Behaviour of juveniles of *Meloidogyne javanica* in salt gradients

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SUMMARY

All juveniles of *M. javanica*, derived from a clone established from a single egg mass, have the same capacity to orient toward the lower concentration in a salt gradient. When the small percentage of juveniles that had migrated to the area of higher concentration were reintroduced into a gradient, the majority again migrated to the lower concentration.

Examination of tracks of juveniles in salt gradients indicated that repulsion from high concentrations is the result of an orientation of their movements. This capacity of orientation is suppressed when the juveniles are introduced into salt gradients at a point where the salt concentration is high.

RÉSUMÉ

Comportement des juvéniles de Meloidogyne javanica *dans des gradients de sels minéraux*

Tous les juvéniles de *M. javanica* issus d'un clone établi à partir d'une seule masse d'œufs ont la même capacité de s'orienter vers la zone ayant la plus faible concentration dans un gradient de sel minéral. Lorsque les quelques juvéniles qui se sont déplacés vers la zone de plus forte concentration sont réintroduits dans un gradient la majorité d'entre eux migrent vers la zone de plus faible concentration.

L'examen des traces, laissées par les juvéniles lors de leurs déplacements dans des gradients de sel, indique que la répulsion due aux sels minéraux est le résultat d'une orientation de leurs mouvements. Cette capacité d'orientation est supprimée lorsque les juvéniles sont introduits dans des gradients de sels en un point où la concentration saline est élevée.

Recently it has been demonstrated that certain mineral salts repelled juveniles of *Meloidogyne javanica* (Prot, 1978) ; i.e. when placed in salt gradients the juveniles moved preferentially toward the region having the lower salt concentration. This repulsion was significant in gradients created in agar by salt solutions of concentrations between 0.125×10^{-2} and 0.5×10^{-2} M/l. However, this repulsion was not observed in a 2×10^{-2} solution of certain salts, i.e. $Ca(NO_3)_2$ and $CaCl_2$, whereas with other salts, eg. NaCl, KCl, the repulsion was significant at this concentration.

In order to further elucidate this phenomenon, three additional experiments were made on certain aspects of this relationship. In the first experiment the subsequent behaviour in a salt gradient of juveniles that moved to the lower salt concentration in an earlier experiment was compared to that of those few juveniles that had moved to the higher concentration. The second experiment was designed to study the behaviour of *M. javanica* juveniles in a salt gradient to determine if there was an effect on the pattern of movement. In the third experiment movement of *M. javanica* juveniles was studied in high and low salt concentrations in relation to the time between establishing the gradient and introducing the juveniles.

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)* in the greenhouse. Only individuals not more than 48 h in age and from the first generation were used.

All necessary manipulations were made under sterile conditions ; all glassware, salt solutions, demineralized water and the agar were autoclaved (120 °C for 20 min).

The procedures followed in the first experiment are diagrammed in Figure 1. Two mineral salts, $Ca(NO_3)_2$ and NaCl, were tested at a

