On the Methodology of Nematode Extraction from Field Samples: Density Flotation Techniques

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Abstract: Density flotation has been frequently used for the extraction of nematodes from field samples. Density flotation curves for four nematode species and five solutes have been prepared. The curves confirm that flotation was governed by several factors: solute density, solute osmotic activity, and physiological properties of the nematode species. Nematode viability and function can be adversely affected by improper selection of solute for density extraction of nematodes; nevertheless, some nematode species can be enriched from mixtures by density and solute selection. *Key words:* osmosis, viability. Journal of Nematology 15(3):444-449. 1983.

Density flotation is one of the more common methods used for the extraction of nematodes. Recommendations (1,4,5,6,7) often involve sugar solutions at a density of approximately 1.2 gm/ml. This method is adequate if the demands made on the process remain modest; i.e., if the need emphasizes cleanliness or comparison of the same kind of nematode from nearly identical sample sources rather than biological quality of the product.

The growing need of the agricultural community for quantitative determinations of nematodes in soil samples, and of the necessity to the science to preserve the biological quality in nematode extractions from soil, requires more circumspection in separatory procedures. It seems naive to expect the thousands of species of nematodes to behave identically in sucrose solutions of specific density 1.2 gm/ml or that their biological quality should remain highly preserved. This report explores the behavioral and sedimentation properties of four species of nematodes in solutions of different densities prepared from five different solutes.

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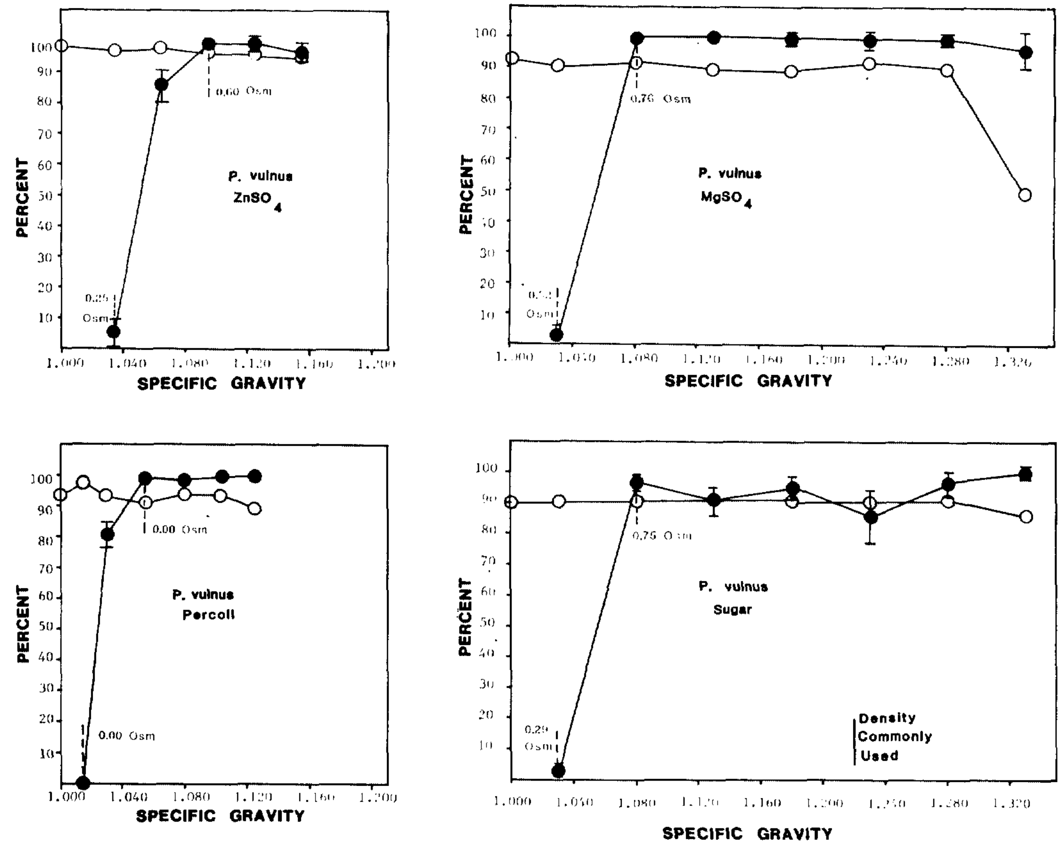
MATERIALS AND METHODS

Clean stocks of *Pratylenchus vulnus* (Allen & Jensen, 1951), *Criconemella xenoplax* (Raski, 1952) Luc & Raski, 1981, *Xiphinema index* (Thorne & Allen, 1950), and *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 were obtained from laboratory or greenhouse cultures by extraction methods other than density flotation. Density solutions were prepared from chemically pure zinc sulfate, magnesium sulfate, commercial household sugar, Ficoll, and Percoll. Ficoll is a synthetic, high molecular weight polymer of sucrose and epichlorohydrin, whereas Percoll is composed of colloidal silica coated with polyvinyl pyrrolidone; both are used in density gradient centrifugation of cells, provide low osmolality, and are available from Pharmacia Fine Chemicals.

