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Effects of the Ionic Composition and Water Potential of Aqueous Solution on the Activity and Survival of *Orrina phyllobia*[1]

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Abstract: The activity and survival of *Orrina phyllobia* fourth-stage juveniles (J4) were examined in aqueous solutions representing 96 combinations of eight predominant soil solution ions at total concentrations of 100, 200, and 1,000 meq/liter. Various water potentials were imposed by the addition of mannitol or polyethylene glycol to ionic solutions. Nematode longevity increased as water potential was decreased. Longevity was approximately doubled at a water potential of -23×10^5 Pa and more than tripled at -60×10^5 Pa. No combination of ions at 200 meq/liter was lethal after a 6-day exposure. Several ion combinations significantly increased longevity at -10 and -23×10^5 Pa. Single cation Na^+ solutions consistently inhibited activity and more than doubled nematode longevity.

Key words: longevity, soil solution, osmolality.

Soil-inhabiting nematodes are essentially aquatic organisms requiring water films for movement. Soil water is highly variable with respect to its chemical potential and ionic composition. Variations are temporal, depth dependent, and geographic. Little quantitative information exists concerning the interactive effects of water

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potential and inorganic ions on nematode activity and survival. Most previous studies either examined nematodes in aqueous solutions with fixed ion ratios or examined nematodes extracted from complex matrices, such as soil, feces, or plant material, where micro-environmental variables were undefined (1,2,10,12).

Systematically varying soil solution ionic composition and water potential is complex. An array of solutions comparing only three concentrations of each of eight predominant soil ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, HCO_3^-, and SO_4^{2-}) may contain as many as 3^8 or 6,561 different ion combinations. The situation is further complicated by the need to control total water potential independently of ion concentrations. The magnitude of the matric component of soil water potential may be 10 or more times that of the solute component (5).

In a previous paper (9), we compared activity levels and survival rates of J4 of *Orrina phyllobia* (Thorne) Brzeski (= *Nothanguina phyllobia* Thorne) in ionic and nonionic solute systems separately at various water potentials. In this paper are described effects on activity and survival caused by differentially controlled variations in water potential and ion concentrations in solutions containing both ionic and nonionic solutes. The simultaneous comparison of numerous test solutions on physiologically identical nematode populations is facilitated by photographic data collection.

Materials and Methods

Nematodes were obtained and stored as previously described (8). Nematodes were transferred to and from solutions via membrane filters. Transfer technique (elimination of electrolyte) was verified by measuring the electrical conductivity of nematode suspensions after transfers from salt solutions to distilled water. All incubations were at 23 ± 0.5 C.

Most experiments simultaneously compared effects of 72 or 96 solutions on separate nematode aliquots (ca. 1,500 nematodes/aliquot) randomly drawn from a stock suspension containing 2×10^6 freshly hydrated juveniles. Large numbers of solutions prohibited the time-consuming techniques of visually counting moves per unit time or the percentage of nematodes moving. As an alternative, a photographic technique was developed for recording the percentage of nematodes moving whereby data with acceptable sensitivity (± 15% at $P = 0.05$) was obtained for 30–50 aliquots/hour.

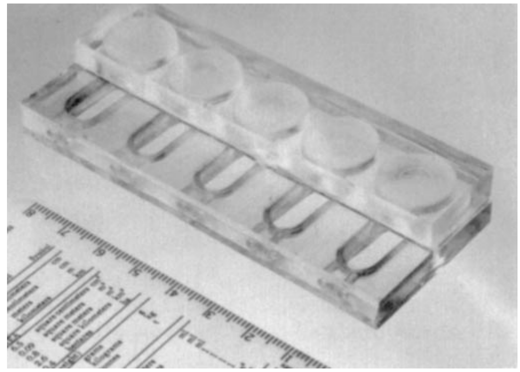


FIG. 1. Container used for photographing nematodes.

