The influence of osmotic pressure on the hatching of *Heterodera schachtii*

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SUMMARY

When free second-stage juveniles of *Heterodera schachtii* were transferred from distilled water to concentrations of trehalose or sucrose greater than 0.1M they lost water. In solutions of about 0.3M the water content became constant at 69 %, the value obtained from unhatched juveniles in eggs equilibrated with water. Compared with juveniles in water, the number of juveniles moving in a range of concentrations of sucrose and inositol did not substantially decrease, unless the concentration was 0.5M or greater. The hatch of juveniles from cysts in 0.3M sucrose and inositol in 2mM picric acid was similar to that in distilled water, whereas a 0.6M solution completely inhibited hatching. These findings are compared with those obtained for *Globodera rostochiensis* (Clarke, Perry & Hennessy, 1978) and it is suggested that a lower osmotic pressure of fluid within the *H. schachtii* egg and the fact that *H. schachtii* juveniles can move in 0.3M sugar solutions may explain why this species hatches readily in water.

RÉSUMÉ

*Influence de la pression osmotique sur l'éclosion d'*Heterodera schachtii

Les juvéniles libres d'*Heterodera schachtii* subissent une perte d'eau lorsqu'on les transfère de l'eau distillée dans des solutions de tréhalose ou de saccharose de concentration supérieure à 0.1M. Dans les solutions de concentration voisine de 0,3M, la teneur en eau des juvéniles atteint une valeur constante de 69 %, valeur égale à celle que l'on obtient pour des juvéniles non éclos se trouvant dans des œufs en équilibre osmotique avec l'eau. Sauf pour des solutions de concentrations égales ou supérieures à 0,5M, le nombre des juvéniles mobiles dans des solutions de saccharose et d'inositol de diverses concentrations n'est pas sensiblement inférieur à celui des juvéniles mobiles dans l'eau. L'éclosion de juvéniles dans le saccharose et l'inositol, en solution d'une concentration de 0,3M dans l'acide picrique à 2mM, est semblable à celle obtenue dans l'eau distillée, alors que l'éclosion est complètement inhibée à la concentration de 0,6 M. Ces résultats sont comparés à ceux déjà obtenus pour *Globodera rostochiensis* (Clarke, Perry & Hennesy, 1978), et il est suggéré qu'une pression osmotique plus faible dans l'œuf d'*H. schachtii* et une résistance plus grande des juvéniles d'*H. schachtii* à l'inhibition du mouvement par la pression osmotique pourraient expliquer le fait que l'éclosion de cette espèce ait lieu aisément dans l'eau.

Unhatched juveniles of *Globodera rostochiensis* seem to be immersed in a fluid with an osmotic pressure equivalent to about 0.4M trehalose (Clarke, Perry & Hennessy, 1978; Perry, 1978) and the egg-fluid of this species contains significant amounts of trehalose (Clarke & Hennessy, 1976). Also, when stimulated with root diffusate, the water content of juveniles increases immediately before hatching (Ellenby & Perry, 1976). Clarke, Perry and Hennessy (1978) suggested that loss of solutes from the egg-fluid enables juveniles to take up water and permits hatching. However, species of cyst-nematodes vary in their dependence on root diffusates to initiate the hatching process (Williams, 1978). *Heterodera schachtii* has a wide host range and hatches readily in water. Some aspects of the tolerance of *H. schachtii* to osmotic stress were studied by Wallace (1956) and Kämpfe (1962). We studied the effects of osmotic pressure on *H. schachtii* in relation to the mechanism of hatching.

Materials and methods

Cysts of *H. schachtii* were raised on pot-grown sugar-beet plants and extracted by usual methods (Shepherd, 1970). Juveniles were hatched in 2mM picric acid, collected 3 days later, washed three times in distilled water and used after storage for a day in distilled water.