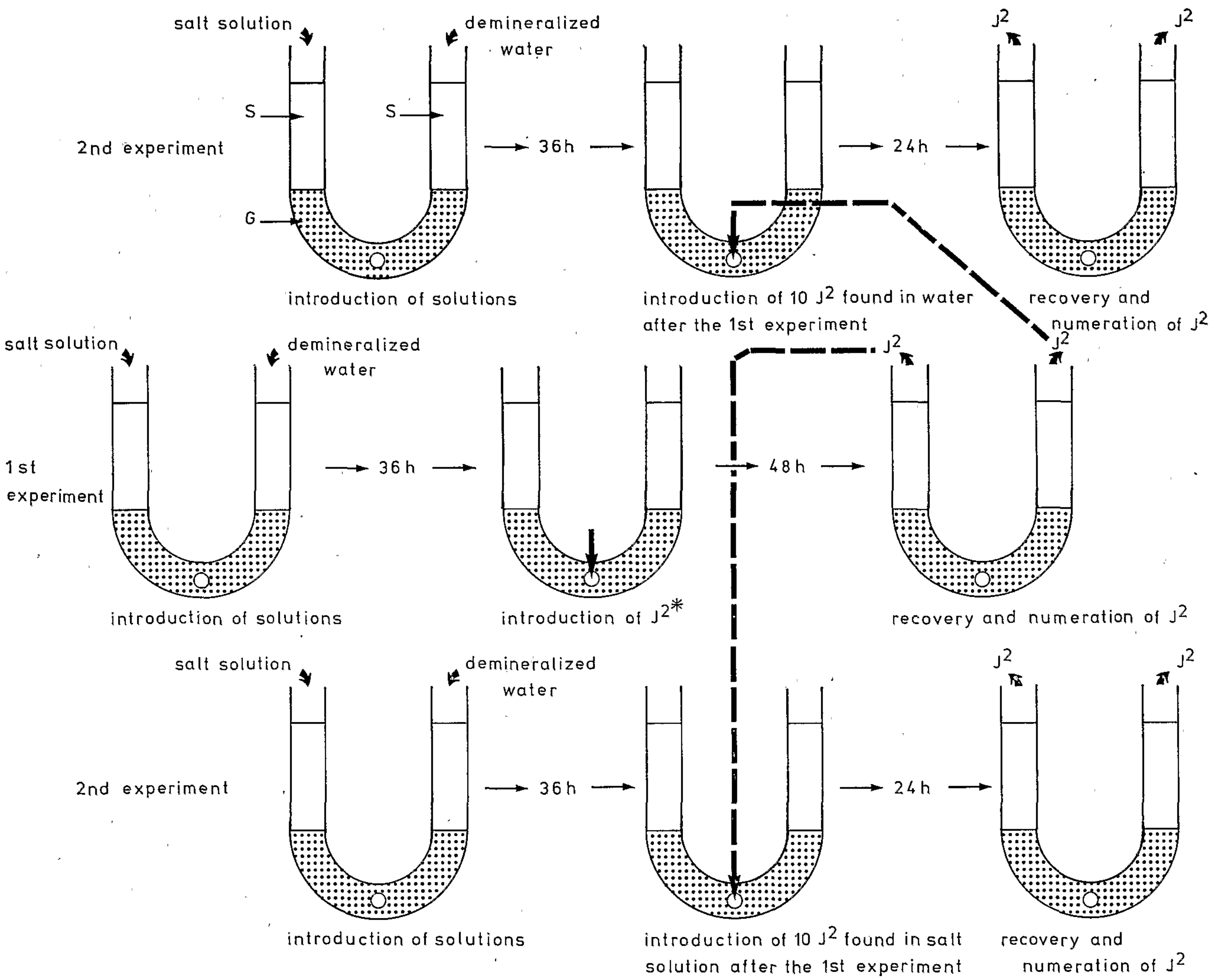

Fig. 1. Experimental apparatus and procedure used to study behaviour of juveniles of a clone of *M. javanica* in a salt gradient. The first part of the experiment is depicted in the middle horizontal row ; the second part in the top and bottom rows. J^2 : introduction of an indefinite number of juveniles of *M. javanica*.

concentration of 0.5×10^{-2} M/l. Juveniles were introduced into U-shaped pyrex tubes 36 h after the salt solution was placed in the tube and 48 h later the juveniles were recovered from the two ends of the tube (see Prot, 1978 for a complete description of the technique). The experiment was made in the dark at 28° C and was repeated three times. In the second part of the experiment, juveniles from the end of the tube having the lower salt concentration were recovered and reintroduced into tubes in which the same salt solution had been placed 36 h before (top row of Fig. 1). Juveniles were recovered from the two ends of these tubes 24 h later and counted. This same procedure was followed with the juveniles recovered from the end with the higher salt concentration in the first part of the experiment (bottom row of Fig. 1). Thirty tubes were used in the first part of the experiment ; the number of tubes used in the second part was dependant upon the numbers of juveniles recovered after the first part of the experiment.

Tracks of juveniles of *M. javanica* in a salt gradient were studied with the method described by Prot (1975) ; tracks were produced by the growth of microbial contaminants left along the path of the nematodes. Experiments were made with gradients created in agar with $Ca(NO_3)_2$ (0.5×10^{-2} and 2×10^{-2} M/l) and NaCl (0.5×10^{-2} M/l). With each gradient 30 tracks of juveniles moving to demineralized water and 30 tracks of juveniles moving to salt solution were analysed. To obtain tracks of juveniles moving to demineralized water two nematodes (not surface sterilized) were introduced into each tube. Since movement of juveniles to the salt solution was more rare, ten juveniles were used in each tube in order to obtain the 30 desired tracks. Experiments were made at 28 °C in the dark. Juveniles were introduced into the center of the gradients 36 h after the start of the diffusion.

