Influence of concentration gradients of salts on the movement of second stage juveniles of *Meloidogyne javanica*

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SUMMARY

The movement of juveniles of *M. javanica* (Treub, 1885) Chitwood, 1949 was studied in salt gradients. Twelve different salts (NaCL ; $CaCl_2$; KCl ; $MgCl_2$; $FeCl_2$; $NaNO_3$; Ca $(NO_3)_2$; KNO_3 ; KH_2PO_4 ; NaH_2PO_4 ; $MgOS_4$; $FeSO_4$) were tested on agar under sterile conditions. With the exception of $FeSO_4$ which failed to exhibit a significant repulsion, all other salts exhibited repulsion at concentration close to those found in the soil. In effect, juveniles placed in gradients created in agar by salt solutions between 0.125×10^{-2} and 0.5×10^{-2} M/l moved preferentially toward the region having the lower salt concentration.

Osmotic pressure, pH and red-ox potential cannot explain the negative tropism exhibited to mineral salts by juveniles of *M. javanica.* It is concluded that this negative tropism is of chemical nature.

RÉSUMÉ

Orientation du déplacement des juveniles de 2e stade du nématode Meloidogyne javanica *(Treub, 1885) Chitwood, 1949 dans des gradients de concentration de sels minéraux.*

Le déplacement des juvéniles de *M. javanica* a été observé en conditions stériles dans des colonnes de gélose supportant des gradients de sels minéraux, les juvéniles étant déposés au milieu du gradient. Douze sels ont été expérimentés : NaCl ; $CaCl_2$; KCl ; $MgCl_2$; $FeCl_2$; $NaNO_3$; Ca $(NO_3)_2$; KNO_3 ; KH_2PO_4 ; NaH_2PO_4 ; $MgSO_4$; $FeSO_4$; la concentration à l'extrémité du gradient où elle est la plus élevée, varie de 0 à 2×10^{-2} M/l.

A l'exception de $FeSO_4$, tous les sels minéraux testés provoquent un effet répulsif significatif sur les juvéniles de *M. javanica,* ces derniers se déplaçant vers la zone la moins concentrée en sel. Ces nématodes sont sensibles à des concentrations salines voisines de celles existant dans les sols, des répulsions significatives étant observées dans des gradients dont la concentration, la plus élevée, varie de $0,125 \times 10^{-2}$ à $0,5 \times 10^{-2}$ M/l.

Par contre, aucune répulsion ni attraction significatives n'ont été observées dans des gradients de sucre (glucose et saccharose) où la concentration la plus forte, varie de 0 à 4×10^{-2} M/l.

Aucune relation n'a pu être établie entre l'intensité de l'effet répulsif et les différences de pH, de potentiel red-ox ou de pression osmotique existant entre les valeurs extrêmes des gradients établis. Il est donc suggéré que la sensibilité aux sels minéraux des juvéniles de *M. javanica* est de nature chimique.

At the present time little information is available on the influence of mineral salts on the migration of phytoparasitic nematodes. Dropkin, Martin and Johnson (1958) demonstrated that high salt concentrations created sufficient osmotic pressure to inhibit hatching of *Heterodera* and *Meloidogyne* species and also inhibited movement of juveniles within the eggs. Ibrahim and Hollis (1967) stated that *Tylenchorhynchus martini* Fielding, 1956 was attracted to 0.1 M $AlCl_3$ and to 0.25 M $CaCl_2$, but was not attracted to other concentrations of these salts nor to NH_4Cl, $MgSO_4$ or $Na_2S_2O_4$. Evans (1969) indicated that $MgSO_4$ influenced the movement of males of *Heterodera rostochiensis* Wollenweber, 1923.

On the other hand, the free-living nematode, *Caenorhabditis elegans* (Maupas, 1900) Dougherty, 1953, has been shown to be attracted to the anions Cl^-, Br^-, and I^- and to the cations Na^+,

Li^+, K^+, and Mg^{++} (Ward, 1973). Dusenbery (1974) classified ions on their relative attractiveness to *C. elegans* and reported the following sequences : Na^+, K^+, 1/2 Mg^{++}, 1/2 Ca^{++} and Cl^-, 1/2SO_4^{--}, NO_3^-, CH_3COO^-.

The present study was undertaken to determine if concentrations of mineral salts close to those observed in cultivated soils influence the movement of 2nd-stage juveniles of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949.