For all experiments, test solutions were prepared as outlined by Clarke, Perry and Hennessy (1978). To test the effects of solutions on juveniles, a suspension (0.2 to 0.4 cm^3) containing a known number was added to the appropriate solution, allowance being made for the volume of solution introduced. All solutions and suspensions were thoroughly mixed. The concentration of sugars resulting was checked with a pocket refractometer. The osmotic pressures of the test solutions (Table 1) were determined using a Wescor Vapour Pressure Osmometer (Model 5100B) calibrated with sodium chloride standard solutions.

The water content of juveniles was measured at set time intervals (Fig. 1) by interference microscopy (Ellenby, 1968) after they had been transferred from water to sugar solutions at 20°. To avoid the ingress of water vapour, the solutions containing juveniles were kept in solid watch glasses covered with Parafilm sealing tissue (Gallenkamp) under a glass lid. Juveniles were transferred in 25 µl of liquid at each time interval to slides and covered with a cover-slip. The water content of twenty individuals in each sucrose solution and fifteen in each trehalose solution was measured on each occasion and that of unhatched juveniles from eggs in cysts soaked for 7 days in distilled water was also determined (Ellenby & Perry, 1976).

The relationship between osmotic pressure and movement of juveniles was examined using freshly prepared suspensions (about 50-60,000 juveniles) in different concentrations of sucrose and inositol (volume, 10 cm^3) in tubes (17.5 × 3 cm) sealed with Parafilm. Samples containing 200 to 400 juveniles were removed at intervals (Fig. 2) and the numbers moving and stationary were counted. After 8 days samples were removed, diluted ten-fold with distilled water and further counts were made after 6 and 30h.

A range of concentrations of sucrose and inositol in 2mM picric acid were used to test the influence of osmotic pressure on hatching. In similar experiments with *G. rostochiensis* (Clarke, Perry & Hennessy, 1978), triethylene glycol was used; however, this substance was toxic to *H. schachtii*. In pilot experiments, microbial growth in the test solution was greater with *H. schachtii* than it had been with *G. rostochiensis* (Clarke, Perry & Hennessy, 1978). Therefore the microbial inhibitor 9-hydroxyquinoline (8 ppm) was added to all solutions, with limited success. Batches of 150 cysts in fivefold replication were soaked for 2 days in distilled water. The water was then removed and replaced by a test solution of appropriate concentration but without the hatching agent; this ensured that the solution replaced the water inside the cyst. After 24h the solution was removed and fresh solution added; after a further 24h the solution was replaced by a comparable test solution containing the hatching agent. The number of hatched juveniles was counted (Shepherd, 1970) after 10 days.

Table 1

Osmotic pressure of experimental solution in mOs/kg. Each value is the mean of four determinations.

	Solution concentration (M)						
	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Inositol	105	206	303	410	508	606	716
Sucrose	114	216	329	459	579	731	872
Trehalose	110	216	324	475	596	739	902