The photographic technique consisted of triple-exposing film on a stereomicroscope modified for photography. The use of slow-speed fine-grained film (ASA 32, Kodak Panatomic X) permitted photography at low magnification (12.5×) of 50–200 nematodes within a single 35-mm frame. Five photographs were taken per treatment. Triple exposure at 1-second intervals was achieved automatically.

Containers that sandwiched 4.5-mm layers of nematode suspensions between plate glass (Fig. 1) were used to maintain nematodes randomly dispersed and forced them to settle quickly into microscope field depth. Each container had five separate 2-ml chambers. Five ml of each nematode suspension was partitioned equally among the five chambers of a container and each chamber was photographed separately. Each moving nematode yielded three light but distinct sequential photographic images, whereas each still nematode yielded a dark image (Fig. 2). The percentage of nematodes detected moving with the photographic technique was referred to as the survival index (SI). Binomial confidence limits were generated about SIs according to Mack (6).

The relationship between SI and the true

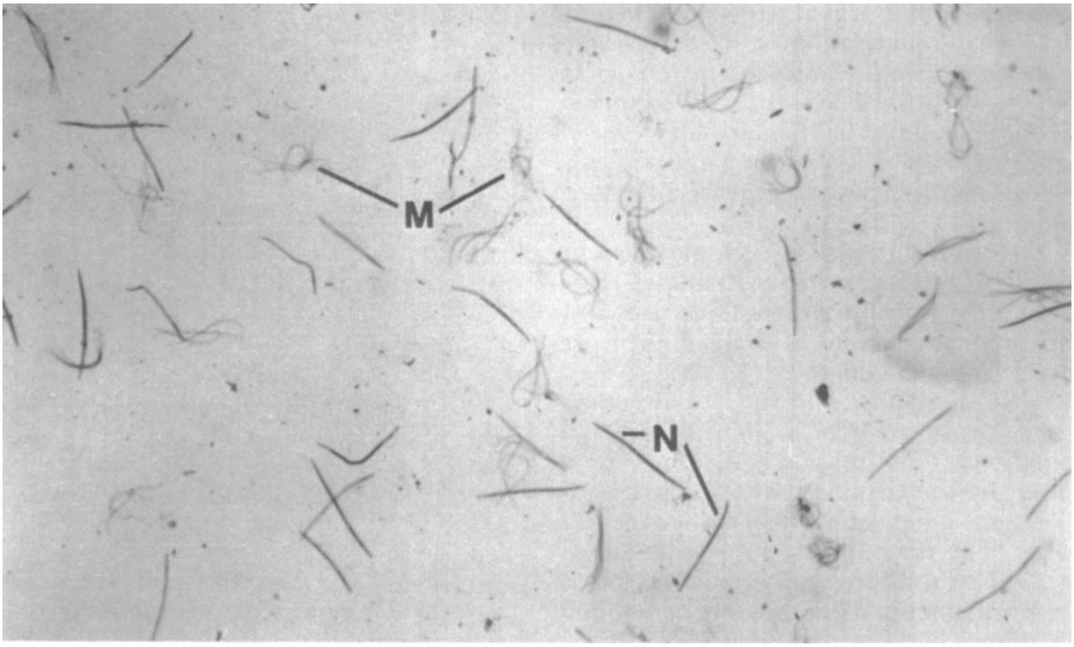


FIG. 2. Typical photograph of *Orrina phyllobia* J4 obtained with the triple-exposure technique. Note the three light but distinct images obtained for each nematode that is moving (M) and the single dark image obtained for each nematode that is not moving (N).

percentage moving was determined by photographing 11 nematode suspensions with known percentages moving between 0 and 100. Suspensions were prepared by mixing 100% heat-killed with 100% active nematodes. Survival index (the photographically observed percentage moving) was a near-linear function of the prepared percentage alive (Fig. 3). Based on data previously collected during the starvation of *O. phyllobia* J4 at 23 C, the survival index was also linearly related to the activity index (average rate of movement for 50 nematodes, Fig. 4) that we used in previous research with *O. phyllobia* J4 (8,9).