Materials and Methods

Juveniles of *M. javanica* (Treub, 1885) Chitwood, 1949 used in these experiments were derived from a clone established from a single egg mass and maintained on egg-plant (*Solanum melongena* L.). Only individuals not more than 48 h in age and from the 1st generation were used.

Effects of salts on the movement of juveniles of *M. javanica* were studied in U-shaped « Pyrex » tubes 18 cm long with an interior diameter of 0.8 cm (Fig. 1). At the middle of tube, an opening was made for introducing the nematodes. The ends of the tubes were closed by an haemolysis tube joined to the main tube by a collar of plastic tubing (« Rhodorsil »). Sufficient 1.5 % agar was poured into the central part of U-tube to form a column 5 cm in length. Adhesion of the agar column to the inner surface of the tube was assured as previously described (Prot, 1975). Into one branch of the U-tube 2 cm^3 of the salt solution to be tested was introduced, whereas 2 cm^3 of demineralized water was placed in the other.

All necessary manipulations were made under sterile conditions ; all glassware, salt solutions, demineralized water and the agar were autoclaved (120 °C for 20 min). $FeSO_4$ and $FeCl_2$ were sterilized by filtration through a « Millipore » filter with 0.22 µm openings.

After the test solutions were introduced, the U-tubes were left for 48 h to allow establishment of a salt gradient in the agar. At this time, 10 juveniles of *M. javanica* (not surface sterilized) were introduced singly into the center of the agar by means of the opening previously described. After introduction of the juveniles, a small plug of « Kleenex » was introduced into the opening ; without this precaution, the nematodes stay in the water film existing on the agar surface.

The number of nematodes present in the liquids in both branches of the U-tube were counted 48 h after their introduction.

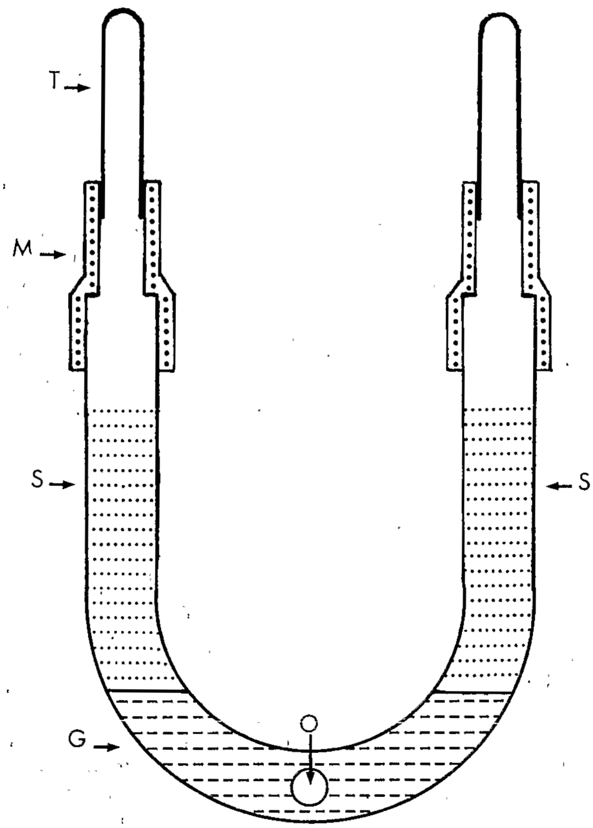

Fig. 1. Diagram of experimental apparatus. G : column of 1.5 % agar, 5 cm in length ; M : collar of "rhodorsil" tubing ; O : opening for introducing nematodes ; S : salt solution or demineralized water ; T : haemolysis tube.

The effects of twelve mineral salts were tested ; the salts used and their concentrations are indicated in Table 1. The pH and red-ox potential of each salt solution were measured with a pH meter and a millivolt meter (« Poly Metron 55B ») at the time they were introduced into the tube ; two measurements were made in the solutions for each concentrations.

Table 1

Concentrations of salts tested for their effects on movement of juveniles of Meloidogyne javanica

Salts	*Concentrations of solutions* ($M \times 10^{-2}/l$)							
	2	*1*	*0.5*	*0.25*	*0.125*	*0.062*	*0.031*	*0*
NaCl	+	+	+	+	+	+		+
$NaNO_3$	+		+		+		+	+
NaH_2PO_4	+		+	+	+		+	+
KCl	+		+		+		+	+
KNO_3	+		+	+	+		+	+
KH_2PO_4	+		+		+		+	+
$CaCl_2$	+		+	+	+		+	+
$Ca(NO_3)_2$	+		+		+		+	+
$MgCl_2$	+		+		+		+	+
$MgSO_4$	+		+	+	+		+	+
$FeCl_2$	+		+		+		+	+
$FeSO_4$	+		+	+	+		+	+

Thirty replications were used for the various solutions of NaCl ; twenty replications were used for all concentrations of the other salts tested.