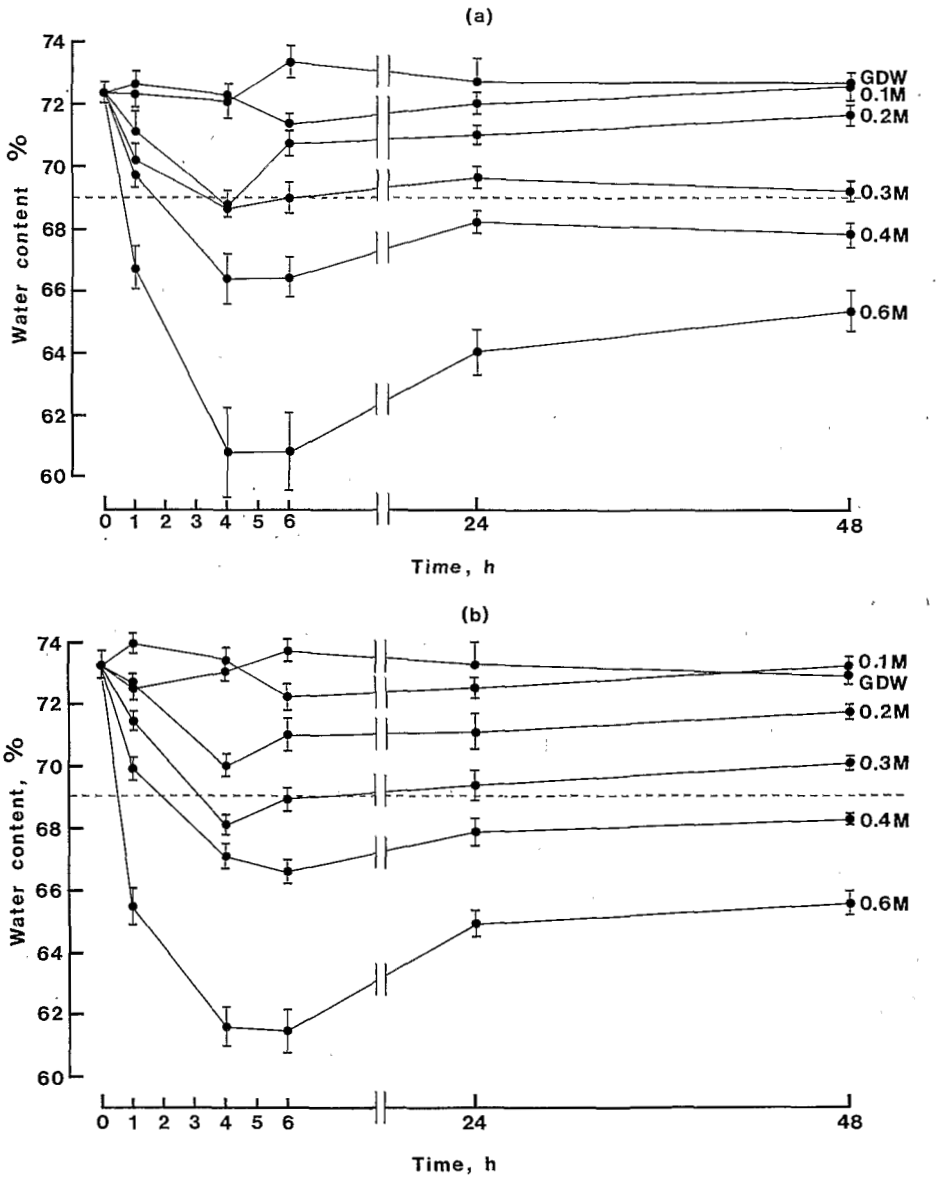

Fig. 1. Changes in water content of second-stage juveniles of *H. schachtii* in glass distilled water (GDW) and after transfer from distilled water to various sucrose (a) and trehalose (b) solutions. The broken line is the water content of unhatched, unstimulated juveniles. Vertical segments represent the limits of standard error of means.

Results

Figure 1 shows the effects of osmotic stress on the water content of free second-stage juveniles of *H. schachtii*. In both sucrose (Fig. 1a) and trehalose (Fig. 1b) solutions of 0.1M, the juvenile water content was similar to that in distilled water. In solutions more concentrated than 0.1M the juveniles lost water, the amount increasing with the concentration of the solute. The water content reached a minimum between 4 to 6h and then increased slightly to a more

