THESIS PRESENTED for the DEGREE of Ph.D.

by

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A NEMATODE DISEASE of POTATOES CAUSED by HETERODERA SCHACHTII (SCHMIDT)

The disease under review is caused by a particular species of the genus Heterodera which we prefer to call Heterodera schachtii (Schmidt) (the beet eelworm)* discovered by Schacht (46) in 1859 and recognised and described by Schmidt (47) in 1871.

HISTORICAL

Although for the past 50 years or more the importance of eelworms as causal agents of disease in plants has been recognised, and the literature on this subject is exceptionally voluminous, it is a remarkable fact that their pathogenic effect on potatoes has not been investigated until within recent years.

As far back as 1881 Kühn (23) recorded the occurrence of H. schachtii on potatoes, but is doubtful if at that time it was a true parasite upon this crop for the same author (26) and Hollrung (20) in 1891 classed plants of the order Solanaceae as being non-susceptible and advocated planting of potatoes on 'beet sick' soils. Some years later (1896)

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* Cobb (8) has placed this species along with Heterodera radicicola in a new genus, Caconema.

Vanha and Stoklasa (71) who, as far as we can trace, next contributed to the subject, actually said that the potato was "just that plant which Nematodes of the genus Heterodera avoid".

Even Marcinowski (31) who, in her admirable paper of 1909, made a great contribution to our present knowledge of Nematology by her recognition of the polyphagous nature of these parasites and of their remarkable power of adaptation to various host plants, quoted Vanha's opinion for the guidance of biologists.

About the same time (1909), however, Hiltner (18) called attention to the fact "that the beet nematode (H. schachtii) can pass over to the roots of the potato". But it was not until 1913 that the potato was deinfitely recognised as a host plant of *H. schachtii*. In that year Zimmermann (76) showed that the parasite was able to adapt itself to the potato to such a degree that it not only injured its cultivation, but in certain localities, as in Mecklenburg, completely prevented it. Especially did he find the trouble important where potatoes were grown in close sequence. He also emphasised the fact that the disease had frequently been ascribed to other causes and in 1920 published further papers (77, 78) dealing with nematode attack (*Heterodera*) on the potato.

It is now a well established fact that, as Baunacke (3) says, the dreaded parasite shows an adaptability which spares few of our agricultural plants. By way of field weeds of different species, he says it passes to cultivated plants belonging to other Natural Orders and finally accustoms itself to these. For example, Black Night-shade (*Solanum nigrum*), which is a very common weed in sugar beet fields in Germany frequently becomes strongly infected by the beet nematode (Baunacke (3) and Wollenweber (74)) and so it is only natural that the parasite soon accustoms itself to the closely related potato plant (*Solanum tuberosum*) and then regularly utilises it as a host plant (2). It is questionable if in view of our present knowledge of the adaptability of this parasite, there is any necessity to assume that the transference occurs through the intermediate agency of weeds.

Within recent years several papers have appeared on this pest of the potato crop but so far no author has attempted to describe in detail the life history of the parasite and the symptoms of the disease and we do not hesitate, therefore, to treat these and such other aspects as have been overlooked and to discuss the effect that the parasite has on the potato plant from an ordinary biological standpoint.

OCCURRENCE

In England the disease has been reported in Lancashire and Cheshire by Smith and Prentice (50), by Smith (51), and by Smith and Miles (52), and in Lancashire by Triffitt (68). In these counties the main damage is done to late varieties of potatoes, in particular to Kerr's Pink. In Lincolnshire and Hertfordshire the disease has been under investigation by Morgan (33, 34, 35), Peters (42), Triffitt (66, 67, 68, 69, 70), and Edwards (10), and in Yorkshire by Strachan and Taylor (59, 60) where it is known as "Dab".

As our experience of the potato eelworm has been obtained mainly from a study of it in the Ayrshire early potato growing districts and as there are several peculiarities in the cultivation of the crop in that area, it will be instructive to give at this stage a short description of the growing of early potatoes in that county. It must, however, be emphasised that the disease is not peculiar to Ayrshire. It has been diagnosed by us in many other counties of Scotland, mainly however in gardens and allotments.

THE EARLY POTATO CROP in AYRSHIRE

The growing of early potatoes on the Ayrshire coast has been a very important industry for many years. Generally about 3,500 acres are grown every year and this acreage extends from Ballantrae in the south to West Kilbride in the north of the county. The fields given over to this crop in most cases border the shore but in isolated cases they may be found about half a mile inland.

No rotation is practised and on many of the fields potatoes have been grown without a break for 30 to 50 years. It is customary, however, after the crop has been lifted in June or early July, to take a catch crop such as Barley, Rape, Italian Ryegrass, or Green Kale.

Barley is quite commonly grown, and it is usually fed off green to sheep in the early autumn but in some cases if the seed has been sown early, it is allowed to ripen and harvested as a grain crop.

Rape, on the other hand, can only be grown at most once every five years because of its susceptibility to Finger and Toe disease (*Plasmodiophora brassicae*). It is invariably fed off to sheep.

When Italian Ryegrass is grown, about 3 bushels of seed are sown per acre and a good crop of green food obtained even in spite of the prevalence of annual weeds which may tend to choke out the young plants.

Green Kale is not often taken, mainly because it is sold for human consumption and there is only a limited demand for this as a vegetable.

In some years the ground may be seeded with Oats and a crop of grain secured. Oats, however, are very liable to be attacked by the eelworm Tylenchus dipsaci in the early stages of growth, and hence the growing of Oats as a catch crop is not extensively practised.

The ground is, as a rule, not ploughed for the potato crop until December or January, and previous to ploughing, either dung (15-20 tons) or seaweed(30-40 loads per acre) is applied. An effort is made to apply dung and seaweed in alternate years where both are available.

Along with the dung or seaweed a liberal quantity, generally 10-15 cwts per acre, of a complete mixed artificial manure is applied containing on an average 7.5-8.25% Nitrogen 8-9% Phosphoric Acid, and 3% Potash.

The seed is planted from the middle of February to the end of March. The variety almost universally grown is Epicure.

The lifting of the crop proceeds from about the middle of June to the end of July. The crop in the Ballantrae area is generally first to be marketed, then that in the Girvan district, which in turn is raised from 10-14 days before that in the West Kilbride area.

It is obvious that when potatoes are grown in close sequence as in Ayrshire, ideal conditions prevail for the propagation of disease in general and for eelworm attack in particular.

Analysis of the soils.

Through the courtesy of Dr McArthur, Professor of Agricultural Chemistry in the West of Scotland Agricultural College, we append the analysis of two soils. Soil No.1 is representative of the majority of the richer soil in the Girvan district, but No.2 in which there is a much higher fraction of coarse sand is typical of the poorer soils predominating in some areas, both north and south of Girvan.

From the mechanical and chemical analysis of the soils it is apparent that there is very little variation in soil type. They are all of the nature of a light sandy loam.

