WATER UPTAKE AND HATCHING IN THE POTATO CYST NEMATODE, *HETERODERA ROSTOCHIENSIS*, AND THE ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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SUMMARY

1. The second-stage larva of the plant parasitic nematode *Heterodera rostochiensis* shows very little movement while confined to the egg; on the other hand, the larva of the closely related *Meloidogyne incognita* is very active

2. Using interference microscopy, it is shown that the water content of the *Heterodera* larva increases immediately it is liberated from the egg while that of the *Meloidogyne* larva remains constant.

3. It is suggested that the *Heterodera* larva is physically constricted by the egg shell and that this restricts both its movement and its water content.

4. Measurement of larval and egg volume in both forms supports this hypothesis. The *Meloidogyne* larva has about 30 % of free space inside the egg shell while the *Heterodera* larva fits it completely.

5. It is suggested that these differences can be correlated with differences in the distribution and life-history of the two forms.

INTRODUCTION

It was suggested in 1957 (Ellenby) that the second-stage larva of *Heterodera rostochiensis* showed little movement inside the egg shell because it filled it so completely. Although the recent observations of Doncaster & Shepherd (1967), using time-lapse photography, have shown that activity is increased under the influence of the hatching factor, it is still true that movement is very limited. The contrast with the behaviour of the larva of *Meloidogyne incognita* is most striking; this shows great activity inside the egg shell and appears to have more room.

Both the second-stage larva and the egg shell are freely permeable to water in both directions (Ellenby, 1968*b*). If the egg shell imposes a physical restraint on the larva, it may also impose a constraint on its water content, like the cellulose wall of the plant cell; rupture of the shell might then lead to an increased water uptake. This view is not new. Wilson (1958) showed that the permeability of the egg shell of *Trichostrongylus retortaeformis* changed some time before hatching; he suggested that this led to an increase in the uptake of water. Observing that the movement of the larva decreased progressively and that movement was minimal when permeability was maximal, he concluded that 'the results suggest that the larva is unable to move

freely just before hatching, and that this is due to the osmotic uptake of water'. His technique, however, was not adequate to demonstrate any significant change in the volume of the larva at this time. The fact that the water content of the individual living nematodes may be estimated by interference microscopy (Ellenby, 1968 a, b) has enabled a direct approach to the problem. A brief account of part of this work has already appeared (Ellenby & Smith, 1969).

MATERIALS AND METHODS

Second stage larvae of *Heterodera rostochiensis* (*sensu strictu*, Stow, 1972) were obtained from cysts derived from the roots of potato-plants (var Golden Wonder) specially grown for the purpose. After a 2-week presoak in tap-water, the cysts were either stimulated with an active hatching factor concentrate at optimum concentration (1 : 250000 in tap-water) or, for some experiments, continued in tap-water. *Meloidogyne* larvae were obtained from egg-masses picked off tomato hosts: they were separated into individual eggs by gentle agitation for 1 h in tap water with no. 5 ballotini beads; a wrist action shaker was used. Further details are given under the appropriate sections.

EXPERIMENTAL

Activity

Even superficial observation makes it clear that the *Meloidogyne* larva is far more active inside the egg than that of *Heterodera*. If a number of eggs of the former sort are examined, it is certain that some of the contained larvae will be showing some movement. On the other hand, in *Heterodera*, even with eggs soaked in a solution containing hatching factor, only prolonged observation, preferably in a hanging drop or similar preparation, will enable movement to be detected. The difference between them, in fact, was so great that our attempts to put it on a quantitative basis were abandoned as being fatuous.

Change in length

Preliminary observations were first made with *Heterodera* larvae. Presoaked cysts were isolated in unit cells (Ellenby, 1943) in drops of hatching factor concentrate so that cysts which were hatching vigorously could be detected. Such cysts were then ruptured and their contained eggs used in the tests.