Only tracks going completely from the starting point to the finishing point in demineralized water or salt solution were taken into consideration. Tracks were classified into three groups :

- straight tracks with no more than two alterations in the principal direction ;
- confused tracks with more than two alterations in the principal direction ;
- reversal of direction, in which, for example, a juvenile started moving to the salt solution but ultimately arrived at the demineralized water. In this case, the track was considered as going to the mineral salt, but with a reversal of direction (see Fig. 2).

Effects of $Ca(NO_3)_2$ and NaCl on the movement of juveniles of *M. javanica* were studied in the same U-tubes as previously described, with three concentrations of salt (0.5×10^{-2}, 2×10^{-2}, 8×10^{-2} M/l). The time between initiation of a salt gradient and the introduction of juveniles in the middle of the latter was varied.

Introduction of 1.5 cm³ of the salt solution into one branch of the U-tube (1.5 cm³ of demineralized water were placed in the other) was considered as zero time of the experiments. Ten juveniles of *M. javanica* (not surface sterilized) were introduced singly into the center of the agar (1.5% agar was poured into the central part of the U-tube to form a column 5 cm in lenght) after 0, 12, 24, 36 or 48 h ; the tubes were maintained at 28 °C in the dark. The numbers of nematodes present in the liquids in both branches of the U-tube were counted 24 h after their introduction. Twenty replications were used for all times and concentrations of the two salts tested.

Results

Table 1 shows the mean percentages of juveniles found in the salt solution and in the demineralized water after two consecutive tests with the same juveniles in gradients created by $Ca(NO_3)_2$ or NaCl at 0.5×10^{-2} M/l. During the two tests a repulsion caused by mineral salts was observed that in most cases was highly significant. During the second test the repulsion was observed with the juveniles found in mineral salts during the first test as well as with those found in demineralized water.

Table 1

Mean percentage of juveniles found in salt solution and demineralized water after two consecutive tests with the same juveniles in salt gradients. ** : significant difference with a Wilcoxon test at a probability of 0.01 using the difference between the numbers of juveniles recovered in the two branches of the tube. * : significant difference at a probability of 0.05 with the same test. In parentheses : minimum-maximum values.

Salt and Concentration	*1st test*		*2nd test using juveniles found in mineral salt solution after the 1st test*		*2nd test with juveniles found in demineralized water after the 1st test*	
	Number of replications	*Mean percentage of juveniles found in salt solution*	*Number of replications*	*Mean percentage of juveniles found in salt solution*	*Number of replications*	*Mean percentage of juveniles found in salt solution*
$Ca(NO_3)_2$ 0.5×10^{-2} M/l	30	12** (2-24)	11	13** (0-38)	19	17** (0-50)
$Ca(NO_3)_2$ 0.5×10^{-2} M/l	30	30** (7-55)	15	22** (0-50)	15	41 (0-100)
$Ca(NO_3)_2$ 0.5×10^{-2} M/l	30	21** (5-47)	10	30* (0-57)	10	32 (0-50)
NaCl 0.5×10^{-2} M/l	30	21** (1-51)	15	30** (0-71)	15	25** (0-83)
NaCl 0.5×10^{-2} M/l	30	22** (0-50)	11	28* (0-57)	15	37 (0-100)
NaCl 0.5×10^{-2} M/l	30	16** (0-78)	7	33 (0-75)	15	34* (12-100)

Table 2

Classification of thirty tracks of juveniles of *M. javanica* that moved to demineralized water and salt solutions in three different salt gradients.

Gradient created in agar by a solution of	*Tracks of juveniles to demineralized water*		*Tracks of juveniles to mineral salts*	
NaCl 0.5×10^{-2} M/l	straight	23	straight	10
	confused	7	confused	14
	reversal of direction	0	reversal of direction	6
$Ca(NO_3)_2$ 0.5×10^{-2} M/l	straight	28	straight	9
	confused	2	confused	12
	reversal of direction	0	reversal of direction	9
$Ca(NO_3)_2$ 8×10^{-2} M/l	straight	16	straight	4
	confused	13	confused	21
	reversal of direction	1	reversal of direction	5