Using the same techniques the effects of sucrose at concentrations of 0.062×10^{-2}, 0.125×10^{-2}, 0.25×10^{-2}, 0.5×10^{-2}, 1×10^{-2}, 2×10^{-2} and 4×10^{-2} M/l and glucose at concentrations of 0.125×10^{-2}, 0.5×10^{-2}, 1×10^{-2} ; 2×10^{-2} and 4×10^{-2} were tested. Thirty replications were used for each sucrose concentration and twenty for each glucose.

Results

Results of the experiments on the effects of twelve salts on the movement of juveniles of *M. javanica* are presented in Figure 2.

A mean of 6.7 juveniles of the 10 introduced per tube were recovered from the test solutions and demineralized water. Juveniles that remained in the agar were not sought.

There was never a significant difference between nematodes recovered in the left and right branches of the tube when the tubes received demineralized water in both branches (semicircles on the right and left of the ordinate of each figure).

With the exception of $FeSO_4$ all the salts tested exhibited a significant repulsion, juveniles moving preferentially toward the region having the lower salt concentration.

No correlation could be made between the repulsive effect and differences of osmotic pressure calculated or differences of pH and red-ox potential measured between the various test solutions and demineralized water.

In the experiments on the influence of sucrose and glucose on the movement of juveniles of *M. javanica*, no significant differences were found for either sugar at the concentrations employed.

Discussion

Among the twelve salts whose effects on the movements of juveniles of *M. javanica* were tested, only $FeSO_4$, failed to exhibit a significant repulsion. For all of the other salts, the repulsion was observed at salt concentrations close to those found in the soil. In effect, juveniles placed in gradients created in agar by salt solutions between 0.125×10^{-2} M/l and 0.5×10^{-2} M/l moved preferentially toward the region having the lowest salt concentration.

Repulsion by the salt solutions could be either chemical in nature or a result of differences in osmotic pressure, pH or red-ox potential between the salts tested and demineralized water. When the results of all the experiments are considered together in regard to differences between osmotic pressure of the test solutions and demineralized water, no influence of osmotic pressure can be demonstrated. This is also confirmed by the lack of significant repulsion by osmotic pressures of the same order obtained with sucrose and glucose. That movement of juveniles is not influenced by these sugars is in agreement with the results previously reported by Bird (1959) who also noted that extremes in pH were repulsive. In the results of this study, no influence of pH, within the range produced, could be detected on the movement of juveniles of *M. javanica*. In the same manner, if red-ox potential influences orientation of nematodes towards plant roots (Bird 1959, 1962), this effect was not shown in our experiments.