Individual eggs were mounted in tap-water under a cover-glass ringed with cellulose paint. The ring was flexible enough to permit pressure on the cover-glass sufficient to rupture the egg. The length of the expelled larva was then measured as soon as possible using a traversing hairline micrometer eyepiece at short intervals until 30 min had elapsed. The procedure was repeated with about 70 other larvae. Almost invariably, the observations indicated that there was an increase in larval length during this time.

The results for the series are presented in Fig. 1, where in general, larval length 30 min after eclosion is expressed as a percentage of the initial length.

Sometimes the larva became so active during the 30 min that further measurement was impossible; in these cases the last observation available was used to calculate percentage length change. Fig. 1 shows that, in an overwhelmingly large proportion
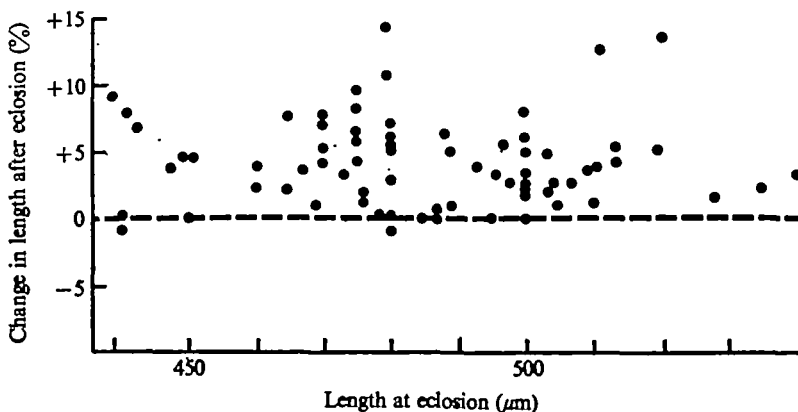
Fig. 1. Change in length of *Heterodera* larvae during the first 30 min after liberation from the egg.

of cases, the observations record an increase in length; in only 2 of the 12 larvae were length decreases recorded.

These results are very suggestive, but it would be wrong to conclude from them that, striking as they are, they prove that the *Heterodera* larva increases in *volume* after eclosion. Measurements of the length of the living larva are not very accurate and the increases in length, apparent in so many, were only of the order of a few per cent. There is also a much more serious criticism.

The nematode body is an anisometric structure, although opinions may vary as to how the anisotropy is achieved in different forms (Harris & Crofton, 1958; Inglis 1964). There is certainly no doubt that the *Heterodera rostochiensis* larva is anisometric; under pressure, its length can be increased by 50 %, with no detectable increase in breadth (Ellenby, unpublished), an operation which the larva survives. But although it is anisotropic, this does not mean that it increases *solely* in one direction, a suggestion which Harris and Crofton never made. To take changing length as related to changing volume, as some workers have done, is therefore of doubtful validity. For example, in a *Heterodera* larva approximately 500 µm long and 20 µm in diameter, a small decrease in diameter of the order of 0·5 µm could hardly be detected. If it is assumed that it decreases in diameter by this amount and that the worm *remains of constant volume*, simple calculation shows that it would increase to a new length of approximately 526 µm. In other words, a virtually undetectable decrease in cross section would be accompanied by a very detectable increase in length of about 5% - without any change in volume at all. Clearly, the results presented in Fig. 1 do not prove that there is an increase in volume on eclosion.

Estimation of water content
(a) Heterodera rostochiensis

Experiments were first carried out with *Heterodera* larvae. An egg from a cyst which had been hatching well was mounted as in the previous experiment; it was then centred under an interference microscope. The slide was then transferred to a stereo microscope, the egg ruptured and the slide quickly transferred back again to the interference microscope. With care and good fortune, the often actively moving larva was located very swiftly, orientated by means of the rotating stage, and photographed through a fringe field eyepiece. Further photographs were taken in rapid succession; from
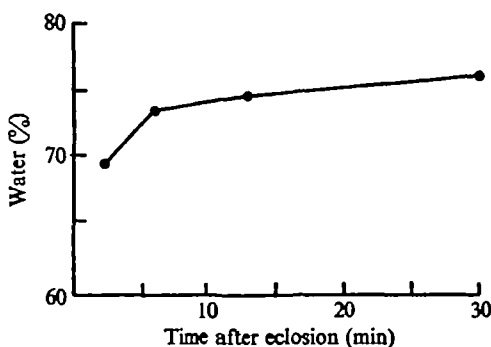
Fig. 2. Estimated water content of *Heterodera* larva on liberation from the egg.