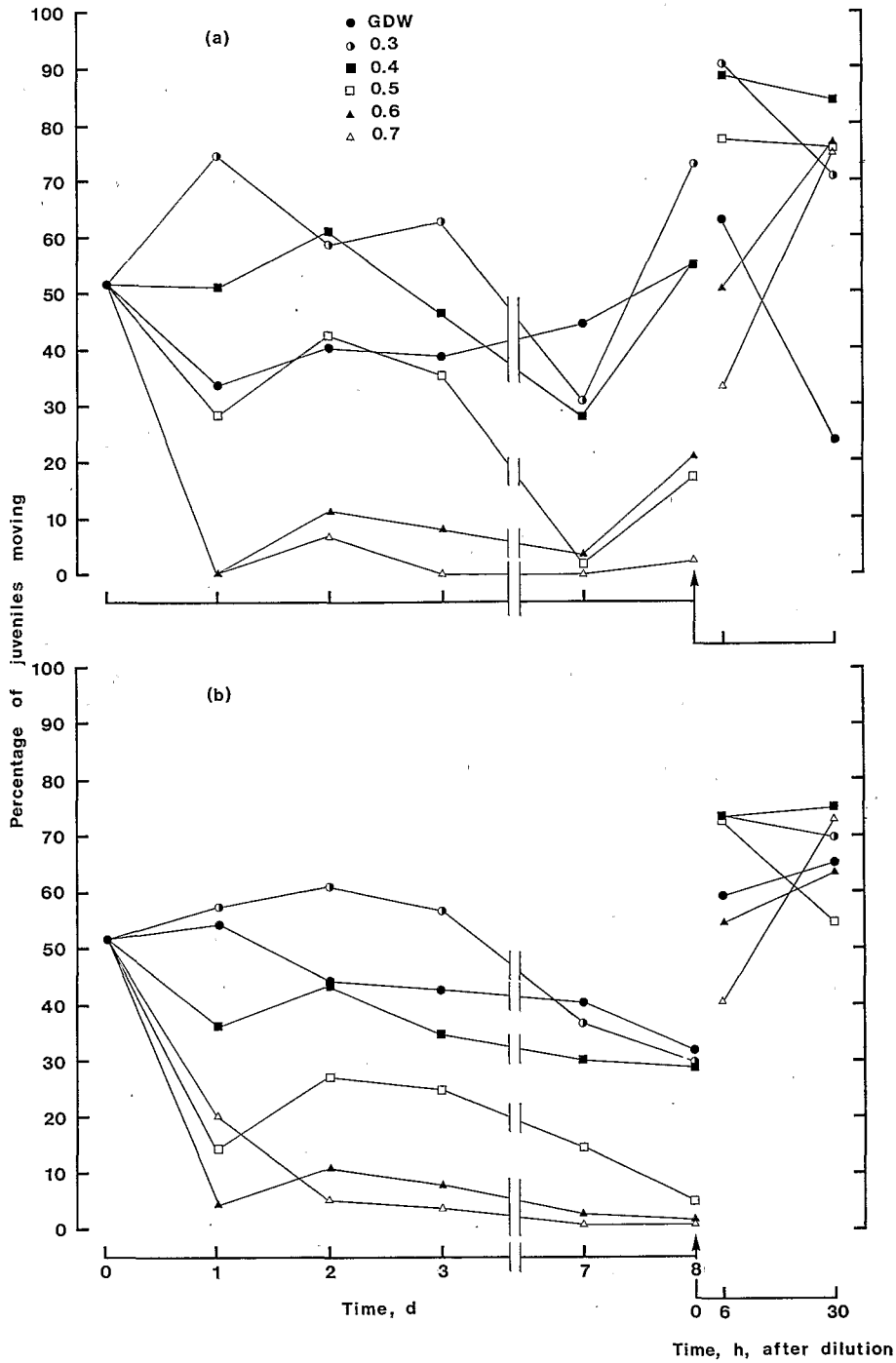

Fig. 2. Percentage of second-stage juveniles of *H. schachtii* moving in glass distilled water (GDW) and 0.3-0.7M sucrose solutions (a) and 0.3-0.7M inositol solutions (b) for periods up to 8 days and then at 6 h and 30 h after addition of water.

constant value after 24h. Usually the increase from the minimum was greater in the more concentrated solutions. The mean water content of twenty juveniles before hatching was 69.1 % ± 0.4, which is close to the value (69.5 %) previously obtained (Perry, 1977*a*) for a different population and similar to the water content of free juveniles in 0.3M sucrose and 0.3 to 0.4M trehalose.