The experimental solutions used to compare the effects of ions were derived from 96 stock solutions (1,052 meq/liter). These solutions comprised all isoequivalent combinations of one, two, three, or four cations combined with all isoequivalent combinations of one, two, three, or four anions that could be generated within solubility limits, of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, HCO_3^-, and SO_4^{2-}. Stock solutions were prepared by volumetrically mixing single salt solutions whose freezing point depressions had been checked against published data (3). Cation concentrations of final stock solutions were verified by atomic absorption spectroscopy. Stock solutions were mixed with distilled water, polyethylene glycol

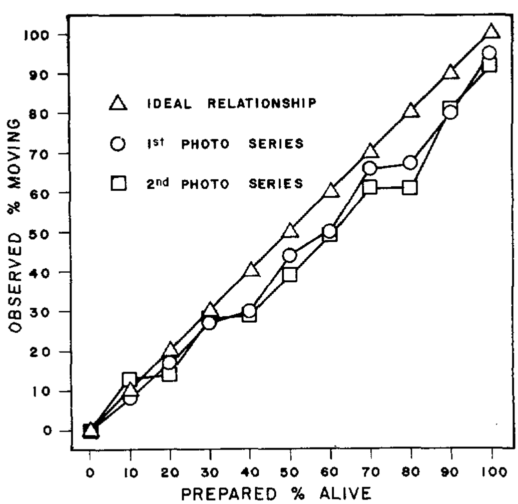


FIG. 3. Relationship between the photographically observed percentage moving and the percentage alive, for 11 suspensions of *Orrina phyllobia* J4 prepared by mixing 100% heat-killed with 100% active nematodes. Data for two photo series of the same nematodes.

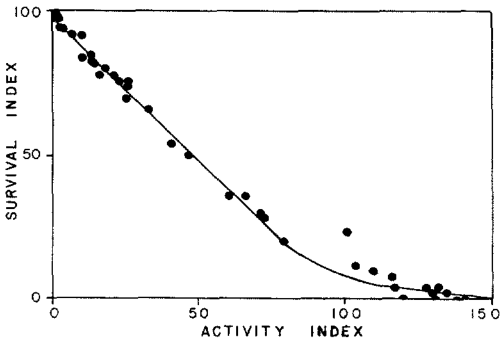


FIG. 4. Relationship between the survival index (percentage moving) and the activity index (average rate of movement, undulations/15 seconds) measured for starving *Orrina phyllobia* (50 J4/datum, 23 C).

(PEG), or mannitol to produce experimental solutions at desired water potentials and desired ion concentrations.

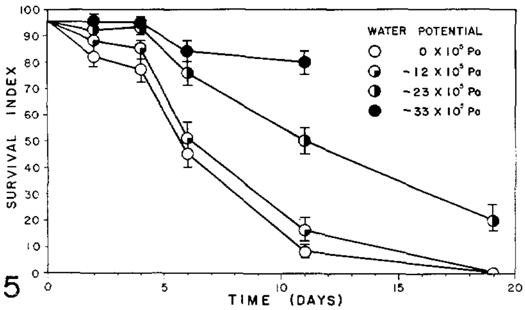
Five experiments were conducted. Cumulatively, they compared nematode activity and survival at water potentials ranging from 0 to -33×10^5 Pa in the absence and in the presence of various combinations of ions at total concentrations ranging from 100 to 1,000 meq/liter. In the first experiment, ionic solutes were not included. Nematodes were placed in distilled water and six PEG 6,000 (6,000 average molecular weight) solutions at water potentials ranging from 0 to -33×10^5 Pa. After time intervals of 2, 4, 6, 11, and 19 days, nematodes were transferred to distilled water for 20–28 hours and photographed.

