Horizontal migrations of second-stage juveniles of *Meloidogyne javanica* in sand in concentration gradients of salts and in a moisture gradient [1]

Jean-Claude PROT

Laboratoire de Nématologie, ORSTOM, B.P. 1386, Dakar, Sénégal.

SUMMARY

Horizontal migration of juveniles of *M. javanica* was studied in columns of sand in which was established either a moisture gradient or a concentration gradient of salt combined with a moisture gradient. The juveniles accumulated in the end of a moisture gradient having the highest moisture content. In concentration gradients of KH_2PO_4, KNO_3, $Ca(NO_3)_2$, $MgCl_2$ and of Hoagland's solution, the behaviour of these nematodes in relation to the moisture gradient was reversed : i.e. the juveniles moved preferentially toward the region having the lower salt concentration which also had the lower moisture.

The hypothesis is presented that at least in soils containing adequate quantities of mineral salts, a concentration gradient of salt created by dilution of the soil solution along a moisture gradient could induce a migration of the juveniles to the region having the lower salt concentration which is also the wet end of the moisture gradient. In this case a chemorepulsion could explain an apparent "hydrotaxis".

RÉSUMÉ

Migrations horizontales des juvéniles du deuxième stade de Meloidogyne javanica *dans le sable, dans des gradients de concentration de sels et un gradient d'humidité*

La migration horizontale de juvéniles de *M. javanica* a été étudiée dans des colonnes de sable où était établi un gradient d'humidité combiné ou non à des gradients de concentration de sels minéraux. Les juvéniles se regroupent à l'extrémité la plus humide d'un gradient d'humidité simple. Dans des gradients de concentration de KH_2PO_4, KNO_3, $Ca(NO_3)_2$, $MgCl_2$ et de solution de Hoagland le comportement de ces nématodes en relation avec le gradient d'humidité était inversé : les juvéniles se déplaçaient préférentiellement vers la région ayant la plus faible concentration en sels qui est aussi celle de plus faible humidité.

L'auteur émet l'hypothèse qu'au moins dans des sols correctement fournis en sels minéraux, un gradient de concentration de sels, créé par dilution de la solution de sol le long d'un gradient d'humidité, pourrait induire une migration des juvéniles vers la région ayant la plus faible concentration en sels, région qui est aussi la plus humide du gradient d'humidité. Dans ce cas, une répulsion d'origine chimique expliquerait une apparente « hydrotaxie ».

Recently, it has been demonstrated that juveniles of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 placed in salt gradients, created in agar by salt solutions of concentrations between 0.125×10^{-2} and 0.5×10^{-2} M/l, moved preferentially toward the region having the lower salt concentration (Prot, 1978 *a* and *b*). In addition, examination of tracks of juveniles in salt gradients indicated that the repulsion from high concentrations was the result of a real orientation of their movements (Prot, 1978 *b*).

In order to determine if concentration gradients of mineral salts were able to influence orientation of juveniles of *M. javanica* in a

(1) Communication 3rd International Congress of Plant Pathology, München, 16-23 August 1978.

column of sand, their migrations in a moisture gradient and in concentration gradients of salts combined with a moisture gradient were compared.

Materials and Methods

Juveniles of *M. javanica* used in these experiments were derived from a clone established from a single egg mass and maintained on kenaf (*Hibiscus cannabinus* L.) in the greenhouse. Only individuals not more than 48 h in age were used.

All experiments were made in polyvinyl chloride tubes 9.5 cm long with an interior diameter of 1.6 cm, closed at both ends by rubber stoppers that extended 1 cm into the tube ; thus, the useful length was reduced to 7.5 cm. At the middle of the tube, a 0.3 cm diameter opening was made for introducing the nematodes. The tubes were cut half way through 1.25 cm on either side of this opening, allowing division of the column of sand in the tube into three fractions of 2.5 cm. During the experiments the cuts and the opening were closed with adhesive tape.

Sterilized soil was washed to eliminate the clay and sieved through a serie of sieves of 100 and 350 µm. Tubes were filled with sand thus obtained with a particle size of 100-350 µm.

Details of experiments made in a moisture gradient and in concentration gradients of salts combined with a moisture gradient are given in in Table 1.