The density separation was carried out in the following manner: an aliquant of 50–100 nematodes were placed into 15-ml conical centrifuge tubes with additional water so that the nematodes were in suspension in a 15-ml volume. The suspension was centrifuged for 1 min at 700 × g after which the supernatant was decanted. Density solution (15 ml) was added to the pellet and



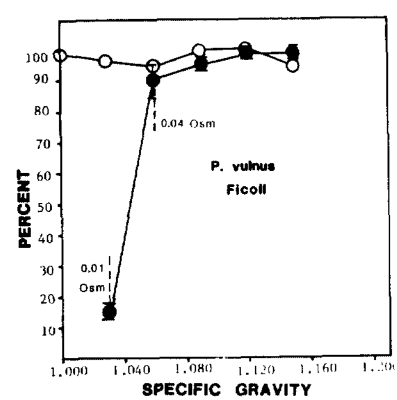


Fig. 1. The responses of *Pratylenchus vulnus* to solutions of different specific gravities prepared with the five solutes indicated. Open circles indicate the motility percentage of the recovered population at each specific gravity tested. Filled circles indicate the percentage of the motile population recovered from suspension. Standard deviation (SD) for averages of the motile population recovered from suspension are indicated; absence of indicators means the SD lies within the point. Osmolarity of solutions are indicated for the rapidly changing portions of the curve.

the suspension stirred vigorously then again centrifuged for 1 min at 700 × *g*. The suspension was poured through a 38-μM mesh screen; the screenings were rinsed off into a petri dish with water and counted. The pellet was washed into another petri dish with water and the nematodes counted. Each nematode was tested for motility; a motile nematode was defined as one which moved spontaneously or responded to a touch stimulus. Osmolality was determined with a freezing point osmometer. The experiment was replicated six times.

RESULTS AND DISCUSSION

The percentage of motile nematodes suspended in five different solute solutions of varying densities and the percentage of motile nematodes in the population at these different densities are summarized in Figure 1 (*Pratylenchus vulnus*), Figure 2 (*Cri-