Figures 2a and 2b shows the influence of 0.3 to 0.7M sucrose and inositol solutions respectively on the movement of hatched juveniles before and after adding distilled water. In the first 2 days, the percentage of juveniles moving was reduced to fewer than 15 % in the 0.6M and 0.7M solutions. The reduction of movement in the 0.5M solution was less marked until days 7 to 8, but movement was little affected by the 0.3 and 0.4M solutions compared with distilled water. The inhibition of movement was reversible after dilution, especially after 30h for juveniles previously in the 0.6 and 0.7M solutions.

Figure 3 shows the influence of increasing osmotic pressure on the hatching of *H. schachtii* Solutions of sucrose and inositol (0.1 to 0.7M) in 2mM picric acid were used. As the concentration increased hatching decreased, the pattern being the same for both compounds. Although the hatch in 0.3M solutions was less than in picric acid alone, it was still considerable and similar to that in distilled water (about 25 % of that in picric acid).

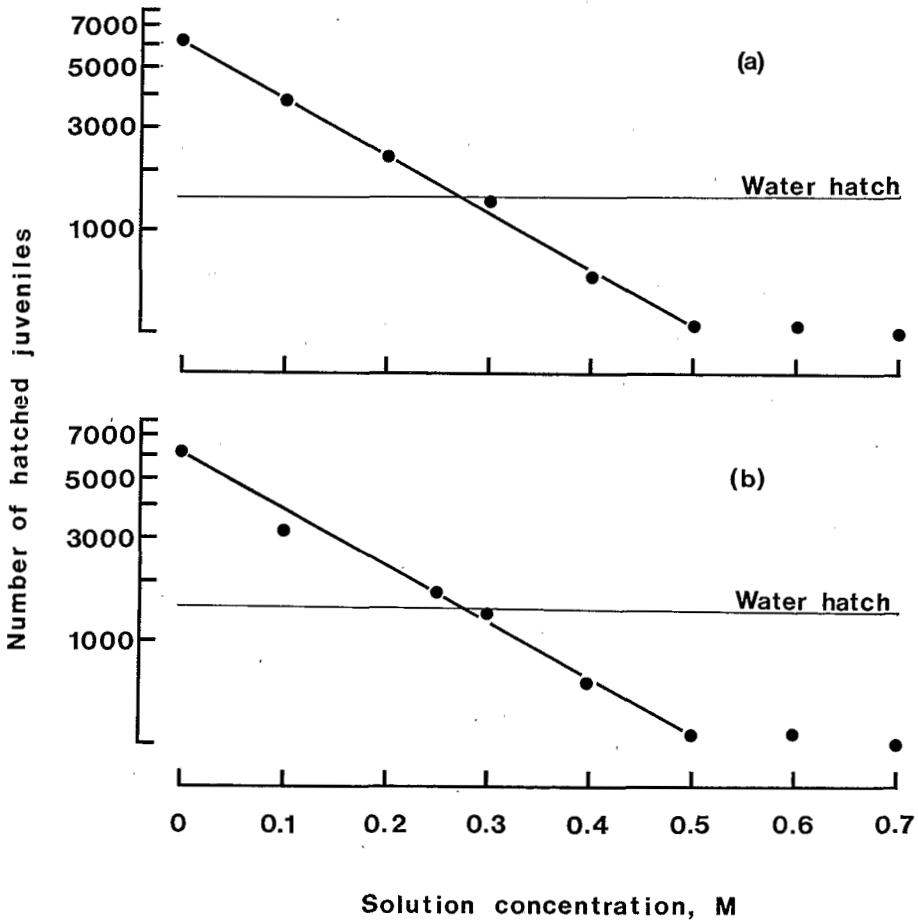

Fig. 3. Emergence of second-stage juveniles from cysts of *H. schachtii* immersed in 0.1-0.7 M solutions of sucrose and inositol in 2mM picric acid. The number of hatched juveniles is plotted on a log scale with the hatch in picric acid alone as the initial point.