Table 1

References, moisture gradients, moisture gradient combined with concentration gradient of salts and gradient of salt tested for their effects on movement of juveniles of *Meloidogyne javanica* in sand

Experiments and number of replications	*Sections of sand column*					
	1		2		3	
	moisture %	*utilized solution* M/l	*moisture* %	*utilized solution* M/l	*moisture* %	*utilized solution* M/l
1 (30)	10	H_2O	10	H_2O	10	H_2O
2 (30)	14	H_2O	14	H_2O	14	H_2O
3 (90)	14	H_2O	10	H_2O	6	H_2O
4 (30)	14	Hoagland 1.3×10^{-2}	10	Hoagland 0.65×10^{-2}	6	H_2O
5 (30)	14	$K H_2 PO_4$ 1×10^{-2}	10	$K H_2 PO_4$ 0.5×10^{-2}	6	H_2O
6 (30)	14	$K NO_3$ 1×10^{-2}	10	$K NO_3$ 0.5×10^{-2}	6	H_2O
7 (20)	14	$Ca (NO_3)_2$ 1×10^{-2}	10	$Ca (NO_3)_2$ 0.5×10^{-2}	6	H_2O
8 (20)	14	$Mg Cl_2$ 1×10^{-2}	10	$Mg Cl_2$ 0.5×10^{-2}	6	H_2O
9 (20)	14	$Mg SO_4$ 1×10^{-2}	10	$Mg SO_4$ 0.5×10^{-2}	6	H_2O
10 (30)	14	$Fe Cl_2$ 1×10^{-2}	10	$Fe Cl_2$ 0.5×10^{-2}	6	H_2O
11 (30)	14	$Fe SO_4$ 1×10^{-2}	10	$Fe SO_4$ 0.5×10^{-2}	6	H_2O
12 (20)	10	$Fe Cl_2$ 1×10^{-2}	10	$Fe Cl_2$ 0.5×10^{-2}	10	H_2O

Immediately after establishing the sand column, a 0.1 ml of a suspension of juveniles, with a determined number, was introduced into the center of the column with a syringe.

The tubes were maintained horizontally at 28 °C in the dark. The sand columns were divided into three parts 72 h after introduction of the juveniles. Nematodes were separated from sand by placing the sand from each section for 48 h on filter paper placed on a support in a Petri dish containing water similar to the method of Oostenbrink (Merny & Luc, 1969).

Results

Figures 1 to 12 present the distribution (per cent recovered nematodes) of the juveniles of *M. javanica* in the sections of the sand column, three days after their introduction. A mean percentage of 60% of the juveniles introduced per tube was recovered at the end of experiments. The main reason of loss was the method of extraction. Results of a "sign test" using all the replications of each experiment are given in each figure.

The following results were obtained.

In a column of sand uniformly moistened with demineralized water, juveniles of *M. javanica* were evenly distributed in parts 1 and 3 of the tube (Fig. 1 and 2).

In a moisture gradient juveniles were concentrated in the region having the highest moisture content (Fig. 3).

When a concentration gradient of nutritive salt solution (Hoagland's) was combined with a moisture gradient, the juveniles moved preferentially toward the region having the lowest salt concentration, which was also the region having the lowest moisture (Fig. 4). This phenomenon was also observed when a moisture gradient was combined with concentration gradients of KH_2PO_4, $K\ NO_3$, $Ca\ (NO_3)_2$ and $MgCl_2$ (Fig. 5, 6, 7 and 8, respectively).

Concentration gradients of $Mg\ SO_4$, $Fe\ Cl_2$ and $Fe\ SO_4$ with a moisture gradient failed to alter the behaviour of juveniles of *M. javanica* in relation to the moisture gradient. The juveniles were concentrated in the region having the highest moisture which was also the region having the highest salt concentration (Fig. 9, 10 and 11).

In a concentration gradient of $Fe\ Cl_2$, without a moisture gradient, juveniles were evenly distributed in parts 1 and 3 of the column (Fig. 12).

Discussion

Juveniles of *M. javanica* accumulated in the end of a moisture gradient in sand having the highest moisture concentration as previously described with *Heterodera rostochiensis* (Wallace, 1960) and *Ditylenchus dipsaci* (Wallace, 1961).

