pratylenchus vulnus* Allen and Jensen, *Meloidogyne hapla* Chitwood, *Hemicycliophora arenaria* Raski, and *Tylenchulus semipenetrans* Cobb occurred at -5, -6, -18, -20, and -20×10^5 Pa, respectively. *Ditylenchus dispsaci* (Kuhn) Filipjev was not permanently affected by NaCl, even at -40×10^5 Pa.

Sucrose stopped the activity of *O. phyllobia* J4 over the range -5 to -30×10^5 Pa. When Steele (11) examined the effect of sucrose on emergence of *Heterodera schachtii* Schmidt J2 from cysts, he found that nematode emergence from cysts ceased between -18 and -32×10^5 Pa. Following the transfer of cysts from sucrose to sugarbeet diffusate, which stimulates hatching, emergence occurred even from cysts that had been held for 96 hours at $< -100 \times 10^5$ Pa. In a similar study on *Heterodera glycines* Ichinohe, Epps (4) found no reduction in the number of juveniles emerging from cysts at sucrose-induced water potentials above -3.5×10^5 Pa. Lower water potentials were not examined. Clarke et al. (2) compared effects of sucrose and triethylene glycol solutions on hatching and posthatch activity of *Globodera rostochiensis* Wollenweber J2. In both solutions, inhibition of hatch began at -3.5×10^5 Pa and a 90% reduction in J2 activity occurred after 24 hours at -10×10^5 Pa. The activity of *O. phyllobia* and other nematodes examined in sucrose ceased over the range of -3.5 to -30×10^5 Pa. The failure of *O. phyllobia* to recover activity when at -30×10^5 Pa in three diverse solute systems suggests that -30×10^5 Pa may be a solute-independent limit for activity.

Activity of *O. phyllobia* J4 in hypertonic solutions probably parallels changes in nematode volume. Inactive *O. phyllobia* J4 at -30×10^5 Pa were noticeably dehydrated in solution. Blake (1) found that loss of activity and loss of volume by *D. dipsaci* in urea occurred over identical water potential ranges (-7 to -22.4×10^5 Pa). Loss of volume regulation by *G. rostochiensis* J2 in balanced salt solutions began between -5 and -10×10^5 Pa (14). Loss of activity by *O. phyllobia* in SSS, which is essentially a balanced salt solution, also began between -5 and -10×10^5 Pa. The greater activity of *O. phyllobia* in SSS than in sucrose and NaCl at the same water potential is in agreement with the need reported for external Ca^{2+} and K^+ for volume regulation in other species (14).

When effects of various modifications of dilute SSS (100 meq/liter) were compared, only NaSSS appreciably affected nematode activity, suggesting that the relative concentration of sodium ions may be important to movement by *O. phyllobia* J4 in wet soil at high water potentials. The comparative survival of *O. phyllobia* in NaCl, SSS, and sucrose suggests that unbalanced NaCl is directly toxic or predisposes nematodes to injury from water stress.

The insensivity of *O. phyllobia* to wide variations of pH in the SSS agrees with the observation of Wallace (13) that there is little evidence that plant-parasitic nematodes are directly affected by pH over ranges that occur in soil.

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