In the second experiment, nematodes were placed in distilled water, mannitol solutions at -23×10^5 Pa, and each of the 96 stock solutions (1,052 meq/liter) adjusted with mannitol to $-23.0 \pm 0.5 \times 10^5$ Pa. After 3 days, nematodes were transferred to distilled water for 20–28 hours and photographed. In the third experiment, nematodes were placed in distilled water, PEG 6,000 at -24×10^5 Pa, and $0.2\times$ dilutions (200 meq/liter) of each of the 72 stock solutions not containing HCO_3^-. Water potentials of salt solutions were adjusted to -24×10^5 Pa with PEG 6,000. After 6 and 19 days, nematodes were transferred to distilled water for 20–28 hours and photographed. The fourth experiment was identical to the third experiment, except that water potentials were adjusted to -10×10^5 instead of -24×10^5 Pa and transfers to distilled water were made at 2 and 12 days instead of 6 and 19 days. In the second, third, and fourth experiments, nematodes were transferred into hyperosmotic solutions through eight equal increases in water potential over a 10-hour period. In the fifth experiment, activity levels were estimated visually after 3 days in $0.1\times$ dilutions (100 meq/liter) of each of the 72 stock solutions used in experiments three and four. Water potentials (ca. -2×10^5 Pa) were not adjusted with PEG, and nematodes were not reacclimated in distilled water.

RESULTS

In PEG solutions containing no added ions, nematode survival was prolonged at low water potentials. Survival indices of nematodes at -23×10^5 Pa decreased half as fast as survival indices in distilled water (Fig. 5). Plots of SI against water potential at each time examined extrapolated to about -50×10^5 Pa, suggesting that death occurs very slowly at that water potential (Fig. 6). The low solubility of PEG 6,000 prevented the examination of survival in PEG at -50×10^5 Pa; however, in two separate trials, 100% survival was obtained after 3 weeks in sucrose at -60×10^5 Pa. Survival at water potentials at and above -10×10^5 Pa was not appreciably prolonged and after 19 days most nematodes were dead.

Harmful effects occurred within 3 days in many solutions with ion concentrations of 1,052 meq/liter (Fig. 7). Survival indices measured after 3 days in the 96 solutions adjusted to -24×10^5 Pa with mannitol ranged from 0 to 95. Each of these survival indices was based on the percentage of 200–500 nematodes photographically detected moving, and binomial confidence limits ($P = 0.05$) of these and subsequent data were within 10 SI units of the values given. Particularly noticeable were a high level of variability in SI not explained by experimental error in SI measurement, generally lower SIs in solutions to which HCO_3^- had been added, lethal effects from single cation Ca^{2+}, high SIs in solutions containing only SO_4^{2-} as anions, and SIs in single cat-



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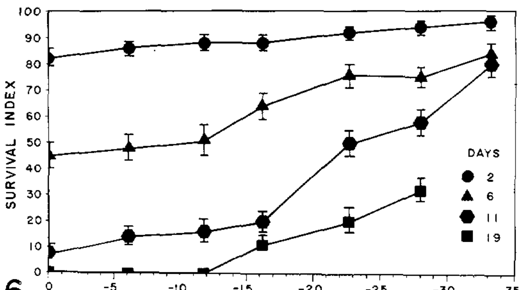

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FIGS. 5, 6. Survival index (photographically measured percentage moving) of *Orrina phyllobia* J4 after 20–28 hours in distilled water. 5) Following various time intervals in distilled water and polyethylene glycol solutions at -12, -23, and -33×10^5 Pa. 6) Following 2-, 6-, 11-, and 19-day exposure to various polyethylene glycol-induced water potentials. Brackets indicate binomial confidence limits at $P = 0.05$.