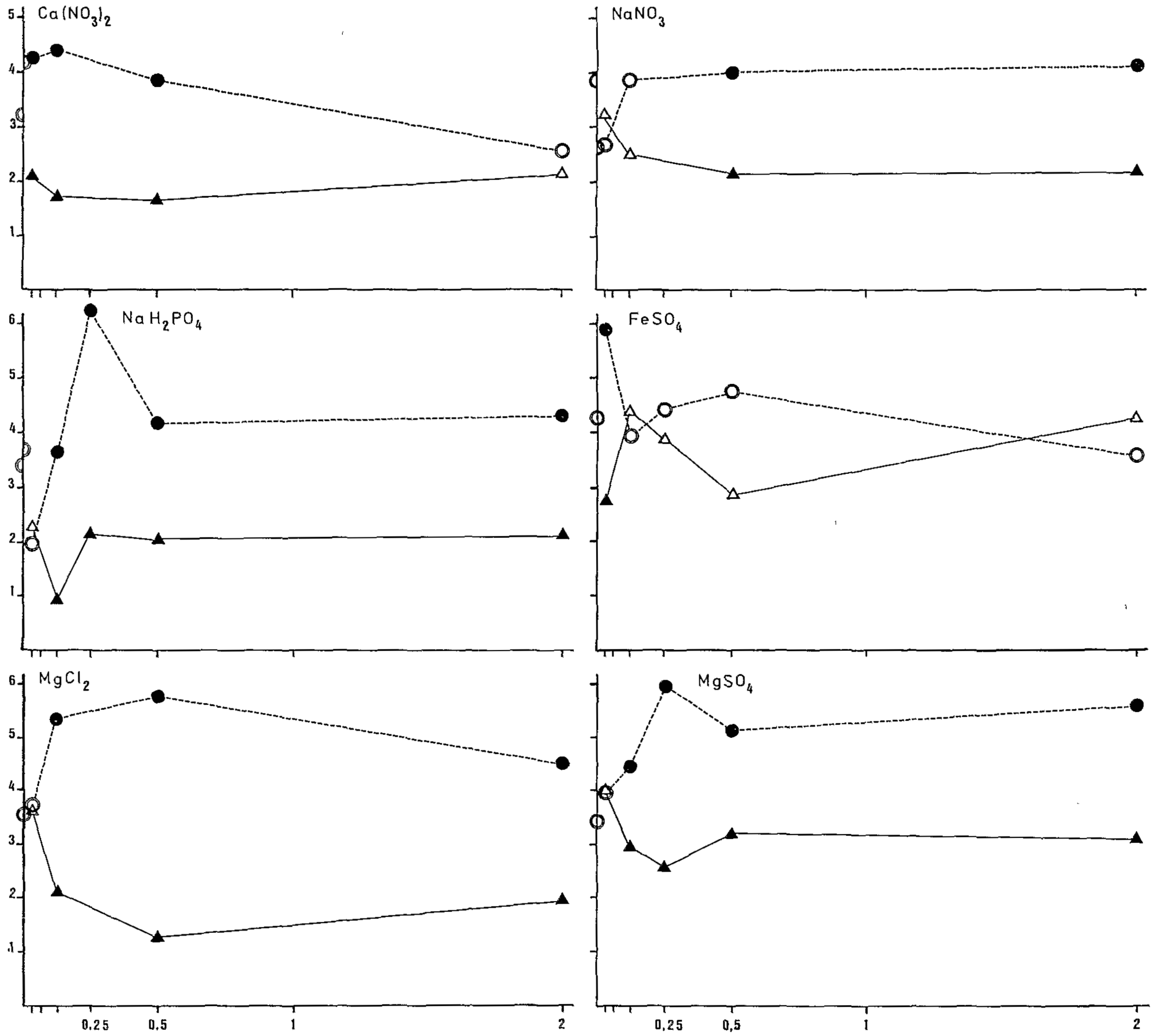

Fig. 2. Influence of twelve mineral salts on the movement of juveniles of *Meloidogyne javanica*. *Abscissa :* concentration of the salt solution as M $\times$ 10^{-2}/l. *Ordinate :* number of juveniles recovered in : dimineralized water (circles and dotted line) salt solution (triangles and solid line). Black circles and triangles indicate existance of a significant difference (Wilcoscon test at a probability of 0.05) between the numbers recovered in the two branches of the tube. On the contrary white circles and triangles indicate that the differences were not significant for that concentration.