	No.1	No.2
Fine Gravel.................	12.11	2.37
Coarse Sand.................	29.93	59.67
Fine Sand...................	16.73	13.07
Silt........................	8.58	7.33
Fine Silt...................	12.35	6.26
Clay........................	1.60	.95
Soluble loss on Mech. Analysis.................	5.86	3.58
Moisture....................	4.06	2.30
Loss on Ignition............	11.19	7.84
Nitrogen....................	.345	.219
Soluble in conc. H.Cl.		
K_2O......................	.333	.403
Do. P_2O_5................	.671	.265
Lime requirement............	.326	.279
pH. value...................	6.1	5.3

Analysis were carried out in accordance with the British Official (1906) method.

SYMPTOMS OF THE DISEASE

Foliage symptoms. The first signs of eelworm attack become evident in the slow rate of growth of the shaws or

PLATE I

Fig. 1 **Fig. 2** **Fig. 3**

Fig. 1.—Potato plant in early stages of infection, showing "feather duster" appearance and leaf roll symptoms.

Fig. 2—A plant badly infected. Lower leaves have all withered. Upper leaves unhealthy and of a yellowish green colour.

Fig. 3.—Plant showing final stage of disease with only four withered leaves still attached.

PLATE II

CIRCULAR DISEASED PATCH SURROUNDED BY AREA IN WHICH PLANTS ARE HEALTHY.

haulms of infected plants, which lag considerably behind normal plants in their development. This can be noted in the very early stages of growth almost as soon as the sprouts appear above ground.

From this point on, symptoms of the disease are fairly well marked and become much more evident as the season advances. The shaws or haulms of infected plants are few in number and remain thin and spindly throughout the growing season. The foliage of the whole plant assumes a dull unhealthy appearance and a tendency to wilt is apparent in the lowermost leaves which eventually hang down on the haulms, wither away, and drop off. (Plate 1, fig.2)

The young leaves at first are not so affected and, in consequence of the loss of the lower ones, form a sort of tufted head to the bare stem. In the field, therefore, infected plants can at once be distinguished by their characteristic appearance, looking like so many feather dusters sticking head upwards out of the soil. (Plate 1. fig.1)

At this stage more or less circular diseased patches are now clearly evident in the field (Plate II.) Even at a distance they may be detected not only by the sickly colour of the foliage but by the general dwarfing of the plants and consequent failure of the crop to meet in the drills. Occasionally the diseased patches are not so well-defined and a

PLATE III

Fig. 1 Fig. 2 Fig. 3

Fig. 4 Fig. 5 Fig. 6

LEAF SYMPTOMS.

Fig. 1.—Triangular shaped withered area at apex of terminal leaflet.

Fig. 2.—Whole apical leaflet withered. The leaflets on the right side of the midrib shew symptoms, while those on the left side are still healthy.

Fig. 3.—Diamond shaped withered area at apex of terminal leaflet. Withering occurring on the leaflets on both sides of the midrib.

Figs. 4-6.—*Later stages in the destruction of the leaf.*

gradual transition may occur between the healthy and diseased areas.

In any one plant the progression of the disease takes place in a well defined and regular sequence.

The lower leaves as has already been noted, are first to show signs of disease and, in general the progression of effect is from below upwards. The central and youngest leaves remain healthy longest and give the characteristic tufted head to the shaw.

In the individual leaf the progression of effect takes place in the opposite way, namely from above downwards.

The terminal leaflet of each leaf is first to show symptoms. Its tip becomes brown, forming a triangular (Plate III. fig.1), or diamond shaped area (Plate III. fig.3) according as the withering develops on one side or equally on both sides of the leaflet mid-rib. Although confined to the tip at first, brown areas, more or less triangular in shape, soon appear along the margins of the leaflet. (Plate III. fig. 1). The brown areas become confluent and the browning extends inwards towards the centre until the whole leaflet withers and dies. About this time the tips of the first pair of leaflets begin to turn brown and the withering proceeds as in the terminal one. (Plate III. figs.2 and 3). Other leaflets are attacked in regular succession. One useful diagnostic character of the disease is that the withering margin always rolls upwards and inwards. Further the dead parts are crisp and dry. (Plate III. figs. 2, 3, 4.)

A cupping or folding inwards of the leaflet towards the mid-rib frequently occurs before any withering has made its appearance. (Plate I. fig.1). This symptom may remind one of Leaf Roll but it must be emphasised that it occurs in plants which are free from this or any other degeneration disease. The explanation is doubtless to be found in a deficiency of water in the leaves due to a lack of functional absorptive rootlets.

One leaf characteristic remains to be noted: that the leaflets on one side of the mid-rib almost invariably begin to wither before the leaflets on the other side. One side, indeed, may be completely withered while the other side is yet green. (Plate III. figs 4 & 5).

Occasionally a certain amount of fasciation may be observed - usually the growing together of two or more, rarely three, leaflets - and this accords with the conclusion of E.F.Smith (53, 54, 55) that fasciation may sometimes be caused by parasites and their products.

The foliage symptoms while always noticeable vary very markedly depending on the degree of infection and upon the stage of development of the plant when attacked. These appearances (except in finer details) may readily be mistaken for potash starvation.

Root Symptoms. It is to the roots that we must turn to ascertain the true nature of the disease for the root is the part of the plant which harbours the parasite; and not only does the root show symptoms of attack but the encysted females are so large that they are easily visible to the unaided eye.

In plants attacked by Heterodera, the females may be found in all stages of development. In the final stage the females break through the cortex and appear on the outside fully nurtured and ready to be fertilised. After fertilisation they swell up into resting cysts and die, although they still remain loosely attached to the rootlets. When mature in the form of cysts they look, both in size and in colour very much like poppy seeds. In the younger stages they are of a milky white colour changing through yellowish white and yellow, to reddish brown as the season advances. The undeveloped cysts will generally be found on actively growing fine rootlets, while the more fully developed ones are usually abundant on the thicker root fibres. This distribution can be accounted for by the growth of the rootlets subsequent to attack, since the position of the females on the root is constant throughout life.

Although the presence of encysted females on the roots is an infallible diagnostic character it must be remembered that, as they represent only a stage in the life cycle of the nematodes, they may not always be found. The cysts, for example, appear in Ayrshire about the end of May or the beginning of June. In their absence the foliage characteristics and the appearance of the roots are of special value in the diagnosis of the disease.

The cysts are more or less numerous according to severity of attack. On the roots of a plant showing slight infection 200 cysts may be found while on badly infected plants as many as 20 mature cysts may be detected on one linear inch of root. In one exceptional case 5 young females were noted on one transverse section of a rootlet. As each cyst contains from 250-300 eggs and larvae, and as, according to Baunacke (3) there may be several generations in one season, the amazing capacity of the eelworm, for rapid increase and mass attack, can be readily gauged. Strubell (61) reckoned the power of increase of a single Heterodera female in the course of a summer under favourable conditions to be 22781 milliards.

Heterodera schachtii, unlike the closely allied species H. radicicola, causes little or no gall formation on the roots, but a certain amount of distortion and swelling does occur especially in the more strongly infected parts immediately after invasion. Later, by further growth of the root, these swellings disappear. Furthermore they occur only where the eelworms are present in an active stage and are not found in roots bearing encysted females.