these the refractive index at intervals during the first critical moments could be determined and thus the water content estimated (Ellenby, 1968*a*, *b*). Observations were continued for 30 min. The procedure was repeated twice more; a typical curve is presented in Fig. 2. Clearly there is an immediate and rapid increase in the water content. It slows quite soon so that, after about 30 min, the estimated water content is about equal to that of larvae which have hatched some hours.

Further observations were made on other eggs, but, since it appeared that water uptake took place very soon after eclosion, only two observations were made in each case: the first as soon as possible after the egg was ruptured, and the second on the same egg exactly 30 min from eclosion. The time of the first observation varied from ½ to 1½ min from rupture, depending on the speed with which the larva could be located; the second could be precisely timed for since the time of rupture was known exactly, the moving larva could be located in the fringe field in good time.

The larvae for these observations were obtained from cysts which had already shown good hatching in single cyst isolation. These cysts were bisected; one half cyst continued to be immersed in a hatching factor concentrate, while its fellow half was maintained in tap-water. Altogether 15 pairs, i.e. initial and 30 min measurements, were made on larvae from eggs in hatching factor and 13 pairs from eggs in tap-water.

In the statistical analysis of the results, the experimental design made it possible to allow for the individual cyst from which the larvae originated. In the event, this was decisive for the differences in water content are small. In fact, the analysis showed that the water content at 30 min was significantly greater than the water content on eclosion ($P = 0.02$). On the other hand, there was no significant difference initially or later between the tap-water and hatching factor treatments, nor was there any difference between cysts – even though removing the variation due to this factor was helpful in the analysis. The results are summarized in Table 1. For the estimates of water content it is assumed, for simplicity, that the specific gravity of the cell solids is about 1; as this is not strictly true absolute values for water content are somewhat higher.

(b) *Meloidogyne incognita*

The results with *Heterodera* demonstrate that water is taken up immediately after eclosion; they strongly support the hypothesis that the lack of movement of the larva in the egg is due, in part at least, to the fact that it is constricted there. Clearly,

Table 1. *Refractive index and* S.E. *for larvae of* H. rostochiensis *from eggs soaked in a solution of hatching factor concentrate in tap-water, and in tap-water alone*

Hatching factor		Tap-water	
Initial	30 min	Initial	30 min
1·3826 ± 0·00052	1·3808 ± 0·00052	1·3836 ± 0·00054	1·3820 ± 0·00054
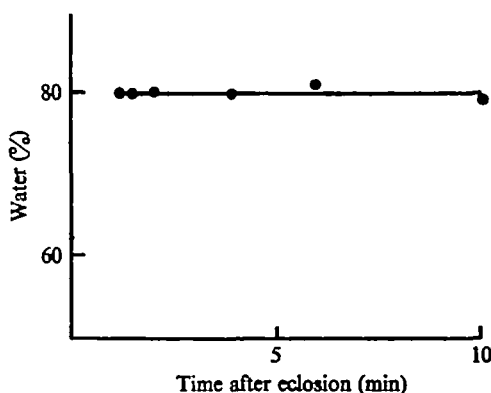
Fig. 3. Estimated water content of *Meloidogyne* larva on liberation from the egg. Compared with Fig. 2, this figure contentrates on the most critical first 10 min.

however, if this hypothesis is correct, the *Meloidogyne* second-stage larva, which is so active inside the egg, should not show any uptake of water immediately after eclosion. Tests were therefore carried out with this form, the procedures followed being exactly as described above for *Heterodera*; for three larvae, however, measurements were made at frequent intervals during the first 10 critical minutes after eclosion.

