The Distribution of Hatching within the Cyst of the Potato Root Eelworm, *Heterodera rostochiensis*

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SUMMARY

1. A serial-sectioning technique is described for cysts of the potato root eelworm. The distribution of hatching among eggs contained in the cysts is investigated by this technique.

2. It is shown that there are significant differences in the numbers of larvae emerging from different parts of the cyst, i.e. hatching is not random. There is a tendency for eggs near the cyst wall to hatch sooner than eggs nearer the centre of the egg mass.

3. The position of eggs in relation to the natural openings of the cyst, at the neck and vulva, is apparently without influence on the distribution of hatching.

4. Although the cyst wall is permeable to the hatching stimulant it is argued that the hatching pattern is more likely to be due to a gradient of oxygen tension within the cyst.

INTRODUCTION

It is well known that larvae are stimulated to emerge from cysts of the potato root eelworm by the action of a substance diffusing from the roots of the growing host-plant (Trifitt, 1930b). Under laboratory conditions once hatching has begun the number of larvae emerging each day reaches a maximum and then decreases to zero even in the presence of fresh root diffusate. If the same cysts are re-stimulated after an interval hatching recommences, showing that the original cessation was not due to the cysts being empty but to some other cause.

Ellenby (1946b) showed that cysts with a small puncture in the wall produced roughly twice as many larvae as unpunctured controls before hatching ceased. Pointing out that the mere presence of another exit cannot itself cause larvae to hatch if they are not ready to do so, Ellenby concludes that the cessation of hatching must be brought about by inhibition. He postulates the production, perhaps by the larvae themselves, of an inhibitor which, on reaching a critical concentration, inhibits further hatching. The puncture provides another exit for the inhibitor which therefore takes longer to build up to the critical concentration. Ellenby suggests that if this is the correct explanation the eggs constitute an ecological community, the behaviour of individuals in the community affecting the behaviour of the others.

If the eelworm cyst is, in fact, an ecological unit, hatching from it may be governed by definite rules. The present paper reports the results of an investigation to determine whether hatching is random or whether the position of eggs within the cyst affects the order in which they hatch.

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Materials and Methods

The cysts used in this work were collected from the roots of the potato, variety 'Redskin', after most of the soil had been removed. The cysts were considered to be full at the start of the work (i.e. no larvae having emerged), but the method of collection does not exclude the possibility of the inclusion of an occasional old cyst from which some emergence might have taken place. All cysts were more than two years old when the work began and the larvae are assumed to be capable of hatching when stimulated. The cysts were stimulated by a modified single-cyst technique (Ellenby, 1943) with potato root diffusate prepared either directly from the roots of a growing plant or from a sample of concentrated hatching factor (Calam, Raistrick, and Todd, 1949). Emerging larvae were counted and removed daily, fresh root diffusate from the same stock being added if necessary. In the majority of cases hatching was stopped when between 40 and 150 larvae had emerged.

The distribution of empty egg cases within the cyst was investigated by cutting serial sections by the technique described below.

Sectioning technique

Owing to the hardness and brittleness of the cyst wall, ordinary wax embedding techniques were a failure. Accordingly, the cyst wall was partially dissolved in sodium hypochlorite (Smedley, 1936); a 1:9 dilution of commercial 'Milton' was found most convenient. There is a good deal of variation in the thickness of the cyst wall which does not appear to be correlated with cyst size. Owing to this variation in thickness no definite time for the Milton treatment can be stated, but 60 minutes is a reasonable average. The cysts are examined under a binocular microscope and transferred to the fixative when the eggs are easily visible through the wall. Unstimulated cysts are soaked in distilled water for 2 or 3 days before treatment. Stimulated cysts are treated immediately after hatching ceases, but if this is inconvenient they are kept in distilled water until sectioning treatment begins.