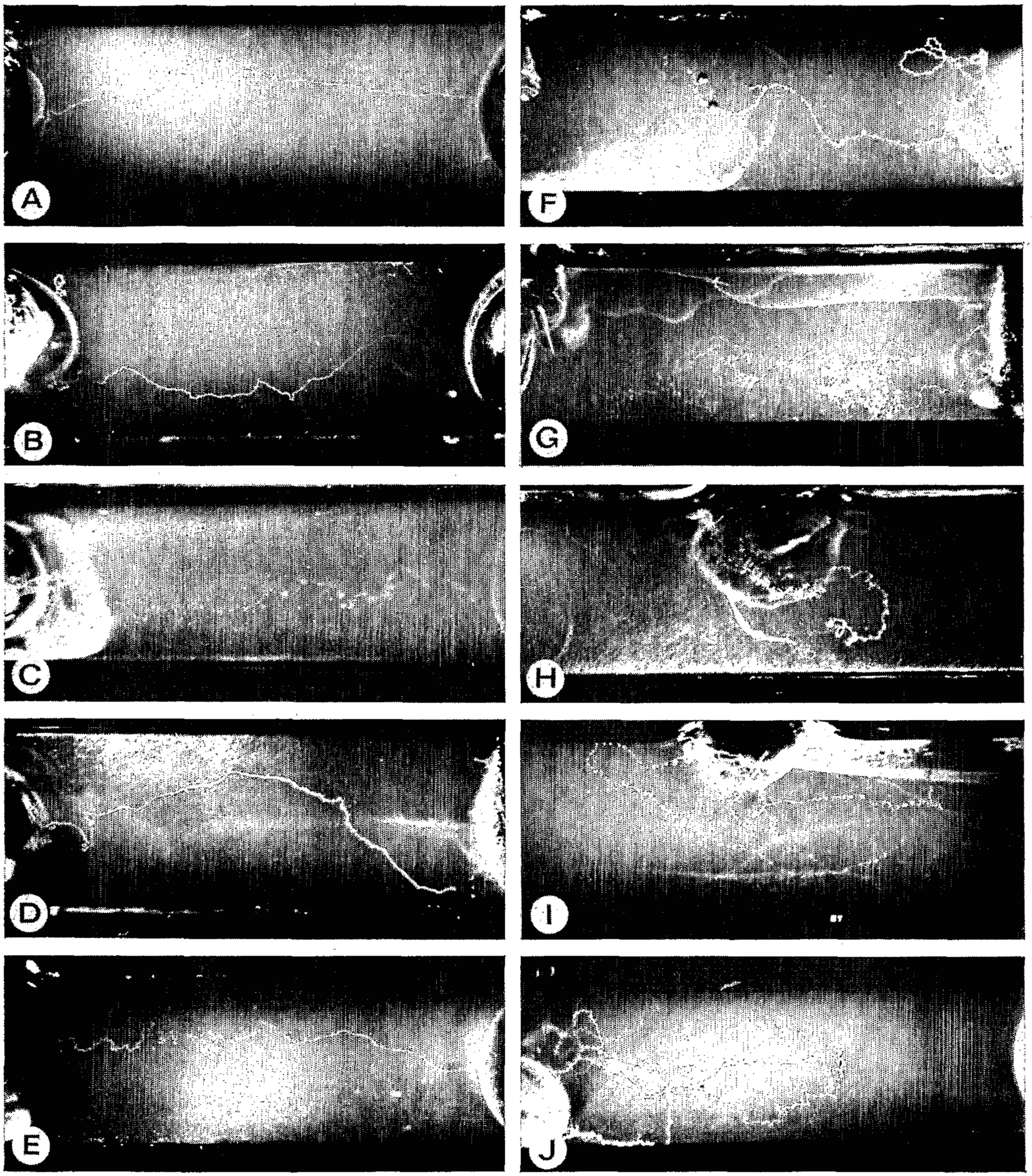

Fig. 2. Examples of tracks obtained during the migration of juveniles of *Meloidogyne javanica* in salt gradients in agar. A : straight track to demineralized water in a gradient created by a solution of NaCl at 0.5×10^{-2} M/l ; B, C : straight tracks to demineralized water in a gradient created by a solution of $Ca(NO_3)_2$ at 0.5×10^{-2} M/l ; D : straight track to a solution of NaCl at 0.5×10^{-2} M/l ; E : confused track to demineralized water in a gradient created by a solution of $Ca(NO_3)_2$ at 8×10^{-2} M/l ; F : confused track to a solution of $Ca(NO_3)_2$ at 8×10^{-2} M/l ; G : confused track to a solution of NaCl at 0.5×10^{-2} M/l ; H : reversal of direction in a gradient created by a solution of NaCl at 0.5×10^{-2} M/l ; I, J : reversals of direction in gradients created by a solution of $Ca(NO_3)_2$ at 0.5×10^{-2} M/l.