Concentration gradients of $K\ H_2PO_4$, $K\ NO_3$, $Ca\ (NO_3)_2$ and $Mg\ Cl_2$ were able to reverse this behaviour of juveniles in relation to a moisture gradient. These results confirm the results previously obtained in column of agar in which juveniles of *M. javanica* exhibited a negative tropism to these salts (Prot, 1978 *a*).

It was noted that $Fe\ SO_4$ failed to exhibit a repulsion on the juveniles of *M. javanica* in agreement with results of experiments made in agar (Prot, 1978 *a*). On the other hand, $Mg\ SO_4$ and $Fe\ Cl_2$, which exhibited a significant repulsion in agar, did not reverse the orientation of the juveniles in regard to a moisture gradient. These results could be explained by an apparent "hydrotaxis" greater than the repulsive effect of these salts or by a lack of repulsion of these salts in these experimental conditions. This latter hypothesis appears corroborated by the fact that juveniles were evenly distributed in a concentration gradient of $Fe\ Cl_2$ (Fig. 12).

It seems possible that mineral salts can influence the behaviour of juveniles of *M. javanica* in the soil. Indeed, the soil solution, containing a mixture of ions and other soluble materials, is certainely more like Hoagland's solution than a pure mineral salt solution. In these experiments Hoagland's solution exercised significant repulsion on juveniles of *M. javanica* since it caused a reversal of their behaviour in a moisture gradient.

Accumulation of juveniles in the end of a moisture gradient having the highest moisture content could be explained several ways.