The cysts are fixed in Duboscq-Brasil's fluid, which is highly penetrating and very suitable for animals with a cuticle; it is warmed to about 40°C to increase penetration. When all cysts have been in the fixative for 20 minutes or more (periods up to 60 minutes are not harmful) they are transferred to 70% alcohol for dehydration. Milton treatment and fixation can be carried out in the ‘single cyst’ Petri-dishes but dehydration and subsequent stages are best done in Stone's trays (Goodey, 1949) or in coverslip dishes (Fenwick, 1943). After 30 minutes the 70% alcohol is replaced by 90% and after a further 30 minutes this is replaced by 95% for 15 minutes. To avoid the hardening effects of absolute alcohol the cysts are cleared in methyl benzoate to which celloidin has been added. This enters the cysts and keeps the contents in place during sectioning. To ensure complete impregnation the cysts are usually left in methyl benzoate / celloidin overnight. (If Stone's trays are used, the plastic of which they are made should be tested to see if it reacts with methyl benzoate.)
The cysts are next transferred from methyl benzoate / celloidin to benzene (15 minutes), a benzene / paraffin wax mixture (30 minutes), and finally to paraffin wax of melting-point 54° C, in which they are left overnight.

Porcelain paint cells (18 × 18 × 10 mm) were found to be better than watch-glasses for blocking cysts. Each cell is filled with molten wax and a cyst is transferred to it by using a cold mounted needle. A warmed needle brought close to the cyst causes it to fall into the cell where it is orientated under a low power binocular and its position marked while the wax is semi-solid. Sectioning is carried out in the normal way with the Cambridge rocker microtome.

Sections were cut at 20 μ; as the approximate size of eggs in the cysts used was 95 × 40 μ, no full egg or empty egg-shell appeared whole in the sections. The distribution of empty eggs, i.e. of hatching, could therefore only be studied by noting the position within the cyst of the cut pieces of empty egg-shells consequent upon sectioning. These are referred to in the text as fragments. As hatching from an egg does not cause the shell to break up and pieces of full eggs contain pieces of larva, the fragments of empty egg-shells are easily distinguishable.

Distribution of fragments

Essentially, the cyst was divided into arbitrary regions and the hatches compared by noting the numbers of fragments in each.

As shown in fig. 1, A, the cysts were divided into three regions, the two ends and the central region. The ends each consisted of five sections; each is therefore a saucer-shaped piece of cyst wall containing eggs none of which is farther than 100 μ from some part of the wall. When the ends contain the neck or vulva owing to the orientation of the cyst, they are known as neck end and vulva end respectively. The central region contains a variable number of sections according to the size of the cyst.

Drawings of the outline of each cyst section were made on squared paper by using a squared eyepiece, and the egg fragments drawn in as accurately as possible. A circle of known radius was inscribed in the centre (as determined by eye) of each section of the central region, dividing it into an inner zone or core and an outer peripheral zone (fig. 1, A and B). The series of drawings made for one cyst is shown in fig. 2.

Cysts were sectioned in one of two ways: either the plane of section was at right angles to the axis of the cysts, thus including the neck and in most cases the vulva in the ends, or the plane of section was parallel to the axis, the two apertures then being incorporated in the central region.

Although the neck will always be part of the end or central region according to orientation, the position of the vulva is not so easily defined, as the vulva is offset from the longitudinal axis of the cyst. An 'end' as defined is a shallow bowl of maximum depth 100 μ. Measurements of 70 cysts from the same stock as those used in the work showed that in 24 cases, had the cysts been sectioned transversely, the vulva would have been excluded from the bowl.
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Fig. 1. A, subdivision of cyst into regions. B, division of each central region section into core and periphery.

Fig. 2. Distribution of fragments in a series of sections from a transversely sectioned cyst.
Estimation of hatches from cyst regions

The areas of all sections were measured with a planimeter. As all sections are of uniform thickness (20 \(\mu\)m) and almost cylindrical, their volumes are proportional to their areas. Dividing the total number of fragments present in a particular region by its total area gave the hatch for the region as so many fragments per unit volume (f / vol).

Since eggs are not spherical, any comparison based on the numbers of egg fragments will be invalid if the eggs are not randomly arranged. Further, it is also necessary to know how accurately the number of fragments represents the number of larvae hatched. Both these points were investigated.

Fragment-larvae relationship and random arrangement of eggs

In fig. 3, A the relationship between fragments and larval emergence is plotted against cyst size for 51 cysts sectioned transversely. In fig. 3, B the same relationship is presented for 21 cysts sectioned longitudinally. (The point 154, 8·09 is omitted from the analysis since with an egg size of approximately 100 \(\mu\)m and section thickness 20 \(\mu\)m the ratio 8·09 is impossible. The most probable explanation is that this was an old cyst from which some hatching had occurred before it was collected.) Regression analysis shows that there is no significant tendency for the fragment/larva ratio to vary with change in cyst size in either case (\(p < 0·1\) in both cases). The regression coefficients and their standard errors are presented in table 1.