Fig. 3, the results for a single *Meloidogyne* larva, show that, unlike *Heterodera*, there was no apparent increase in water content on eclosion. The results for the two other larvae, studied in the same way, were similar. In addition, the water content of 13 larvae was estimated as soon after eclosion as possible and then, for each of them, water content was again estimated 30 min after eclosion. The mean values for 'initial' and '30 min' refractive index from which water content is estimated were identical – 1·3743 ± 0·0024 and 1·3743 ± 0·0025 respectively.

Larval and egg volume

The *Heterodera* larva, then, takes up water immediately on eclosion, while the *Meloidogyne* larva does not; it is suggested that only the former is constricted inside the egg-shell. The hypothesis could be tested by examining the relationship of larval and egg volume in the two forms. Does the former have detectably more 'living space'?

For both species, eggs, freed from their cysts, were set up in cells, *Heterodera* in a solution of hatching concentrate in tap-water, *Meloidogyne* in tap-water.

In the following procedures only larvae which had hatched in a previous 24 h period ʃere used. They were mounted in tap-water and photographed using flash illumination.

Where possible they were then transferred to an 0·5% solution of the narcotic pro-pylene phenoxetol in tap-water (Ellenby & Smith, 1964) and photographed about 30 min later, when they were relaxed in the narcotic. The empty egg shells were also photographed, but because of the technical difficulties involved, it was not possible to obtain photographs of egg shells belonging to particular larvae.

Photographs for ten individual *Heterodera* larvae and eggs were printed at × 280 diameters; to equalize the error involved in measurements, prints of ten *Meloidogyne* larvae and eggs were enlarged to a linear size about equal to that of the *Heterodera* larvae. Volumes were evaluated by a sector method. For the larvae, the photographic images were divided into 1 mm sectors throughout their length and total volume obtained by summing the calculated volumes of the separate sectors, assumed cylindri-cal. For the egg shells, two methods were used: a sector method identical with that for the larva, and a mathematical method based on the assumption that the eggs of both forms are prolate spheroids.

RESULTS

The measurements show conclusively that the *Meloidogyne* larva has substantially more room inside the egg-shell than the larva of *Heterodera*. The mean volume of the *Meloidogyne* larva, $5·7 \times 10^4 \mu m^3$, is about 70% of that of the egg-shell, $7·9 \times 10^4 \mu m^3$; that is, there is about 30% of free space.

On the other hand, the measurements of the *Heterodera* larvae indicate a larva which fits its egg shell very tightly indeed, in fact the results indicate a negative free space! Mean larval volume, $11·5 \times 10^4 \mu m^3$ is about 104% of egg volume, $11·1 \times 10^4 \mu m^3$! It is tempting to suggest that this somewhat bizarre result is in keeping with findings that water is taken up on eclosion. However, although this may be involved, it is more probable that it is due to the limitations of the methods by which the volumes were estimated. Nevertheless, it is perfectly clear that contrasted with the *Heterodera* larva the *Meloidogyne* larva has considerable free space.

DISCUSSION

The *Heterodera* species are found largely in temperate regions and have a life-history which is geared to the seasonal growth of their hosts. The *Meloidogyne* species, on the other hand, are predominantly tropical and sub-tropical and most of them do not have a more or less dormant stage. The fact that the body of the mature female is converted by a tanning process (Ellenby, 1946) into a resistant cyst in the former species while the body wall of the latter remains untanned and decays very easily is undoubtedly correlated with this difference in life-history. Crofton & Whitlock (1965 a, b) suggested that nematode egg size is related to environmental factors and their experimental findings support the hypothesis. The egg of *Heterodera rostochiensis* is far larger than that of *Meloidogyne incognita* and it is very likely that this is correlated with the difference in life history. The larger egg contains a larger larva – one, perhaps, able to survive a long period in the quiescent state. Possibly the fact that the *Heterodera* larva fits its egg-shell so tightly is merely an economical answer to the storage problem; the limitation of movement might also be advantageous in limiting the utilization of food reserves. Limitation of movement might also form part of a complex of factors involved in the evolution of the cyst as a hatching unit (Ellenby, 1956).

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