Discussion

The effect of osmotic pressure on the water content of *H. schachtii* differs from that reported for *G. rostochiensis* (Clarke, Perry & Hennessy, 1978). Although the overall pattern is similar, minimum water content being reached in 4 to 6h followed by an increase to a more constant value after 24h, the water content of *H. schachtii* juveniles is greater in solutions of comparable concentration. 0.1M trehalose and sucrose solutions have little effect on the water content of second-stage juveniles of *H. schachtii* and hatching is far less inhibited at this concentration in this species. In eggs equilibrated with distilled water, unhatched juveniles of *H. schachtii* contain more water (69 %) than those of *G. rostochiensis* (67 %) (Ellenby & Perry, 1976; Clarke, Perry & Hennessy, 1978). The water content of hatched juveniles of *H. schachtii* becomes constant at about 69 % in 0.3M trehalose and sucrose solutions, suggesting that the egg-fluid surrounding the *H. schachtii* juvenile has a smaller osmotic pressure than that of *G. rostochiensis* (= 0.4M trehalose).

The percentage of *H. schachtii* juveniles moving in sucrose and inositol solutions did not substantially decrease over 8 days (compared with juveniles in water) unless the solution concentration was 0.5M or greater; 0.4M solutions had little effect. This contrasts with *G. rostochiensis*, where 0.4M reduced the percentage moving to fewer than 9 % (Clarke, Perry & Hennessy, 1978). The ability of *H. schachtii* juveniles to move in sugar solutions < 0.5M and the smaller osmotic pressure of the fluid within the egg may explain why *H. schachtii* hatches readily in water.

Clarke and Perry (1977) suggested that potato-root diffusate altered the permeability if the egg-shell of *G. rostochiensis* allowing egg-fluid solutes, such as trehalose, to escape thus permitting the juvenile to take up water and become active. The physical constraint of the egg-shell provides an upper limit to the water content of the juvenile and only when it escapes from the egg is it able to become fully hydrated (Ellenby, 1974). The increase in water content before hatch is important, for a water content of at least 69 % is necessary for *G. rostochiensis* to become fully mobile. In contrast, Perry (1977*a*) failed to detect water uptake by *H. schachtii* juveniles before hatching whether cysts were treated with beet root diffusate or not. The unhatched juvenile already contains enough water (69 %) to permit movement. The hatching of the trichostrongylid nematode, *Nematodirus battus*, is similar; there is no water uptake before hatching but the juvenile becomes fully hydrated on escaping from the egg (Perry, 1977*b*). So, the *H. schachtii* juvenile is in a condition suitable for hatching without the application of root diffusate. However, this fails to explain the additional hatch induced by root diffusate. Even a slight dilution of the egg-fluid may, however, be important. Fig. 2a, b shows that when the concentration of the surrounding medium was decreased from 0.3M to 0.03M by dilution, the percentage of juveniles moving increased from 73 to 91 % in the sucrose solution and from 30 to 73 % in the inositol solution. However, juveniles in distilled water also showed increased movement on addition of water, from 55 to 63 %, and from 32 to 59 %, respectively in the two experiments. The results suggest that an appreciable amount of this increase in activity may be attributed to something other (e.g. change in oxygen tension) than the change in osmotic pressure. In consequence the inhibition of movement by osmotic pressure and its removal on dilution was only clearly demonstrated in solutions $\geqslant$ 0.5M.

An alternative explanation for quiescence of the unhatched juvenile is that inhibitors may be present in the egg-fluid that prevent hatching of a proportion of the juveniles. The dilution of the egg-fluid following a change in permeability of the egg-shell might remove this inhibition.

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