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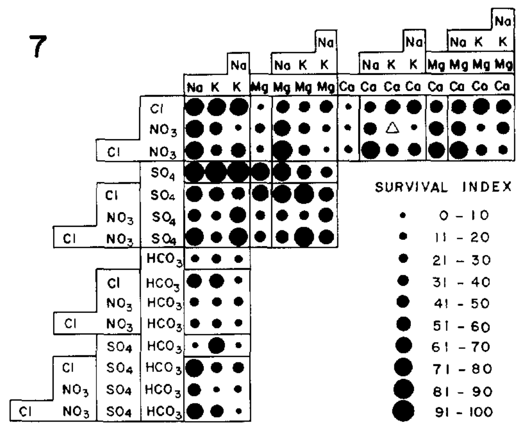


FIG. 7. Survival index (photographically measured percentage moving) of *Orrina phyllobia* J4 after 20–28 hours in distilled water following 3-day exposure to various isoequivalent ion combinations (1,052 total meq/liter) that were adjusted to -23×10^5 Pa with mannitol. Survival indices for nematodes that had been held in distilled water and in mannitol solution without ions at -23×10^5 Pa were 75 and 100 survival index units, respectively. Each survival index is derived from photographic images of 200–500 nematodes. Triangle indicates a missing datum.

ion Na^+ solutions similar to those in the controls.

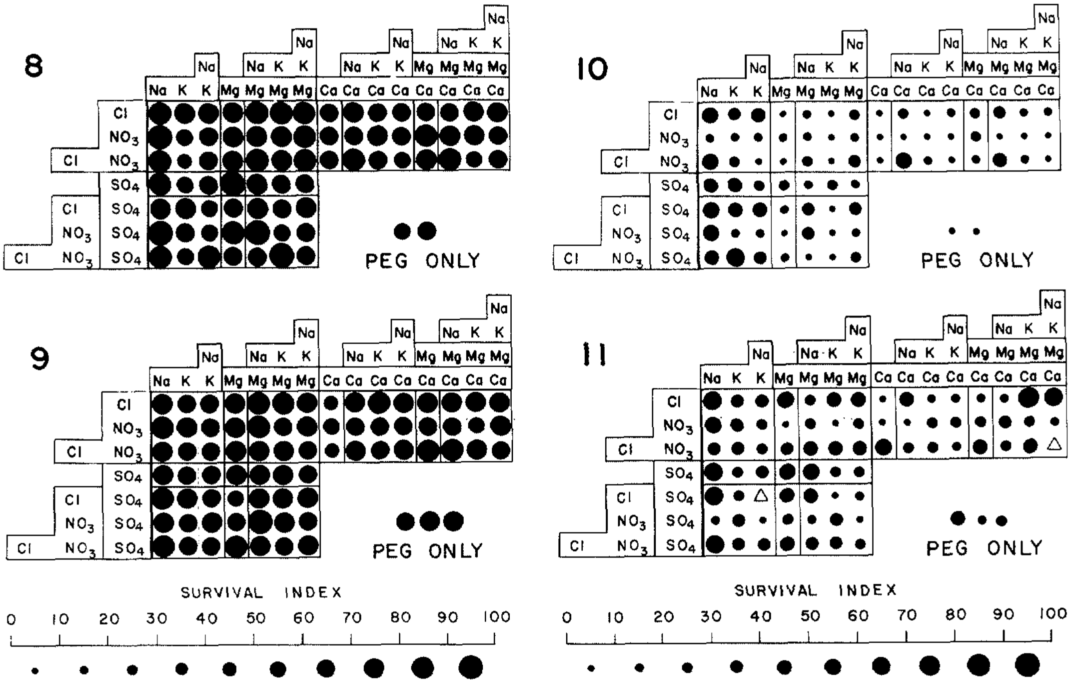
When the total salt concentration was reduced from 1,052 to 200 meq/liter, all ion combinations yielded high survival rates after 2 and 6 days at -10 and -24×10^5 Pa, respectively (Figs. 8, 9). Small decreases in SI were discernible only in single cation Ca^{2+} and K^+ solutions.