It is characteristic that, in the early stages of the disease, an infected plant shows an abnormal development of fibrous roots. These have been described by Marcinowski (31) on sugar beet as "Hunger roots". The fibrous roots are not only long, but are profusely branched, giving a characteristic feathery appearance to each single rootlet.

Of this beard-like mass, the finer branches already rendered functionless by the nematodes, are of a dry brittle nature and readily break to pieces in the hand. By delicate handling it will be found that the outer layers of each root fibre are loosely attached and can be pulled off, like a sheath, leaving the central woody cylinder intact. This condition results in particular from the destruction of the cortical layers of the rootlet by the eelworms. A similar condition has been described by Thomas (62) on the Citrus nematode (Tylenchus semipenetrans).

Despite the fact that the plant appears to have a good root development, this is not the case. The finer roots are no sooner produced than they are killed by the invading nematodes. The root mass observed, therefore, is, to a very large extent, composed of dead and dying fibres of no use to the plant for absorptive purposes. It will be readily understood from this that affected plants will suffer very much from drought, the effects being exaggerated by the presence of the eelworms in the root.

Such nutriment as the plant can produce is used up, at the expense of foliage and tubers, in the production of new roots. These new rootlets are produced above the point of attack hence in plants which have been attacked for a long time they are to be found as a ramifying mass immediately below the surface of the soil. This cannot go on indefinitely and eventually the plant, unable to compete with the nematodes, produces no more roots. The dead ones drop off, and in the final stages of attack therefore the fibrous roots have almost completely disappeared, and from sheer starvation, death of the plant may ensue. (Plate I, figs. 2 and 3). If, as is more usual, the plant survives, it grows unduly late into the autumn until it is cut down by frost.

<u>Secondary infection of the rootlets</u>. When a nematode-

attacked plant does die, its death is most frequently due to secondary infection. Such mechanical injuries as are caused to the roots by the eelworms serve as a ready means of entrance for secondary parasites.

Of particular importance in the case of the potato is the common association of the fungus *Rhizoctonia solani* with nematode infected roots. We have almost invariably found a profuse growth of *Rhizoctonia* mycelium, particularly in those parts of the root which have suffered most from the eelworm, but we have definitely established that *Rhizoctonia* infection is subsequent to eelworm invasion. In the young sprouting tubers, we could find the larvae of the eelworms in the rootlets in 14 days after the setts were planted; (see p.27) whereas the fungus could not be isolated from the roots until a further period of 14 days had elapsed. Further the rootlets invaded by the fungus were always in a dead or dying condition as a result of the destruction of the root tissues, particularly the cortex, by the eelworm larvae. This observation is important because there still seems to be considerable doubt on this point. Morgan (33) states that "It is impossible to make out a clear case of eelworm damage ---- It seems as if *Rhizoctonia solani* contributed largely to the failure of the crop". On the contrary Smith and Prentice (50) definitely prove that there is a positive association of intensity of disease in the plants and the cyst content of the soil.

Often in the field certain areas will be found where most of the plants are in a very unhealthy condition. Many of them die off prematurely, others remain very stunted in growth with few foliage leaves. In such patches a few plants attain a normal size and are apparently quite healthy (Plate IV.)

These areas are spoken of as "Nematode Nests" and according to Baunacke (3) are the result of a very heavy eelworm infestation along with strong secondary infection. Where these nests do occur in our area we find Rhizoctonia solani to be the principal secondary parasite, although on occasion Bacillus solanacearum as the accessory micro-organism has been isolated.

It is significant that in Ayrshire these patches tend to occur on the very light sandy areas which may be found in many of the fields. Such soils contain a very low percentage of organic matter, and on that account the plants even apart from parasitic infection and under the best of conditions tend to suffer from drought and to be stunted in growth; but when the root systems are deficient as they are in attacked plants, extreme dwarfing of the plant is to be expected. Further a light sandy soil is relatively warmer

"NEMATODE NEST."

Most of the plants are very stunted, but some are normal in size. Note the tall healthy appearance of the plants in the background, outwith the "nest."

in the early spring than one with a higher content of organic matter, consequently the larvae become active and attack the young plants at an earlier date in the sandy soil than they would in a soil with more humus. The result is that, as the plants are attacked at an earlier stage of their development, they suffer to a greater extent than those invaded at a later date.

In the case of the Ayrshire potato soils, we have no evidence that in the "eelworm nests" there is a relatively higher eelworm content, and from our counts it would appear that the cyst content in the eelworm nests is rarely if ever higher than in the rest of the field. Consequently the reason for the greater damage to the plants is not traceable to an excessive concentration of eelworms in such areas but to an earlier invasion of the plants by the larvae due to the soil conditions. This earlier invasion will result in fewer cysts being found on the plant and consequently in the soil since all plant parasitic nematodes, providing they are still in the initial stages of development, have a power and readiness to leave their host plant. First stage larvae have been frequently observed leaving rootlets which had been already invaded: a plant therefore, which has suffered severely for a long period from eelworm attack will generally be comparatively free from eelworms while a vigorous healthy plant probably recently infected will harbour a very large number of the parasites. This has been commented on by numerous observers including Hollrung (20) and Liebscher (29). Hollrung finds no nematodes on the roots of beets in the final stage of the disease. Liebscher shows that there are no nematodes on dead and dying beets but that they are common on beet with strong green colour.

The Yield from Infected Plants. From our observations of many affected potato fields extending over five years, we have ascertained that the presence of the eelworm in the rootlets causes a very serious reduction in yield, but that the loss varies from year to year. Erroneous conclusions therefore, may be drawn from data secured from one or two years' experience such as has apparently been done by Smith and Miles (52) who say - "The results obtained lead to the conclusion that eelworm infestation is not of primary importance in determining the yield of potatoes".

The reduction in yield in some years specially favourable for the growth of the crop may not be very marked; but in unfavourable years the reduction may be so serious as to render the crop unprofitable with the result that the field in question may be thrown out of early potato cultivation for a varying period. Such fields are described by the farmer as being "potato sick".

The weather in the months of March and April to a great extent determines what influence the eelworms will have upon the crop yield. If these months are moderately cool, but favourable for growth, the plants will be well established before they are invaded to an appreciable extent by eelworm larvae, provided the crop gets no serious set back from late frosts. Further if the months of May and June are moist and cool, the damage done to the roots may to a great extent be repaired. Affected plants, as we have seen, produce a profusion of fine rootlets which are functional for some time at least. An unduly dry period, especially in April or May, is more or less fatal to the securing of a good yield as the plants suffer in an intensified degree from drought, both natural and artificial.

The yield from a badly infected plant is always low. The produce consists at most of one or two marble-sized tubers with a large number of tubers the size of peas. It is rarely that the yield exceeds 1 lb. of potatoes per plant, unless when the plant has become infected late in the growing season. The yield is not diminished to the same extent in the case of early as with late varieties. The average yield of earlies on badly infected soil is from 2-3 tons per acre, that of lates 15-20 cwts per acre. This difference is undoubtedly due, in the case of earlies in Ayrshire, to the optimum temperature for hatching of the larvae from the cysts never being reached during the growing period of the crop.