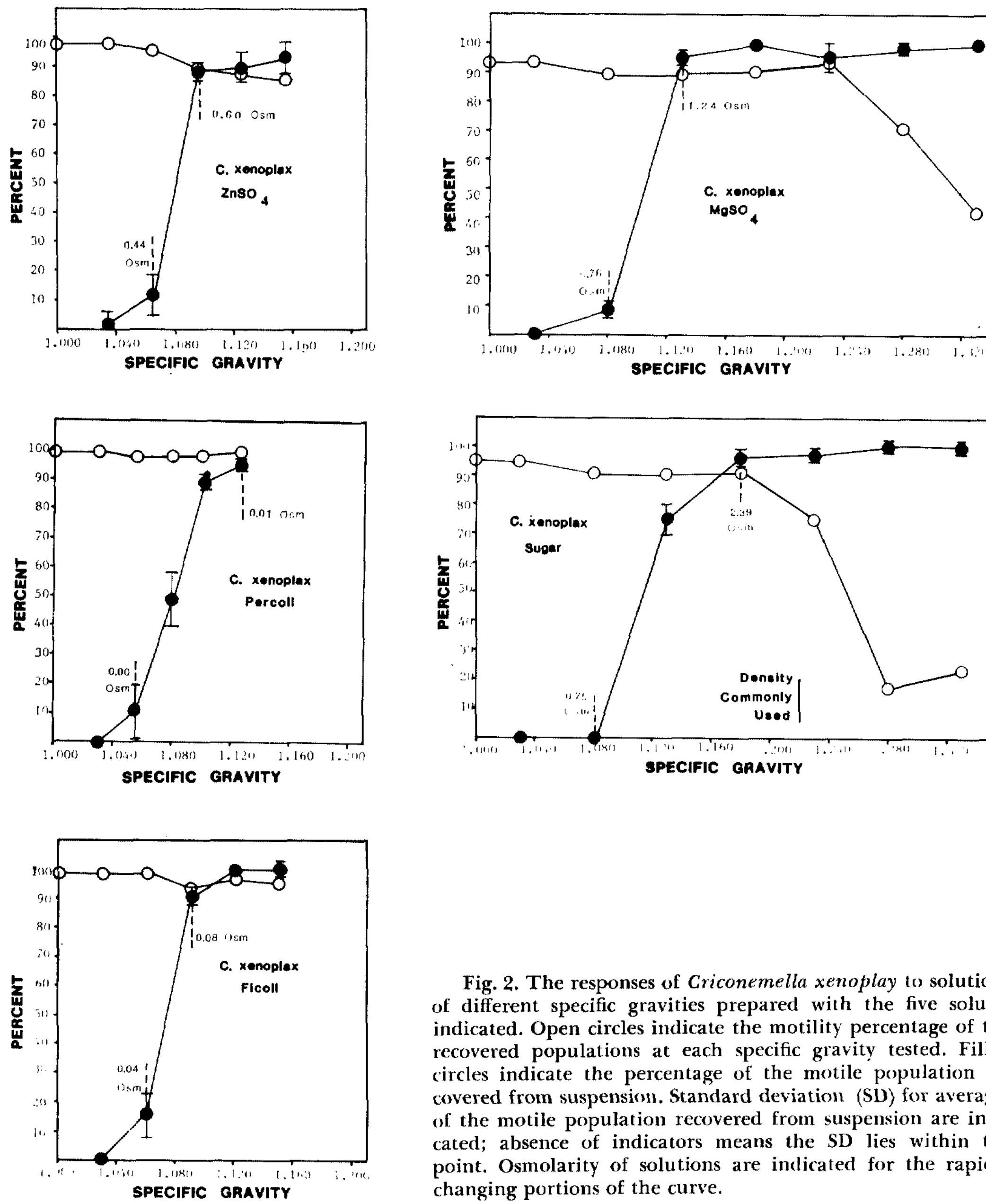
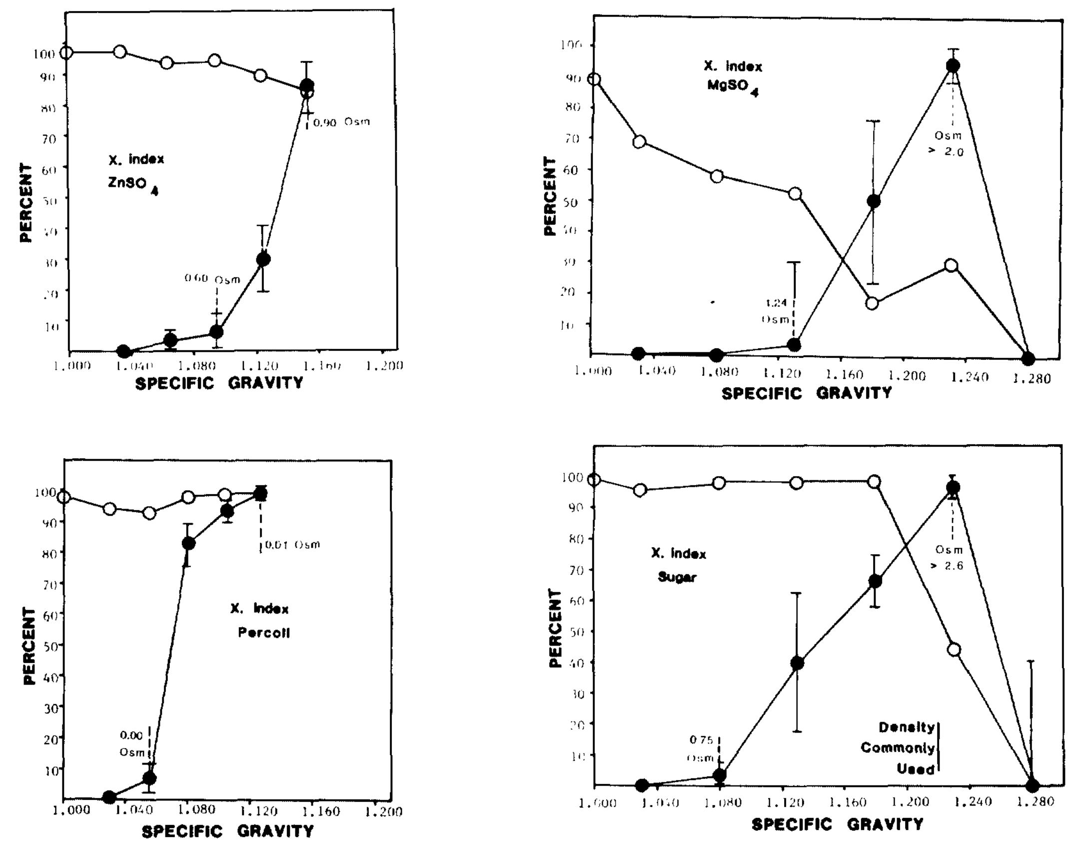