There is, however, considerable variation in the fragment counts. For example, for cysts with 80 larvae hatched the counts vary between 150 and 300. Some of this variation might be due to errors in counting but this cannot be wholly responsible. It was considered that variation in cyst size might affect the fragment counts for if there is a large hatch from a small cyst the fragments will be crowded together and difficult to distinguish. The possibility was investigated.

The volumes of 72 cysts, assuming them to be spherical, were calculated and larval hatch per mm\(^3\) and fragments per mm\(^3\) were evaluated. The former were arranged in ascending order of size in groups of six and the variance of fragment densities for each group was evaluated. In fig. 4 variance is plotted against mean larval hatch density for each group. There is an obvious tendency for the variance to increase as larval hatch density increases. Fragment counting is accurate for fairly low larval hatch densities but where larval hatch density is high there is considerable error. It was for this reason that hatching was stopped, in the majority of cases, when between 40 and 150 larvae had emerged (p. 496).

Many hundreds of sections were examined in the course of the work. At no time was there any evidence that the eggs were not randomly arranged. This is confirmed by the mean values of the fragment/larva ratio for the two groups of cysts described above; for cysts sectioned transversely the mean value is 2·26, and for longitudinally sectioned cysts 2·44. Cutting sections in two planes at right angles therefore has little apparent effect on the numbers
Fig. 3. A, fragment/larva ratio and cyst size for cysts sectioned transversely. B, fragment/larva ratio and cyst size for cysts sectioned longitudinally.
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of fragments produced. Arrangements giving equal numbers of fragments in two planes at right angles are possible; there was, however, no evidence of any such arrangement and it is considered reasonable to assume that eggs are randomly orientated.

**Table 1**

*Fragment/larva relationship and cyst size. Regression coefficients and standard errors of regression*

<table>
<thead>
<tr>
<th>Cysts sectioned transversely.</th>
<th>Regression coefficient</th>
<th>Standard error of regression</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysts sectioned longitudinally</td>
<td>+0.007</td>
<td>±0.004</td>
<td>1.59</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td></td>
<td>+0.005</td>
<td>±0.007</td>
<td>0.73</td>
<td>&gt;0.4</td>
</tr>
</tbody>
</table>

If hatching from the cyst is random the fragment density will be the same in all regions. If, however, hatching is not random, fragment densities will be highest where hatching is greatest and the fact that errors in counting will also be greatest there (fig. 4) will tend to minimize the differences between regions.

The mean values of the fragment/larva ratio are rather small. The thickness of the sections and the size of eggs suggest that the mean values should be in the region of 3.50. Presumably the difficulties of counting fragments when their densities are high accounts for the low values. Further, the ease with
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which fragments are missed probably varies inversely with their size. Eggs cut in L.S. or near L.S. give two or perhaps three large fragments while eggs cut in T.S. give four or five much smaller fragments. While all of the three larger fragments may be counted, some of the latter may be missed, thus lowering the value of the ratio.

Sections were assumed to be cylindrical and their areas to represent their volumes. They are in fact most like cylinders near the centre of each group and least like them at the ends. Volume estimates based on area will be too large and hatch estimates therefore too small, the errors being greatest at the ends. As the core of each central region section is cylindrical the errors in

![Diagram of area corrections](image)

FIG. 5. Diagram of area corrections. E.P.A. and E.C.A. are the effective peripheral and core areas respectively.

the central region will affect the periphery only. If hatching is not random, the differences will clearly be affected by this assumption.

Some of the egg fragments lie on the line dividing the periphery of central sections from the core. As the core is smaller than the periphery in the vast majority of cases, the number of fragments it could contain is less than the maximum for the periphery. If the border-line fragments are ignored the core hatch estimate will be affected to a relatively greater extent. Some correction was therefore necessary.

The effect of ignoring the border-line fragments is compensated for by subtracting from the areas of both core and periphery an amount equal to $\frac{1}{2} \pi rl$, $r$ being the core radius and $l$ the mean fragment length. This represents an area in which the centres of eggs cannot lie without the eggs projecting over the line dividing periphery and core. An amount equal to $\frac{1}{2} \pi Rl$, where $R$ is the section radius, is also subtracted from the area of each periphery and from the area of each end section; this represents a region at the outer boundary of each section in which the centres of eggs cannot lie without the eggs projecting through the cyst wall.
Fig. 5 shows the area corrections for a central region section.