Rensch (44) has shown that some of the larvae of <u>Heterodera schachtii</u> (beet strain) begin to hatch out whenever the soil temperature reaches 6°C (43°F) and that the numbers emerging from the cysts gradually increase up to the optimum temperature for hatching which he puts at 25°C (77°F) x Further the motility of the hatched larvae increases as the temperature rises from 6°C to 25°C.

In Table I. we give the average soil temperature for the year 1929 at a depth of one foot obtaining at Kilmarnock for the months, March, April, May, June, the period during which the early potato crop is growing and for purposes of comparison the average temperature for July, August and September.

TABLE/

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x Baunacke (3) states that a soil temperature not below 18°C. and not above 29.1°C. is necessary for the larvae to hatch from the cyst.

TABLE I.

Average Soil Temperature at Kilmarnock at depth of one foot for the Year 1929.

1929			
March	38°F.	=	3.3°C.
April	43.7	=	6.5
May	50.5	=	10.3
June	57.1	=	13.9
July	60.2	=	15.7
August	58.3	=	14.6
September	56.8	=	13.8

If Rensch's figures for the beet strain apply to the potato eelworm, the latter has only a relatively short period (9-12 weeks) during which it can be active and parasitise the crop which is generally raised in June. In the case of late potatoes planted in April or May the eelworm can attack the plant during practically the whole period of growth of from 5-6 months and cause greater damage to the plant especially in the early stages of growth.

We have never seen a good crop of a late variety of potato grown on a heavily infested soil. This observation does not accord with that of Zimmermann (77) in Germany, or Morgan (33) in Lincolnshire, who record more damage to earlies than to late varieties as a result of eelworm attack. It seems from our observations that the extent of the damage on, and consequently the yield from, diseased potatoes will be dependent upon the incidence of the eelworm attack in the early stages of growth. In Scotland there is a relatively low soil temperature obtaining when early potato seed is planted in February, and the organism does little or no damage to the crop until the plants are well developed, whereas in Germany or in England with a much higher soil temperature at planting time the eelworm will become active much sooner and will parasitise the plant almost from the commencement of growth.

Through the courtesy of the Air Ministry we append a table (Table II) of soil temperatures at Wisbech[x] in Cambridgeshire for the 4 months, March, April, May and June of 1929.

TABLE II.

Mean Soil Temperature at Wisbech at depth of 8" for the year 1929.

March	40.3°F.
April	45.2
May	45.3
June	60.2

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[x] Wisbech is the nearest meteorological station to Kirkton, Lincolnshire.

It will be noted that the soil temperature in March at Wisbech is 2.3°F. higher than that at Kilmarnock and over the whole period from March to June it is higher than the Kilmarnock soil temperature by 2.7°F. Soil temperatures at Dresden, given by Baunacke (3), are, for the corresponding 4 months, from 1 to 4°F higher than those at Kilmarnock.

The observations of Zimmermann and Morgan - that early varieties of potatoes suffer more than late potatoes from Heterodera attack - may be further explained on the assumption that early potatoes, on account of their rapidity of growth, especially in the early stages, would attract a greater number of active eelworm larvae than later varieties planted at or about the same date, since the so called excretions from the roots of a rapidly growing early potato would be more potent than those from a slower growing late variety. This explanation assumes that the soil temperature at the time of planting of the early variety is sufficiently high to activate the larvae present in the soil or in the cyst.

The tubers from infected plants are perfectly sound, and show no injury or gall formation on their surface, but encysted females can generally be found attached to their surface, either free or in the adhering earth.

Occasionally tubers occur with cavities immediately underneath the skin. These cavities are filled with a whitish crumbling material which on microscopic examination is found to contain numerous eelworms as well as eggs in a matrix of loose cells and starch grains. Such nematodes are purely saprophytic in their nature and of the many hundreds of potato tubers which have been examined, the presence of H. schachtii has not been demonstrated.

Briefly reviewing what has been said as to the effect of this eelworm on the potato plant, we can say that Heterodera Schachtii acts as a true parasite, and makes the living plant serve for its nutrition as well as for its development. It does not usually kill the plant but on the contrary tends to prolong its life. By continual withdrawal of nutriment from the plant, growth is inhibited and ripening delayed, and as the plant expends its energies in the production of lateral roots little or no reserve materials are stored in the form of tubers. The eelworm injures the plant secondarily but distinctly by weakening it for combat against external conditions such as drought and by leaving behind wounds which render it liable to secondary infection of all kinds.

LIFE/

LIFE HISTORY

The life history as worked out by us will be dealt with under the following headings.

(a) Methods adopted in the investigation.

(b) Attack of the parasite on the host.

(c) Development of the parasite in the host.

(a) Methods adopted in investigation.

Well sprouted tubers of the variety Epicure were planted in boxes containing soil which was estimated to contain 96 cysts of Heterodera schachtii per 10 cc. and kept at a temperature of 18°C. Then at intervals of 3½ days, beginning on the tenth day after planting, two plants were lifted and their roots fixed in Carnoy's acetic alcohol* for 3 minutes, after which they were washed in several changes of absolute alcohol and preserved in the same fluid.

Examination of the fixed material was invariably done after clearing in Lactophenol. Rensch's (44) method of staining with an alcoholic solution Iodine in Potassium iodide and clearing in Clove Oil, while suitable for demonstrating the presence of larvae in the roots, was discarded owing to the tendency of the clearing agent to cause

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* Composition.

Glacial acetic acid	1	part.
Absolute alcohol	6	"
Chloroform	3	"