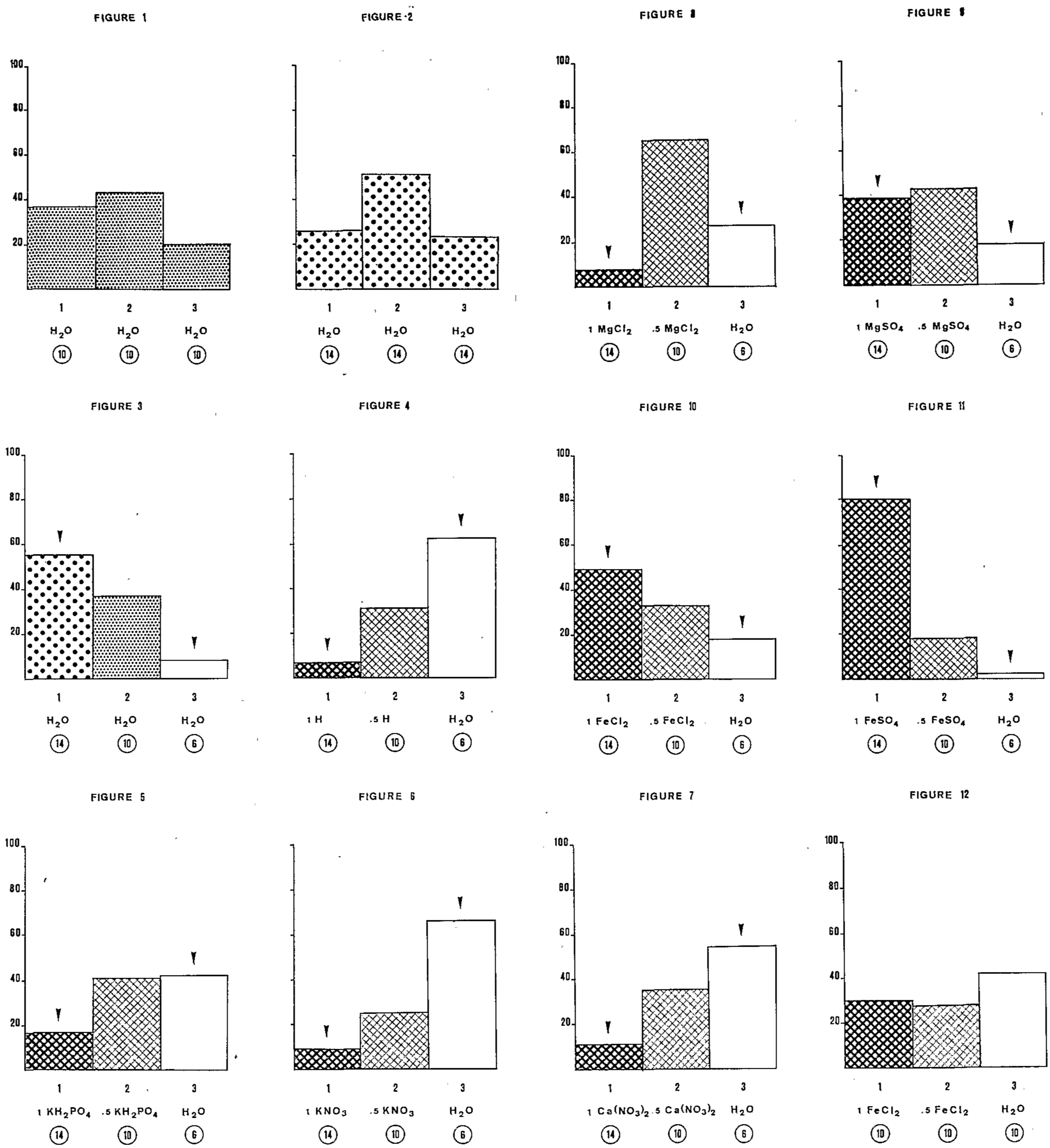

Fig. 1-12 : Distribution of juveniles of *M. javanica* in sand columns in which were established different moisture gradients or salt gradients combined with a moisture gradient. *Abscissa :* fractions of the column ; the solution used to moistened the sand is indicated under the number of the fraction, the moisture of the section is indicated in the circle. *Ordinate :* juveniles found in each section in per cent of recovered nematodes. Arrows indicate a significant difference between the numbers of juveniles found in sections 1 and 3 after a "sign test".

Accumulation occurs in the region having the highest water film thickness corresponding to the optimal conditions for mobility, after random movement.

A "rheotaxis" was induced by flowing water from the most moist end to the driest end of a moisture gradient, however, Wallace (1959) demonstrated that *Aphelenchoides ritzemabosi* moved upwards on a stem in a stationary water film when a downward current tended to carry the nematodes down.

Sensitivity to another gradient (pH, gas, osmotic pressure or soluble materials) was created simultaneously with the establishment of a moisture gradient.

It is likely than the apparent "hydrotaxis" is the result of several factors. Among these, repulsion due to mineral salts can contribute. Indeed, in soils containing adequate quantities of mineral salts, an increase of moisture can dilute the soil solution. Data (Fig. 4) indicate that juveniles react more strongly to the mineral salt gradients than to the moisture gradient ; i.e. the presence of a salt gradient overcomes the reaction of the juveniles to move to a moisture gradient. What appears to be a reaction to a moisture gradient (migration to the region of highest moisture concentration) may actually be a negative reaction to a salt gradient since the region of lowest salt concentration corresponds to the region of highest moisture concentration. In this case a chemorepulsion could partly explain an apparent "hydrotaxis".

Accepté pour publication le 20 septembre 1978.

References

LANE, C. (1930). Behaviour of infective hookworm larvae. *Ann. trop. Med. Parasit.*, 24 : 411-421.

MERNY, G. & LUC, M. (1969). Les techniques d'échantillonnage des peuplements de nématodes dans le sol. In : Lamotte, M. & BOURLIÈRE, F. (Eds) *Problèmes d'écologie : l'échantillonnage des peuplements animaux des milieux terrestres.* Paris, Masson & Cie : 257-292.

PROT, J.-C. (1978 *a*). Influence of concentration gradients of salts on the movement of second stage juveniles of *Meloidogyne javanica. Revue Nématol.*, 1 : 21-26.

PROT, J.-C. (1978 *b*). Behaviour of juveniles of *Meloidogyne javanica* in salt gradients. *Revue Nématol.*, 1 : 135-142.

WALLACE, H. R. (1959). Movement of eelworms. V. Observations on *Aphelenchoides ritzema-bosi* (Schwartz, 1912) Steiner, 1932 on florist's chrysanthemums. *Ann. appl. Biol.*, 47 : 350-360.

WALLACE, H. R. (1960). Movement of eelworms. VI. The influences of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.*, 48 : 107-120.

WALLACE, H. R. (1961). The orientation of *Ditylenchus dipsaci* to physical stimuli. *Nematologica*, 6 : 222-236.