I am grateful to Mr. J. M. Hammersley of the Department for the Design and Analysis of Scientific Experiment, University of Oxford, for suggesting the above treatment of the problem. He derived the expressions for the effective areas of both core and periphery after making the following assumptions:

1. That egg fragments could be considered to be straight lines.
2. That these lines may lie in any direction; all directions, in three dimensions, being equally likely.
3. That the length of an egg fragment is independent of the direction in which it lies.
4. That all fragment lengths between zero and $2l$ are equally likely, $l$ being the mean fragment length.

![Fig. 6. Core hatch and peripheral hatch for cysts sectioned transversely.](image)

The mean length of eggs from cysts used in the work was 95 $\mu$ and a regression analysis showed that there was no significant tendency for egg length to vary with changes in cyst size ($p > 0.6$). The mean fragment length was
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47·5 μ. The corrections derived from these values amounted to 18° of the core and 25–30% of the peripheral area.

RESULTS

1. Hatching from periphery and core

In fig. 6 values for the hatches from the periphery are plotted against corresponding values for the core of each of 36 cysts sectioned transversely, i.e.

![Graph](image)

Fig. 7. Core hatch and peripheral hatch for cysts sectioned longitudinally.

with the plane of section at right angles to the longitudinal axis. Clearly, if the hatches in the two regions were equal, all values would lie on the line of slope 45° drawn in the diagram. In fact, no less than 32 of the 36 points lie above this line and the remaining four quite close to it. A similar diagram for 32 cysts sectioned longitudinally, i.e. parallel to the axis, is presented in fig. 7. In this case, 31 of the 32 points are above the 45° line. Clearly for both lots of cysts the peripheral hatch is greater than the core hatch in the majority of cases.
Comparison of the hatches by pairing, a test based on the differences between the core and peripheral hatches of single cysts, showed that in both cyst lots the differences between the hatches of core and periphery are very highly significant ($p < 0.001$ in both cases). There was considerable variation in the data; for transversely sectioned cysts, for example, the standard deviation for the differences between members of individual pairs was $\pm 3.96$ frag./vol. The results of the comparisons are shown in table 2a.

2. Hatching from the ends and periphery

In fig. 8 values for the hatches from the neck end are plotted against corresponding values for the peripheries of cysts sectioned transversely. The points are fairly evenly distributed about the $45^\circ$ line, i.e. the hatches from the two regions are very similar. Comparison by pairing shows that, in fact, there is no significant difference between them ($p > 0.6$). Fig. 9 is a similar diagram for the vulva end and peripheral hatches of the same cysts. Unlike fig. 8, fig. 9 shows clearly that, in general, the vulva end hatches are larger than the peripheral hatches, 15 of the points being above the $45^\circ$ line and 7 below but fairly close to it. Comparison by pairing showed that the differences between the hatches of the vulva end and the periphery are, in fact, highly significant ($p < 0.01$).

In longitudinally sectioned cysts, the neck and, in the majority of cases, the vulva are incorporated in the central region, leaving the ends without apertures. Both ends were therefore considered to be alike and only one was
Onions—Distribution of Hatching within the Cyst of the investigated. In fig. 10, values for the hatches from the end investigated are plotted against corresponding values for the peripheral hatches of 32 cysts sectioned longitudinally. Twenty-three of the 32 points lie above the 45° line, showing that on the whole the end hatches were greater than the peripheral hatches. Comparison by pairing confirmed that the differences between the end and the peripheral hatches are highly significant \( p < 0.01 \). The results of all end-periphery comparisons are presented in table 2b.

Eelworm cysts may differ from one another in a variety of ways. For example, there may be genetical differences (Gemmell, 1940), differences between cysts from various potato varieties (Gemmell, 1943; Ellenby, 1946c), and differences in age, size, total emergence, and emergence per day, i.e. hatch rate. All cysts used were taken from potatoes of variety 'Redskin', and, although this is unlikely, the results may apply only to cysts of this variety.
Fig. 10. End and peripheral hatches for cysts sectioned longitudinally (ends devoid of apertures).
Table 2

Differences between hatches from various regions of cysts sectioned either transversely or longitudinally