PLATE V

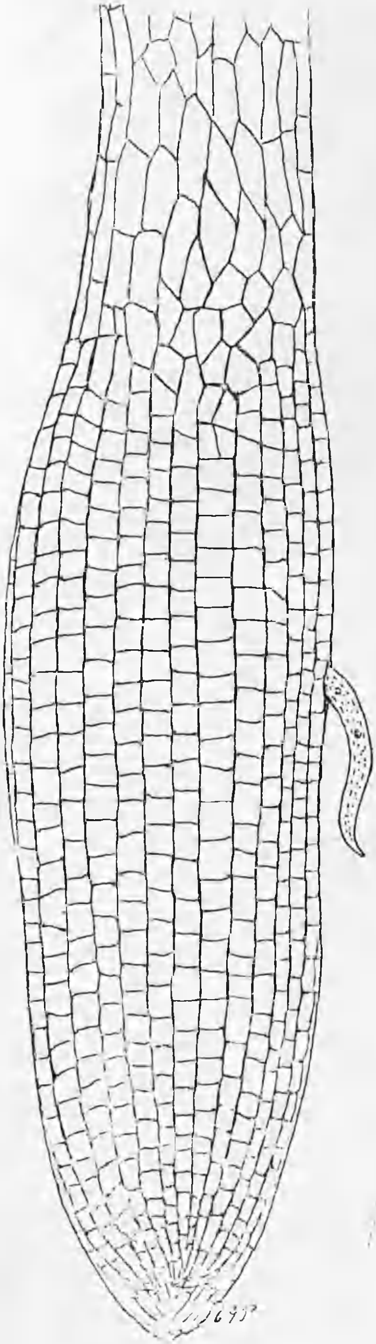


Fig. 1

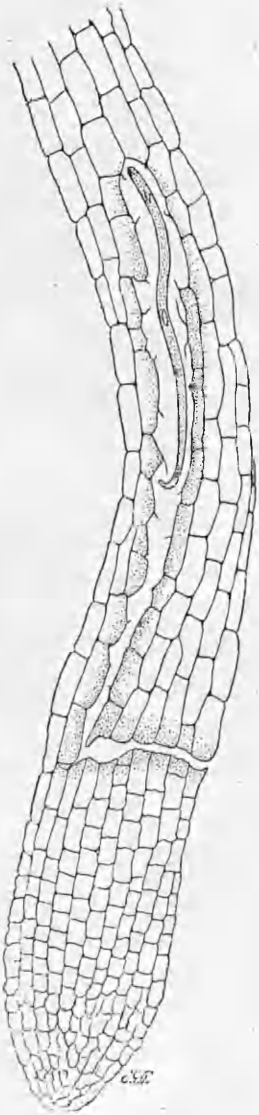


Fig. 2

Fig. 1.—Larva penetrating healthy rootlet—17 days. (x180.)

Fig. 2.—Larva inside rootlet drawn in optical section. Shows track of larva within the ruptured cortical cells—18 days. (x90.)

the material to shrink and to render it so brittle that only with extreme difficulty could the larvae be dissected out of the root tissues without breaking.

In preparing sections the best results were obtained by imbedding the material in clove-oil-celloidin and wax as described by Bolles Lee (27) and staining in Delafield's Haematoxylin and Eosin.

(b) Attack of the parasite on the host.

The larvae of Heterodera schachtii can be readily demonstrated actually entering the rootlets or completely inside the rootlet tissue of potato plants which have been growing for about 17 days in infested soil. This represents about 14 days of actual root growth in the case of early varieties, well sprouted before planting. [x]

In his paper of 1920 Zimmermann (77) says that "the invasion by the eelworms of the inside of the root-parts has up to now not been demonstrated and that further observations must be made to decide this point". They can be easily detected in the cortex of the finer rootlets if the plants are examined in the early stages of growth.

The point of entry to the rootlet is almost invariably subapical (Plate V. fig.1), but cases have been observed where entry has been gained into the older and thicker parts

[x] Throughout this paper a 17 day root will refer to a root from a potato which was planted 17 days previously and so on.

of the root system. The invasion continues throughout the whole growing season, and even late in the summer it is possible to find young newly formed rootlets into which larvae have just entered or are in the process of entering.

Baunacke's (3) investigations on *Heterodera schachtii* on beet, led him to support Berliner and Busch's* (4) belief that the larvae could not enter a rootlet unless the latter had suffered from some previous injury. While such injury would no doubt facilitate invasion by the larvae our observations accord with those of Reinmuth (43) that it is not necessary that the larvae can penetrate a perfectly healthy root by the action of its mouth spear (Plate V.fig.1)

Once inside the root, the larva confines itself to the cortex. It moves away from the root-tip toward the parent root and in doing so kills the cortical cells through which it passes (Plate V. fig.2). As these dead cells quite often take on a brownish tinge, the path of the larva is easily traced from its point of entry, especially if the rootlets be cleared in lactophenol or stained in an alcoholic solution of iodine in potassium iodide and cleared in clove oil.

Since the hole through which the larvae enter the root remains patent, micro-organisms of all kinds are furnished with an open passage into the tissues. This no doubt

PLATE VI

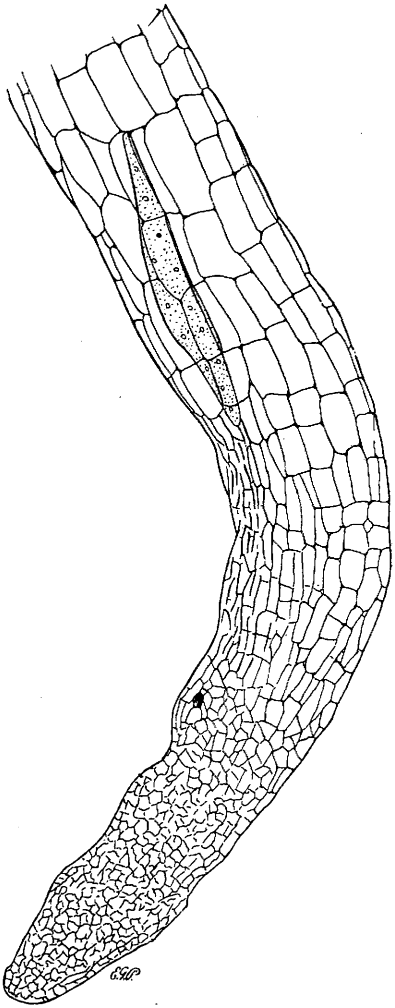


Fig. 1

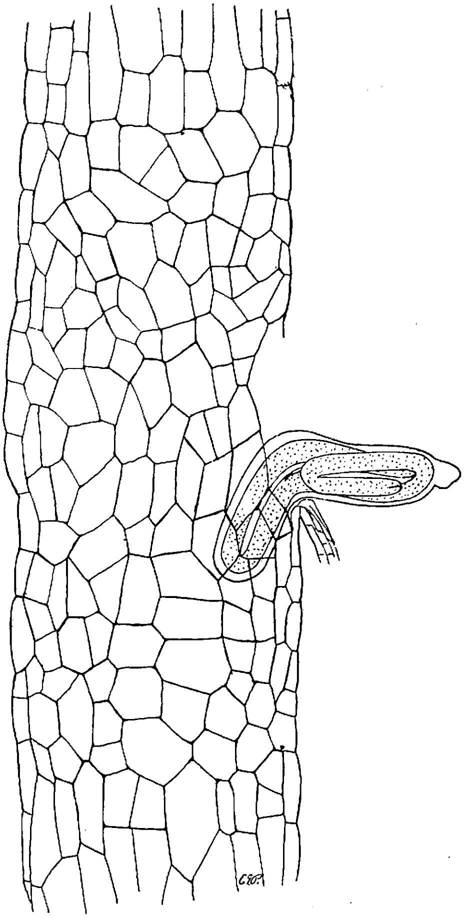


Fig. 2

Fig. 1.—Rootlet showing point of entry of larva (represented by black dot), dead apex of rootlet, and collapsed cortical cells between point of entry and position taken up by larva—35 days. (x90.)

Fig. 2.—Mature male bursting through cortical cells of rootlet—48 days. (x90.)

accounts to a large extent for rootlets invaded by larvae being frequently attacked by secondary fungi (see p.16).

The distance which a larva travels before settling down, prior to feeding and moulting, varies greatly. If only one larva has entered a rootlet, it seldom travels more than about 1 mm. and then ceases movement. But in the more usual cases where several have entered the same rootlet, they tend to travel up into the parent root before coming to rest - a distance of about 1 c.m. - This is possibly due to the fact that such a heavily infected rootlet - which is only about .14 mm in diameter - succumbs fairly readily to the attack and shrivels up, whereas the parent root, which has a diameter of about .5 mm is better able to tolerate the invasion.