Fig. 2. The responses of *Criconemella xenoplay* to solutions of different specific gravities prepared with the five solutes indicated. Open circles indicate the motility percentage of the recovered populations at each specific gravity tested. Filled circles indicate the percentage of the motile population recovered from suspension. Standard deviation (SD) for averages of the motile population recovered from suspension are indicated; absence of indicators means the SD lies within the point. Osmolarity of solutions are indicated for the rapidly changing portions of the curve.

conemella xenoplax), Figure 3 (*Xiphinema index*), and Figure 4 (*Meloidogyne incognita*).

For a nematode to remain in suspension or to float, the density of the bathing solution must be equal to, or greater than, the average density of the nematode particle. The body wall separates the nematode from the environment; within the wall an effective semipermeable membrane serves to preserve the solute balance between the body fluids and the bathing solution. It is known that nematodes respond differently, depending upon the solute used in making up the hypertonic solution (3,8). In solutions of low hypertonicity, the nematode shrinks more longitudinally than radially; with increasing hypertonicity of salt solutions, the nematode continues to exosmose and shrink so that the body wall takes on an accordion-like appearance after which the body distorts and collapses. In hypertonic solutions



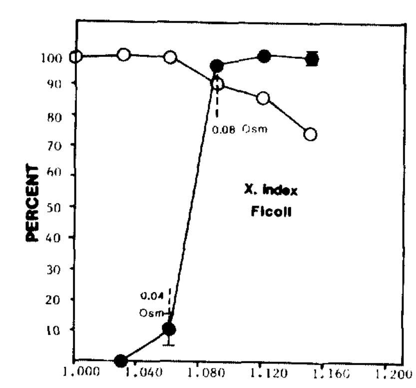
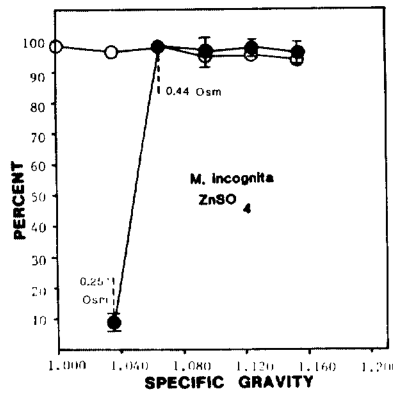
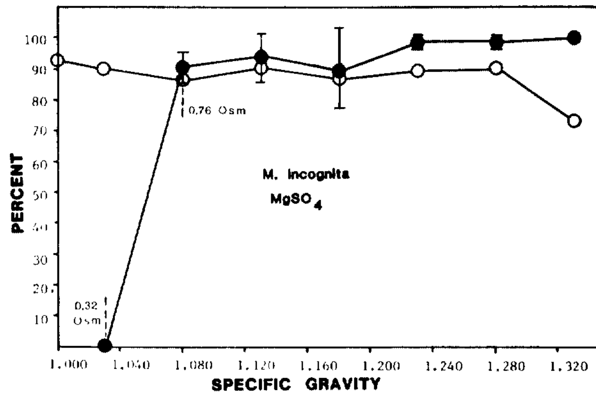


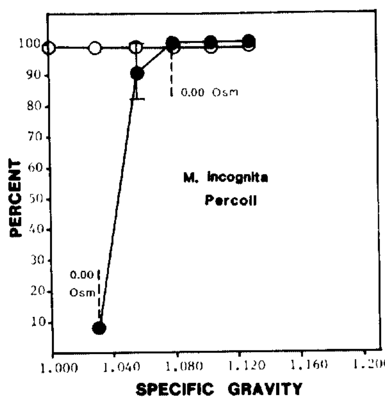
Fig. 3. The responses of *Xiphinema index* to solutions of different specific gravities prepared with the five solutes indicated. Open circles indicate the motility percentage of the recovered populations at each specific gravity tested. Filled circles indicate the percentage of the motile population recovered from suspension. Standard deviation (SD) for averages of the motile population recovered from suspension are indicated; absence of indicators means the SD lies within the point. Osmolarity of solutions are indicated for the rapidly changing portions of the curve.