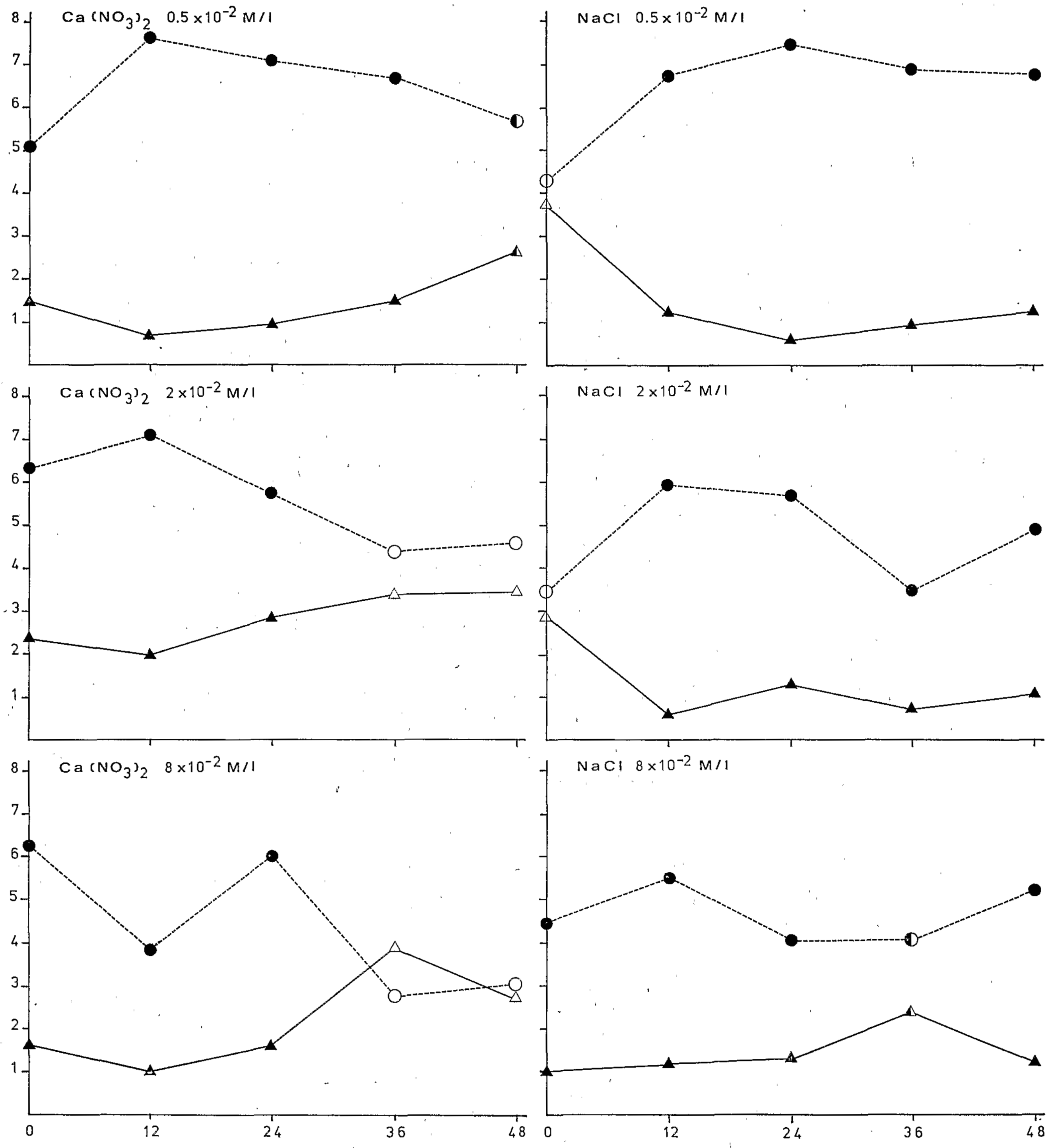

Fig. 3. Influence of $Ca(NO_3)_2$ and NaCl at 0.5×10^{-2}, 2×10^{-2} and 8×10^{-2} M/l as a function of elapsed time between the initiation of the gradient and the introduction of 10 juveniles of *M. javanica* into the center of the gradient. Abscissa : time in hours. Ordinate : mean number of juveniles recovered in demineralized water (circles and dotted line) and salt solution (triangles and solid line). Black circles and triangles indicate existence of a significant difference between the numbers recovered in the two branches of the tube (Wilcoxon test at a probability of 0.01) ; black and white circles and triangles indicate a significant difference at a probability of 0.05 with the same test. White circles and triangles indicate that the figures were not significant.