The type of damage which a single larva produces on an infected rootlet is depicted in Plate VI. fig.1. The track left by it in passing from the point of entry to its present position is indicated by a line of collapsed cortical cells and the whole apical parts of the root have died and shrivelled. The cells in the immediate vicinity of the growing larvae remain apparently normal.

In the more common type of invasion where several larvae enter one rootlet and then pass into the main root - the same kind of damage is done but it involves and destroys the whole rootlet from its origin to its tip.

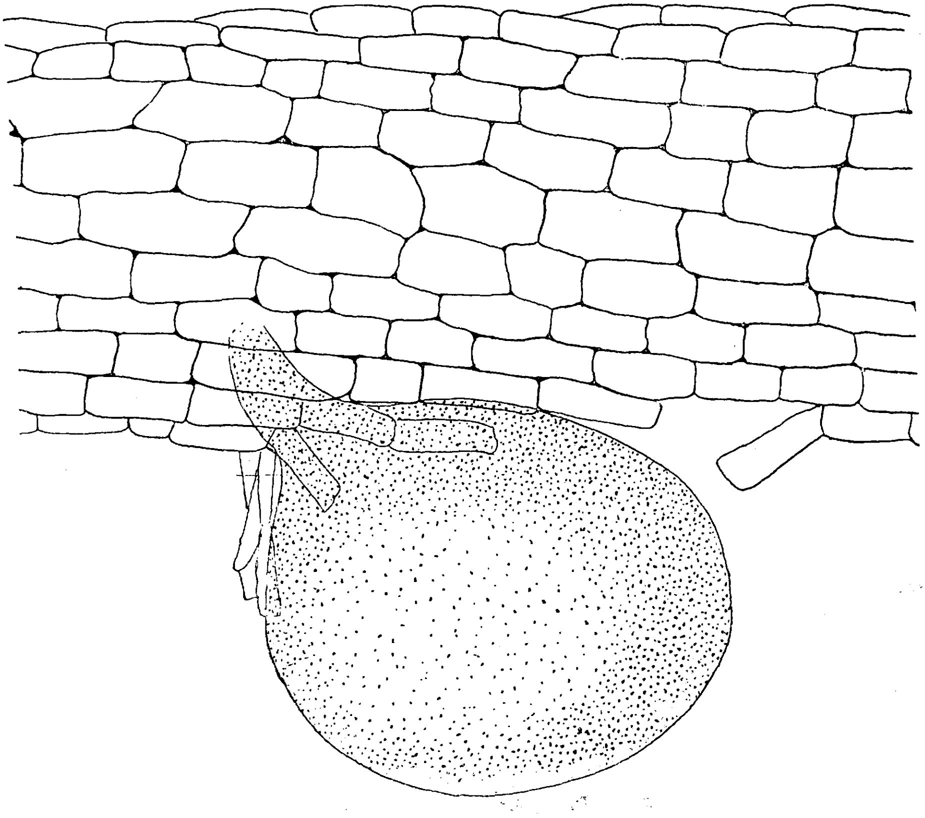


Fig. 2

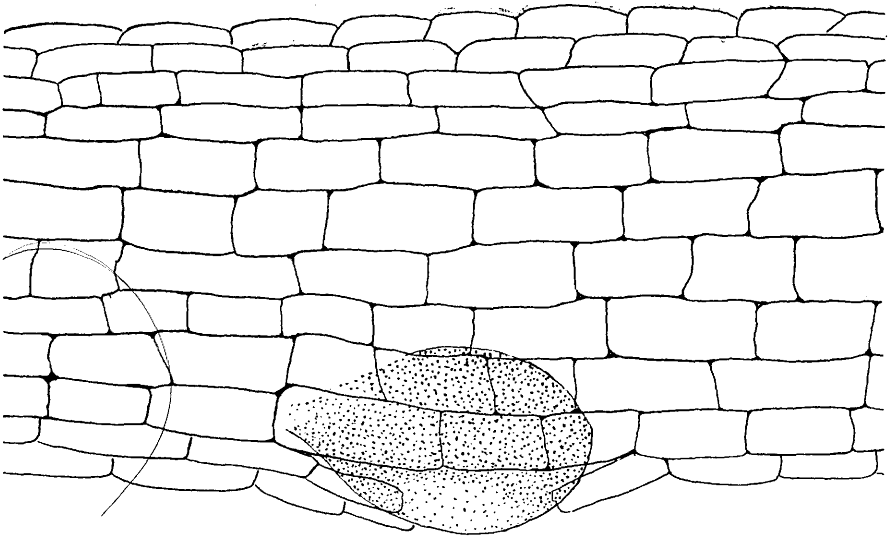
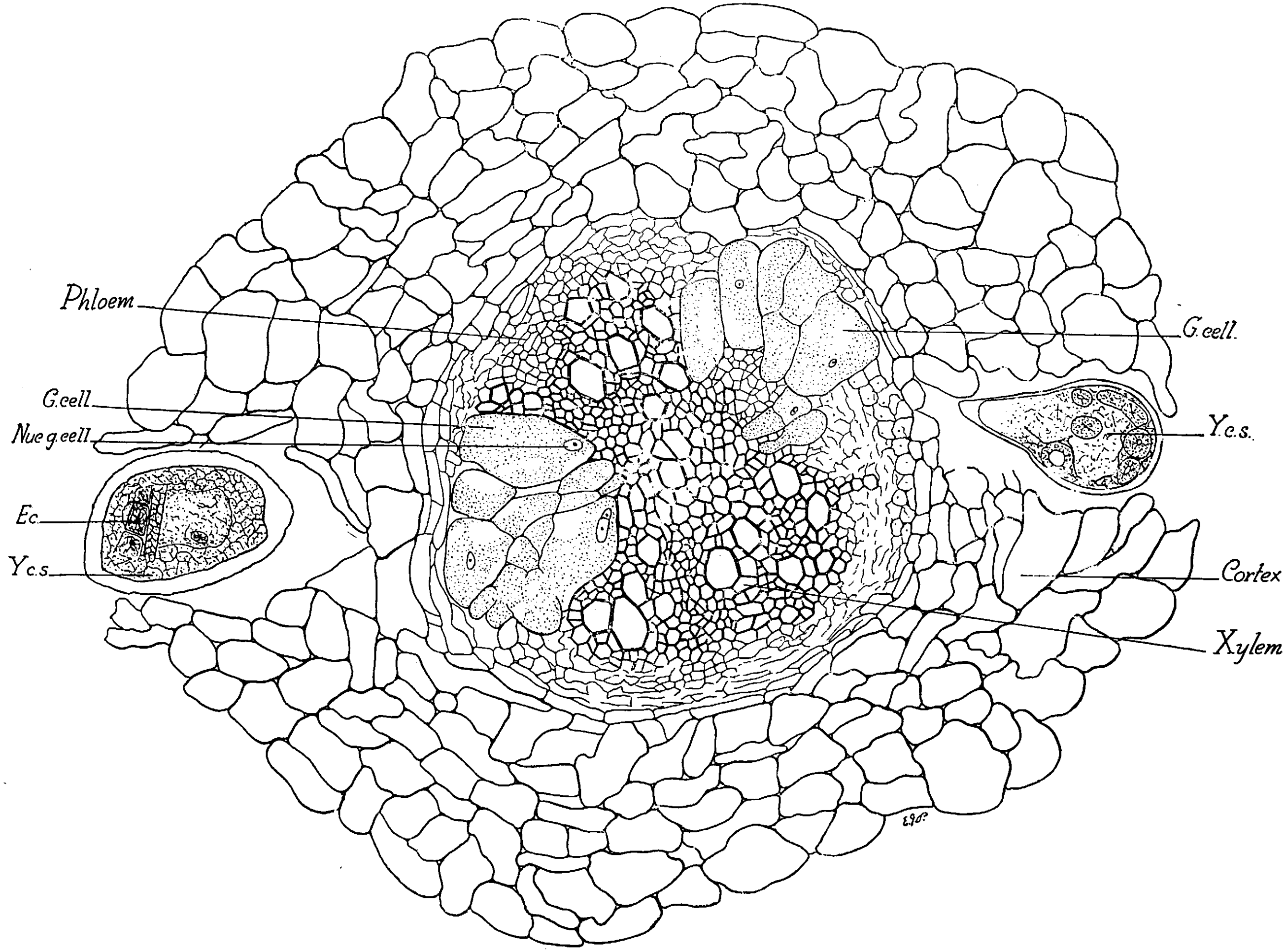