(a) Comparison of peripheral hatch and core hatch

<table>
<thead>
<tr>
<th>Cysts sectioned</th>
<th>Mean value of ratio peripheral-hatch/core-hatch</th>
<th>Mean difference between peripheral hatch and core-hatch and S.E. (f/vol)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>transversely</td>
<td>1.59/1</td>
<td>+4.75, ±0.66</td>
<td>7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>longitudinally</td>
<td>1.77/1</td>
<td>+6.13, ±0.84</td>
<td>7.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

(b) Comparison of ends and peripheries

<table>
<thead>
<tr>
<th>Cysts sectioned</th>
<th>Mean values of ratios</th>
<th>Mean difference between hatches and S.E. (f/vol)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>transversely</td>
<td>Neck-end-hatch/peripheral-hatch 1.03/1</td>
<td>+0.40, ±1.00</td>
<td>0.4</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td></td>
<td>Vulva-end-hatch/peripheral-hatch 1.24/1</td>
<td>+3.04, ±0.98</td>
<td>3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>longitudinally</td>
<td>End-hatch/peripheral-hatch 1.19/1</td>
<td>+2.80, ±0.90</td>
<td>3.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Fig. 11. End/periphery hatch ratio and total emergence for cysts sectioned longitudinally.

They were all more than two years old when the work began. As the other factors might well affect the hatching pattern, their influence was examined. In figs. 11, 12, and 13 the values of the ratio end-hatch/peripheral-hatch are
plotted against total emergence, hatch rate, and cyst size for cysts sectioned longitudinally. Regression analyses showed that total emergence and hatch rate had no significant effect on the ratio \((p > 0.2\) in both cases); however, there is a significant tendency for the ratio to increase as cyst size increases \((p < 0.01\).

The ratios peripheral-hatch/core-hatch, neck-end-hatch/peripheral-

hatch, and vulva-end-hatch/peripheral-hatch for transversely sectioned cysts, and the peripheral-hatch/core-hatch ratio for longitudinally sectioned cysts were also tested. In no case did any of the three variables, total emergence, hatch rate, or cyst size have any significant effect.
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DISCUSSION

The results show that hatching from cysts is not random. On the whole, eggs near to the cyst wall, i.e. in the periphery, tend to hatch first compared with eggs nearer the core. Apparently a 'hatching gradient' is set up.

Two factors are known to affect hatching; there may be others. Triffitt (1930a) showed that no hatching takes place in the absence of oxygen, and she also showed (1930b) that although a few larvae will emerge in water, large-scale hatching does not begin until the cysts are stimulated with root diffusate. Either of these factors might be capable of explaining the 'hatching gradient'. The possibility of root diffusate being the critical factor could be tested by examining the hatching pattern of cysts like those of the beet eelworm, from which there is some hatching in water alone, or by examining cysts from the field if the findings of Oostenbrink (1950) and Fenwick (1950) are of general validity. However, the balance of evidence already suggests that oxygen is the more important factor.

The cyst wall has been shown to be permeable to root diffusate (Ellenby, 1955); this might be thought to explain why the eggs near the wall hatch first. However, there is always some early hatching from the core even though it is shown to be less than from the periphery. Presumably, therefore, root diffusate reaches the core fairly quickly. Moreover, if root diffusate were responsible for the difference between periphery and core, the periphery-hatch/core-hatch ratio might be expected to decrease with time as hatching proceeds. In fact, as shown by figs. 8 and 9, neither total emergence nor hatch rate has any significant effect on the ratio. This suggests very strongly that some factor other than root diffusate is responsible for the hatching gradient.

The fact that cyst size does not affect the periphery/core ratio is at first rather puzzling, for when the core becomes farther from the wall the gradient should become steeper, i.e. the ratio should increase. However, as the size of the core is constant, increase in cyst size leads to an increase in the size of the periphery alone and therefore to the number of eggs contained in it. If hatching is reduced in the core because it is farther from the wall, it will also be reduced in the periphery because, as cyst size increases, there will be more and more eggs at a greater distance from the wall than there are in the peripheries of smaller cysts. The effects on the core and periphery may therefore balance and the ratio remain fairly constant with increasing cyst size. This, in fact, is found to be the case.

In the present work, total emergence had to be restricted to about 150 larvae per cyst because of technical difficulties associated with dealing with large numbers of fragments in the sections. Therefore in the majority of cases hatching was not allowed to proceed to the cessation of emergence. Thus it is by no means clear whether the factors bringing about the cessation of emergence are similar to the factors producing the gradient demonstrated in this work. But, clearly, root diffusate can have nothing to do with the cessation of emergence as this occurs even in the presence of freshly supplied root
diffusate; on the other hand, oxygen deficit could quite easily form part of the complex situation resulting in inhibition of hatching. And the present results are in agreement with the hypothesis that a gradient of oxygen tension is responsible for the hatching gradient.