After 12- and 19-day exposures to -10 and -24×10^5 Pa, respectively, nematode survival rates were highly variable (Figs. 10, 11). Survival indices in solutions containing PEG but no added ions were similar to those after 12 and 19 days in the previously described experiment comparing survival in PEG solutions (Fig. 5). For nematodes from solutions containing ions in addition to PEG, many statistically significant differences between SIs occurred; however, only one pronounced relationship between ionic composition and SI was noted. At -10×10^5 Pa (Fig. 10), nematodes from six solutions containing only Na^+ cations had very high SIs (40–70) compared with nematodes from solutions containing only PEG (SI < 10). Nematodes in Na^+ solutions were motionless until transferred to distilled water. After 24 hours in distilled water, they had the optical refractivity and exhibited the smooth and rapid movements that are characteristic of freshly extracted *O. phyllobia* J4; nematodes from other solutions did not.

After 3 days at 100 meq/liter (-2×10^5 Pa), the complete cessation of nematode movement in Na^+ solutions was once again observed (Fig. 12), with a partial inhibition of activity also occurring in single-cation Mg^{2+} and two-cation Na^+/Mg^{2+} solutions.

DISCUSSION

Orrina phyllobia J4 at 23 C survive in distilled water (9) and in numerous salt solutions shorter times (1 or 2 weeks) than do juveniles of many nematode species. Short survival by *O. phyllobia* J4 has been noted in greenhouse pots and ground beds and in field plots (personal observations by the senior author). We have indirect evidence



FIGS. 8–11. Survival index (photographically measured percentage moving) of *Orrina phyllobia* infective juveniles after 20–28 hours in distilled water following 2-, 6-, 12-, or 19-day exposure to various isoequivalent ion combinations (200 total meq/liter) that were adjusted to -10 or -24×10^5 Pa with polyethylene glycol 6,000. Each survival index is derived from photographic images of 200–500 nematodes. 8) After 2-day exposure to -10×10^5 Pa. 9) After 6-day exposure to -24×10^5 Pa. 10) After 12-day exposure to -10×10^5 Pa. 11) After 19-day exposure to -24×10^5 Pa. Triangle indicates a missing datum.

that short survival by *O. phyllobia* J4 results from starvation. During 1 or 2 weeks at 23 C, *O. phyllobia* J4 undergo a marked decrease in optical refractivity, as has been associated with the depletion of lipid reserves in other species. Rapid starvation suggests a high metabolic rate and could be related to temperature, ionic or osmotic stress, or a high level of motility. *Orrina phyllobia* J4 are especially motile tylenchids. Body undulations in water at the thermal optimum for motility (ca. 24 C) are continuously propagated as fast as two undulations per second.

Our detection of numerous effects on nematode survival from variations in the ionic composition of aqueous solution gives credence to the hypothesis that ion concentrations in soil solutions are important to the suitability of soils for the survival of *O. phyllobia* and other nematodes. We emphasize that each SI was based on the motility of more than 200 nematodes and where each nematode is considered a statistical replicate, many effects were significant. However, most of them were not duplicated experimentally and should be re-examined. Effects that resulted when-

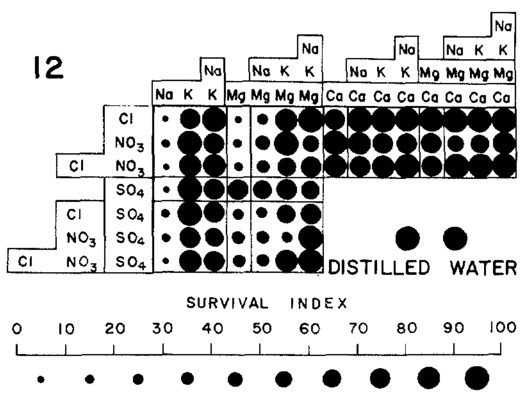


FIG. 12. Survival index (visually estimated percentage moving) of *Orrina phyllobia* in solutions with various isoequivalent ion combinations (100 total meq/liter). Each survival index given was estimated by observing > 1,000 J4.