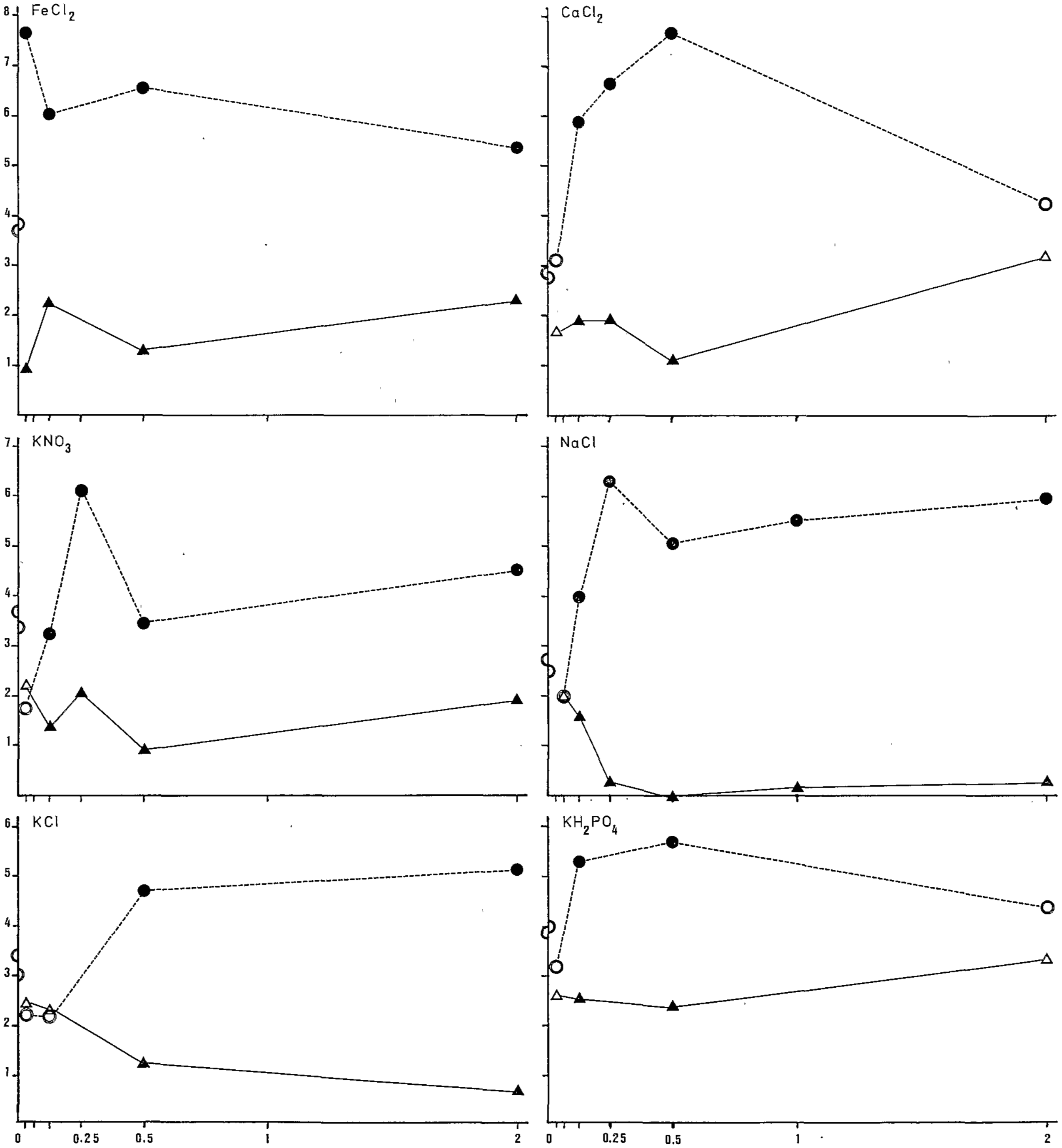

Fig. 2. Continued.

If osmotic pressure, pH and red-ox potential cannot explain the negative tropism exhibited to mineral salts by juveniles of *M. javanica*, it seems logical to conclude that the cause is of chemical nature. Perhaps the influence of the later masks the effects of the three others. This chemical sensitivity could explain why certain salts (NaCl, $FeCl_2$, $CaCl_2$) appear to be more repulsive than the others and why no significant repulsion was observed with $FeSO_4$. This chemical repulsion can also explain the observed significant repulsion of weak concentrations ($CaCl_2$ from 0.125 to 0.5×10^{-2} M/l) whereas there was no further effect at higher concentrations (2×10^{-2} M/l). At these higher concentrations, it is possible that the concentration at the point of introduction of the nematode was too high to permit orientation to occur.

Further investigations on this phenomenon are in progress.

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REFERENCES

BIRD, A. F. (1959). The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica*, 4 : 322-335.

BIRD, A. F. (1962). Orientation of the larvae of *Meloidogyne javanica* relative to roots. *Nematologica*, 8 : 275-287.

DROPKIN, V. H., MARTIN, G. C. & JOHNSON, R. W. (1958). Effect of osmotic concentration on hatching of some plant parasitic nematodes. *Nematologica*, 3 : 115-126.

DUSENBERY, D. B. (1974). Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by counter-current separation. *J. exp. Zool.*, 188 : 41-47.

EVANS, K. (1969). Apparatus for measuring nematode movement. *Nematologica*, 15 : 433-435.

IBRAHIM, I. K. A. & HOLLIS, J. P. (1967). Nematode orientation mechanisms. I. A method for determination (Abstr.) *Phytopathology*, 57 : 816.

PROT, J. C. (1975). Recherches concernant le déplacement des juvéniles de *Meloidogyne* spp. vers les racines. *Cah. ORSTOM, Sér. Biol.*, 10 : 251-262.

WARD, S. (1973). Chemotaxis by the nematode *Caenorhabditis elegans* : identification of attractants and analysis of the response by use of mutants. *Proc. natn. Acad. Sci. USA*, 70 : 817-821.