Whether or not the gradient is one of oxygen tension, a gradient of some kind is clearly demonstrated. After stimulation there will be an increase in larval movement within the cyst; nevertheless the gradient exists in spite of this, indicating that the stirring effect caused by larval movement is negligible. This is not surprising, for the cysts are packed with eggs and egg shells and this baffles the effect of the movement.

A high oxygen tension at the outside of the egg mass would decrease towards the centre of the cyst even in the absence of any stimulation. Increase in larval activity could quickly steepen this gradient, helped possibly by proximity to the wall and therefore to the stimulus in addition to proximity to the oxygen. Hatching presumably ceases when the oxygen tension falls below a certain minimum level even in the presence of root diffusate. After stimulation, more oxygen may be required by the larvae, owing to their increased activity: if this oxygen is not replaced quickly enough, a deficit might develop causing inhibition of hatching. Further, the increased larval metabolic rate would result in increased production of CO₂, which, although it might escape fairly quickly, could help to inhibit hatching by increasing the acidity of the medium. Ellenby (1946b) has shown that pre-treatment with acid solutions does cause reduction in larval emergence. During the interval between inhibition and re-commencement of hatching the oxygen deficit could be made good and the CO₂ concentration revert to normal.

The above hypothesis is in agreement with Ellenby's suggestion that inhibition occurs as a result of stimulation with root diffusate, i.e. as a result of increased larval metabolism within the confines of the eelworm cyst. The punctures in his cysts could speed the entry of oxygen and exit of CO₂ and thus delay the onset of inhibition. An hypothesis of oxygen deficit might also explain the difference in the levels of infection of heavy soils and light sandy soils. Triffitt (1930a) suggests that the higher degree of infection of the latter is due to its better aeration, i.e. to the availability of more oxygen. Further, Wallace (1954) suggests that high emergence from beet eelworm cysts in water is correlated with high oxygen concentration of the surface films.

The fact that, in general, the highest hatches are found in the ends of cysts is not inconsistent with the oxygen tension hypothesis. No egg contained in an end is ever more than 100 μ from some part of the cyst wall; whatever the size of the cyst the eggs in the ends, taken as a group, would always be in a region of higher oxygen tension than those in the periphery. The end hatches would therefore tend to be higher. As cyst size increases, ends and peripheries both get bigger and their hatches would therefore, on hypothesis, decrease. But because of the relative distances from the wall of eggs in the ends and the peripheries, the end hatches would decrease more slowly and the end-hatch/periphery-hatch ratio might tend to increase. In fact, this has been shown to
be the case for cysts sectioned longitudinally (fig. 13, p. 509), in which the neck and vulva are incorporated in the central region.

Except as exits for the larvae, the part played by the natural apertures in hatching is not clear. The hatch from the neck end is not significantly greater than that from the periphery, but this may be due in some way to its abnormal shape. The end hatch has been shown to be significantly greater than the peripheral hatch and the mean value of the end-hatch/periphery-hatch ratio does not change significantly whether the vulva is included in the sections of the end or not. Both apertures are probably very small; accurate measurements are difficult but the apertures appear to be considerably smaller than the diameter of larvae. The apparent difficulty which the larvae have in emerging from either aperture under in vitro conditions supports this view. Eelworms are able to pass through holes considerably smaller than their own cross section; for example, the chrysanthemum eelworm, *Aphelenchoides ritzema-bosii*, enters leaves through the stomata (Stewart, 1921). The apertures of potato eelworm cysts are apparently unimportant in relation to the hatching pattern and the results of the present work are consistent with the view that root diffusate enters the cyst equally through all parts of the wall.

Little is known about what actually happens inside the cyst. The present paper gives some idea of the state of the cysts after varying periods of hatching. It enables some suggestions to be made about the causes of the distribution of hatching inside the cyst but much more evidence is needed before anything definite can be said on the matter.

I am grateful to Dr. C. Ellenby for his advice and encouragement at all stages of the work; also to Professor A. D. Hobson for his interest, and to all others who helped in any way. The work was financed by a grant from the Agricultural Research Council.

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