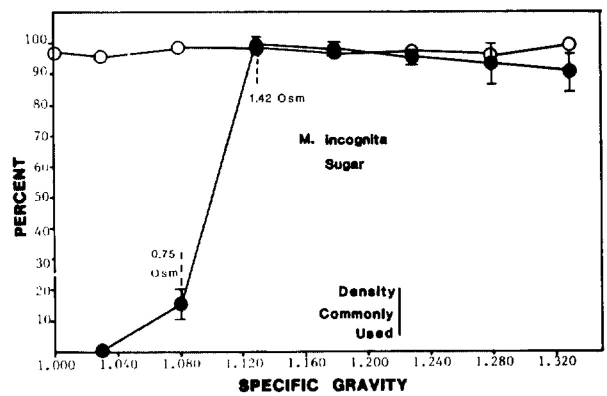
of sugar, the nematode, after the initial shrinkage, exosmoses and collapses to a ribbon form.

Nematodes treated with hypertonic solutions may or may not recover when transferred back into water, depending upon the solute used to make up the hypertonic medium, the strength of the hypertonic medium, the length of treatment, and the nematode species involved. Ficoll and Percoll constitute a different class of compounds in that they can be dissolved in water to increase density but are osmotically much less active than the other compounds used. Nematodes immersed in solutions of these substances exosmose very little; more of the animals remain spontaneously active, not only after transfer to water, but while









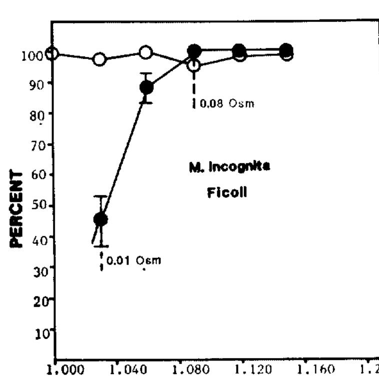


Fig. 4. The responses of *Meloidogyne incognita* to solutions of different specific gravities prepared with the five solutes indicated. Open circles indicate the motility percentage of the recovered populations at each specific gravity tested. Filled circles indicate the percentage of the motile population recovered from suspension. Standard deviation (SD) for averages of the motile population recovered from suspension are indicated; absence of indicators means the SD lies within the point. Osmolarity of solutions are indicated for the rapidly changing portions of the curve.

immersed in density solutions. Unfortunately, the motility evaluation is an imprecise determination; normally when nematodes immersed in osmotically active solutions are transferred into water, the proportion of animals that are spontaneously active to those that are responsive to touch stimulus decreases with increasing solute density. The decrease in motility for all four nematodes at high densities of magnesium sulfate, as well as for *C. xenoplax* and *X. index* in sugar solutions, is reflective of increasing injury to nematodes with increasing hypertonicity. Furthermore, the different responses illustrate the differential sensitivity of nematodes to density and, perhaps more important, to hypertonicity and other solute properties. It is clear that in all cases, except for *X. index* immersed in solutions of approximately 1.28 sp gr prepared with magnesium sulfate or sugar, membrane function existed. In both exceptions, motility and buoyancy fall to zero. This suggests a collapse in membrane function thereby allowing free exchange of immersion solution through the *X. index* body wall; under centrifugation the dead nematodes precipitate and collect in the pellet. The motility determination constitutes a crude approximation of nematode injury with increasing density solutions. The density at which other nematode properties (e.g., dispersion, sensory function, penetration, development, and reproduction) become impaired have yet to be determined. If density flotation is to be used as an extraction procedure for a target nematode, it appears prudent to utilize the lowest effective density using a solute producing the lowest osmotic activity. Nevertheless, other factors are involved; e.g., *X. index*-Ficoll, where a polymer at low density and very low osmotic activity reduces motility as does $MgSO_4$ at the same density but at higher osmolality. The mechanism by which a supposedly benign synthetic polymer preparation reduces motility of *X. index* but not the other nematodes is open to speculation. These results have been based upon the use of pure nematode populations, pure solute solutions, and one operator faithfully endeavoring to reproduce each operation. It remains to be established to what degree various soil types, associated debris, or different operators have on the reliability of the extraction process. In summary it appears that there may be more appropriate solute solutions than the conventional sucrose solution for the extraction of certain nematodes (2). Furthermore, results suggest that certain nematode species may be separated to over 75% purity by the appropriate selection of solutes and densities.

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