Fig. 1

Fig. 1.—Female bursting through cortical cells of rootlet—52 days. (x90.)
Fig. 2.—Swollen female protruding from rootlet—65 days. (x90.)

PLATE VIII



TRANSVERSE SECTION OF POTATO ROOTLET—45 DAYS. (x90.)

E.c. = Egg cell; G.cell = Giant cell; Nuc.g.cell = Nucleus of giant cell; Y.c.s. = Young cyst in section.

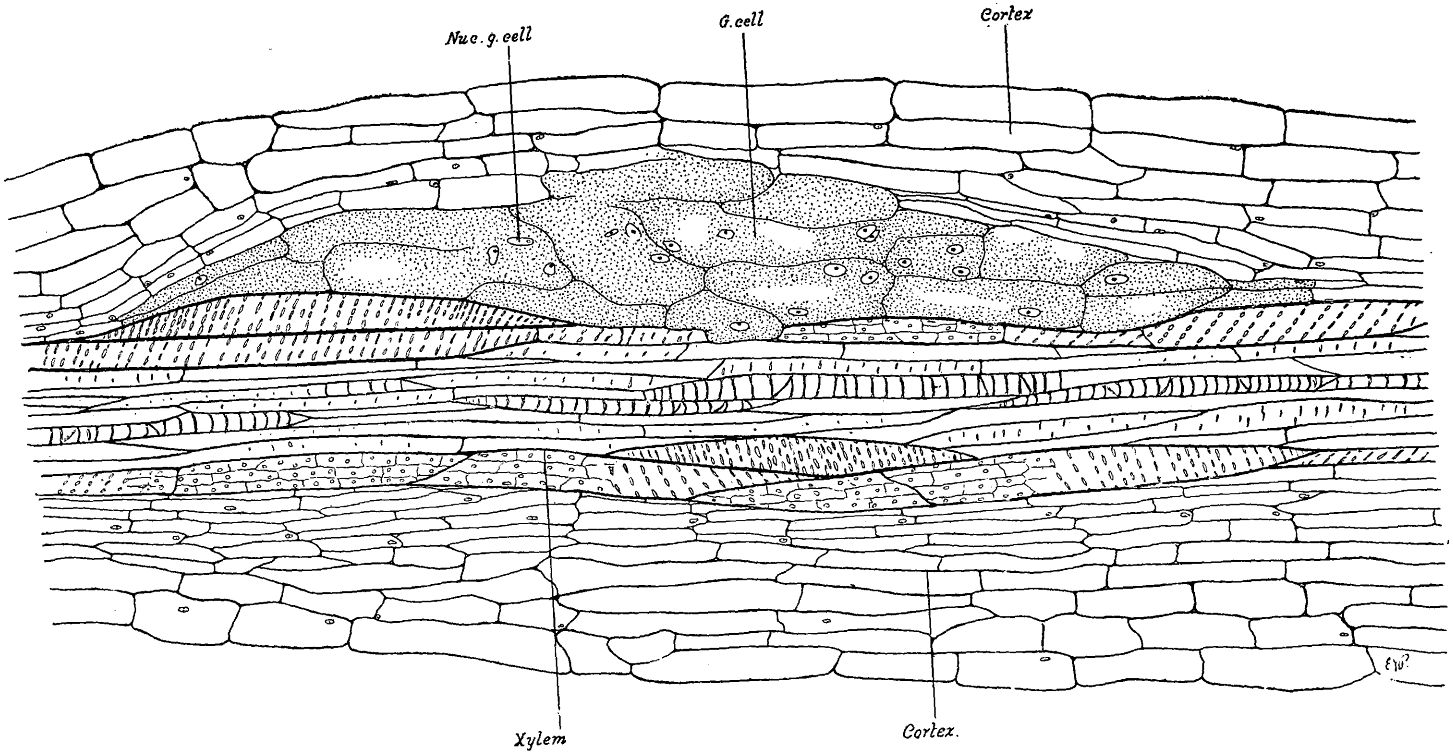
The larvae, meanwhile, having passed into the cortex of the main root become stationary and proceed to undergo the first stages of post larval development. Even in these thicker roots, a very heavy infection may lead to the death of the cortical tissue, but in less severe cases, the presence of the larvae is indicated only by a slight browning of the tissue in their neighbourhood. As many as 8 larvae in various stages of development may occur in one linear centimeter of a 45 day root .6 mm in diameter.

Once the larvae have become stationary they cause little obvious damage to the root tissues in their immediate vicinity until they become sexually mature. Then they burst through the cortical tissues and either escape directly into the soil - male - (Plate VI. fig.2) or remain attached to the rootlet only by their headward end, while their rounded posterior part protrudes through the rupture - female - (Plate VII. figs. 1 and 2).

Within the root tissues, however, the larvae set up serious disturbance which is believed to be related to their mode of feeding. On coming to rest within the cortex, they orientate themselves in a direction parallel to the root, and with their heads abutting on the endodermis, enter upon a feeding period which varies from about 25 days in the case of the male to over 60 in that of the female.

The reaction on the part of the host is confined

PLATE IX



LONGITUDINAL SECTION OF POTATO ROOTLET—45 DAYS. (x90.)

G. cell = Giant cell; Nuc. g. cell = Nucleus of giant cell.

to the tissues lying within that part of the endodermis adjacent to the head of the nematode and apparently originates in the parenchyma cells, the elements of which become replaced by a series of very large cells to which Nemec (40) working on sugar beet, has given the name 'Giant Cells'. In transverse section one of such cells may measure .14 mm in diameter and a whole group may reach .23 x .18 mm and occupy almost quarter of the vascular bundle (Plate VIII). In a longitudinal section the complex of giant cells may attain to a size of 1.2 mm (Plate IX). These measurements are much smaller than those given by Nemec.