ever Na^+ was the only cation in solution were duplicated several times. For other effects, there is necessarily uncertainty concerning possible contributions from unknown variations in transfer technique, microbiotic contaminants, etc. There is also uncertainty concerning the biological significance of the short time used to transfer nematodes from distilled water through increasingly concentrated solutions to final test solutions (10 hours). Comparable loss of soil moisture is likely to occur over a longer period (days). Ten hours was used in our experiments because it was already known that *O. phyllobia* J4 die within several days in synthetic soil solutions and because the motility of *O. phyllobia* J4 previously had been found to stabilize within 8 hours after transfer from distilled water to NaCl and synthetic soil solutions.

Solutions which contained only Na^+ cations consistently inhibited motility and markedly prolonged survival. Since the concentrations of other cations were essentially zero, sodium ion-induced survival prolongation may not be important in nature. However, relative Na^+ concentrations in soil solutions increase during soil drying, through cation exchange and the precipitation of calcium and magnesium carbonates. Other species may be more sensitive to Na^+. The effects of Na^+ on nematode survival in solutions with low concentrations of other cations should be examined. It would be desirable, in fact, to expand the entire collection of solutions we used to include solutions that would be more realistic in terms of low ion concentrations. It is noteworthy, nonetheless, that changes in solution ionic composition at edaphically realistic total ion concentrations (100 and 200 meq/liter) were associated with large changes in nematode motility and survival.

Effects from variation in total water potential were bigger than effects from variations in solution ionic composition. The latter effects might be augmented under conditions where the survival of *O. phyllobia* J4 is prolonged appreciably, such as at low temperature or at very low water potentials. At 4 C, *O. phyllobia* J4 in distilled water survive more than 100 days (8). Low water potential appreciably prolonged survival only at water potentials below the classical permanent wilting point for plants (-15×10^5 Pa). Such water potentials would be typical only in the surface layer of soil in many agronomic situations. It can be argued that at these water potentials, effects from dissolved ions are not likely to be important, since nematodes would be in contact with only minute quantities of liquid water. At -15×10^5 Pa, the maximum effective soil pore diameter for hydraulic conduction should be 0.2 μm, according to the capillary rise equation (7). Nematodes are generally thought to migrate through soil with little or no disturbance of soil particles (13) and should lie within channels with diameters 100 times greater (25 μm). A nematode might be in contact with individual clay crumbs with larger radii, and a clay crumb is comprised of numerous micelles separated by thin water films. However, at -15×10^5 Pa, the likelihood of capillary continuity between crumbs is small. Consequently, the total quantity of dissolved ions to which a nematode would be exposed also should be small.

Wright and Newall (14) were hesitant concerning the importance of matric potential to water balance in soil nematodes. Conversely, plant physiologists have long recognized matric potential to be the most important component of total soil water potential governing plant water regulation. Such contrasting viewpoints between zoologists and botanists are understandable since zoologists traditionally studied water movement in animals that were not soil inhabitants, where water movement across membranes could be reasonably well explained in terms of differences in osmotic pressure. The use of PEG to simulate soil solution matric potential is novel in this regard. Polyethylene glycol has been used for many years to control or simulate matric potential in the study of plant water relationships (5), and the use of PEG for investigating microfloral growth in relation to soil moisture has also been encouraged (4). Polyethylene glycol suspensions in water are not true solutions. They exhibit the Tyndall effect characteristic of colloids, and when various molecular weights are compared, they generate water potential as a function, not of molar concentration, but of percentage by weight. Steuter et al. (11) proposed that PEG is, in fact, a matricum, as is the colloidal fraction of soil, rather than an osmoticum. Polyethylene glycol suspensions may approximate in some important respects conditions to which nematodes are exposed in the soil.

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