Table 2 summarizes the characteristics of the 30 tracks of juveniles moving to demineralized water and to the salt solution, in salt gradients created in agar by NaCl at 0.5×10^{-2} M/l and $Ca(NO_3)_2$ at 0.5×10^{-2} and 8×10^{-2} M/l. In experiments with NaCl and $Ca(NO_3)_2$ at 0.5×10^{-2} M/l tracks of juveniles moving to demineralized water were generally straight (Fig. 2 : A, B and C) and no reversal of direction was observed. To the contrary tracks of juveniles moving to salt solutions were generally confused (Fig. 2 : G) and reversal of direction occurred frequently (Fig. 2 : H, I and J). With a gradient created in agar by $Ca(NO_3)_2$ at 8×10^{-2} M/l the straight tracks to demineralized water were still in the majority but confused tracks (Fig. 2 : F) were numerous and one reversal of direction was observed.

Figure 3 summarizes the results of the effect on *M. javanica* juveniles of $Ca(NO_3)_2$ and NaCl at three concentrations as a function of time between the initiation of gradients in agar and introduction of nematodes into the center of the gradients. A significant repulsion was observed with all solutions when the juveniles were introduced in the center of the gradient less than 36 h after initiation of the experiments. The repulsion remained significant when juveniles were introduced 36 or 48 h after the start of establishment of gradients with the three concentrations of NaCl and the lowest concentration of $Ca(NO_3)_2$. However no significant repulsion with $Ca(NO_3)_2$ at a concentration of 2×10^{-2} and 8×10^{-2} M/l was observed after 36 or 48 h.

Discussion

Examination of tracks of *M. javanica* juveniles in salt gradients indicates that the observed repulsion from high concentrations is the result of actual orientation under these conditions. The tracks of juveniles moving toward the area of lower salt concentration were generally straight ; directional changes were common only in a gradient created by a high salt concentration ($Ca(NO_3)_2$ at 8×10^{-2} M/l). On the other hand, tracks of juveniles moving toward the higher concentration were generally tortuous and both confused tracks and direction reversal were common. If migration of juveniles toward the area of lower concentration was the result of random movement combined, for example, with a reduction in rate of movement by salts, the tracks would have had the same appearance as those going toward the region of higher concentration. This was not the case.

Although repulsion to mineral salt was statistically significant a certain percentage of juveniles migrated to the region of higher concentration. When these latter juveniles were reintroduced into a gradient, the majority migrated to the lower concentration. Similar results were obtained with those juveniles that had originally migrated toward the lower concentration, although a certain percentage moved to the higher concentration. From these observations it is concluded that all individuals in the population tested have the same capacity to orient toward a lower concentration in a salt gradient, but because of random movement or some unknown factor a very small percentage of individuals react in the opposite manner.

In certain cases the capacity of orientation in a salt gradient is suppressed as observed in high salt concentration, i.e. $Ca(NO_3)_2$ at 2×10^{-2} and 8×10^{-2} M/l. Also when juveniles were placed in the center of salt gradients 48 h after introduction of the salt solutions, they appeared incapable of orientation. However, when placed there less than 36 h after initiation of the gradient, the repulsion was significant. On the basis of these observations, it appears that the juveniles are incapable of orientation when the salt concentration is high at the point at which they are introduced. Additional evidence to support this point is provided by the nature of tracks left by juveniles in a gradient created by $Ca(NO_3)_2$ solution at 8×10^{-2} M/l (Fig. 2 : E and F). In this case, the tracks of the juveniles moving to the lower concentration were frequently "confused" suggesting random movement rather than directional orientation.

Therefore, it can be concluded that juveniles of *M. javanica* exhibit real orientation in gradients of mineral salts. Further, all juveniles in a clone have the same capacity of being repelled by higher salt concentrations, except that when high salt concentrations are used the juveniles are incapable of orientation.

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