Each giant cell is richly granular, and contains several very large nuclei, one or more of which may be seen in transverse and many in longitudinal section. Their walls are fairly thick but those running longitudinally are pitted and permit of the more easy transfer of their cell contents.

A group of giant cells, therefore, constitutes a large syncytium similar to those found in plant tumours.

As the complex of giant cells develops, it presses upon and actually displaces the xylem. It seems to act like a wedge which breaks or seriously inhibits the conduction of nutritive materials by the xylem vessels. The consequence is that very little water can pass from the small absorptive rootlets to the plant and conversely no plastic substances can pass from the plant to the apex of the young growing rootlet. The plant in an endeavour to rectify matters proceeds to produce fresh rootlets which are no sooner formed than they are invaded and rendered functionless. An affected plant, therefore, always possesses an abnormal development of fine rootlets which have arisen through an attempt on the part of the plant to obtain sufficient water and minerals in solution (see p. 14).

The cells round the head of the eelworm remain intact - a fact also recorded by Nemec (39) on sugar beet - and consequently it is obvious that the passage of food into the mouth is not brought about by rupturing the cells with the spear and then sucking in their protoplasmic contents. It seems rather that the giant cells are intimately concerned with the nutrition of the parasite. The latter probably secrete a substance, possibly from the salivary glands as suggested by Steiner (56), and Smith (55), which is toxic in nature and which stimulates the giant cells to develop. These act like a trap on the vascular bundles preventing the passage of water and minerals upward and the passage of plastic substances downward. They become filled with an extremely rich protoplasm which inclines Nemec (40) to look upon them as being glandular in nature and functioning like a "food preparing" nectarium as described by Schniewind Thies (48). The nematode by the action of its oesophageal bulb merely requires to suck in the food through its hollow spear.

The results arising from the blocking of the vascular bundles by the giant cells are (a) a profusion of lateral rootlets, (b) an inhibition of growth at the apices of invaded roots, (c) an insufficiency of water supply to the stem and leaves, (d) an insufficiency of mineral nutrients to the whole plant.

In connection with the mineral content of an invaded plant, it has been shown that in the case of the sugar beet a deficiency in potash is most marked (15), so much so that at one time the trouble was mistaken for potash starvation. This probably accounts to some extent for the beneficial effect, frequently commented on by numerous authors, of superfluous potash manuring on plants invaded by eelworms. Steiner (56) is inclined to believe that the presence of potash in the plant tissues may serve to neutralise the toxic substances which may be secreted by the eelworms. These may be products of the salivary glands which are well developed, or they may be soluble excretory substances passed outwards either through the excretory pore or the anus. It is held that the toxins, possibly those from the salivary glands, cause the formation of giant cells in the bundles (p.31). Further these substances as well as the excretory products, will ultimately be transported by the vascular bundles to the leaves where they will accummlate by concentration in those regions of the leaves where transpiration is most rapid. In this respect Lutman (30) points out that there are numerous special stomata or hydathodes round the margins and especially towards the apices of potato leaflets. At the margins, therefore, and especially at the apex where the hydathodes are present in large numbers we would expect to find, as we do find, that the effects of the concentration of these toxins first become evident.

It has also been noted by Lutman (30) that a conducting strand - the marginal vein - is to be found following closely the periphery of the leaflet which could readily account for the distribution of the toxins round the margins. The injury to the leaves reminds one very strongly of the potato disease known in America as Tipburn or Hopperburn. According to Fenton and Hartzell (11) the cause of one form of this leaf injury is a toxin or toxins injected into the potato leaflets by the Potato Leafhopper - Empoasca mali Le Baron.

Further the leaves of sugar beet, the roots of which have been invaded by H. schachtii, show abnormalities in their mineral content. Stoklasa (58) has found that leaves of infected beets contain only about half as much lime as healthy plants but an enormous amount of soluble oxalic acid. Smith (55) who has conducted elaborate experiments on tumour formation on plants concludes that tumour formation, as also the formation of giant cells, is due to the liberation into the tissues by the parasite of either weak acids or weak alkalies. He has experimentally determined that acetic acid, formic acid, ammonia and aldehyde are all capable of producing tumour development, and that these substances are formed by the crown gall micro-organism and probably by a variety of other tumour producing parasites. It seems likely that in the case of the potato eelworm the acid secreted is oxalic acid. Its presence in weak concentration would act as a stimulus and cause the formation of giant cells. Its presence in the leaf in considerable concentration would result in the leaf symptoms already mentioned and also in the fasciation commented on as commonly occurring in potatoes the roots of which have been invaded by eelworm. (See p.11). It might be argued that oxalic acid is commonly found in the leaves of plants but in such cases it is generally present as calcium oxalate and in that form it is harmless.

(c) Development of the parasite in the host.

The newly hatched larva (Plate XII. fig.1) varies in size but has a mean length of .435 mm. and a mean breadth of .019 mm. The striations on the cuticle are distinct and the genital rudiment is in the form of two closely apposed uninucleate cells.

This early first stage larva is very common on rootlets up to 31 days, after which time it becomes relatively rarer owing to the other and later stages of the parasite beginning to make their appearance (Plate XII. figs. 2 and 3).

The first of these, the fully mature first-stage larva (Plate XII. fig.2) is to be found in 31 days roots. It is not longer but is much broader than the newly hatched larva and measures .420 x .028 mm. The cuticular striations are difficult to see except at the extremities but the gonad remains in its original 2-celled state.

Stages in the first moult can also be found in 31 day roots. In this process the larva shrinks from its old cuticle (Plate XII. fig.3) and then emerges from it as a young second-stage larva* (Plate XII. fig.1). This larva, which occurs in 35 day roots, measures .373 x .035 mm. and has a rounded tail by which it is readily distinguished from the late first-stage larva. It resembles the latter in that the cuticular striations are difficult to see. Cell division may now be noted in the genital rudiment.

The young second-stage larva becomes a mature second-stage larva (Plate XIII. fig.2) in about 10 days and can be found in 45 day roots. This form differs from the early second-stage larva only in size (.414 x .049 mm) and in the growth of

* Cobb (7) states that the second-stage larva of the beet strain with the rounded tail can hatch directly from the egg.

the gonad, which now stretches about half the length of the larval body. From this point onward, the male and female follow different courses of development.

Development of the Male. The mature second-stage larva ceases to feed and shrinks from its cuticle to which the spear and the lining of the rectum remain attached (Plate XIV. figs. 1, 2, & 3). Then gradually the oesophagus, salivary glands, intestine and gonad, become indistinguishable while at the same time the larval body begins to increase in length (Plate XIV. fig.3. Plate XV. figs. 1 & 2). When it has reached about half the length of a fully developed male, the spear, gonad and spicules appear. (Plate XV. fig.3. Plate XVI. fig.1). With further increase in length, the oesophagus and intestine become evident (Plate XVI. figs. 2 & 3) and by the time the male has reached its full length all the internal organs are complete (Plate XVII. figs. 1 & 2). The male then leaves the root, either still contained within the second larval cuticle (Plate VI. fig.2) or as a free moving active eelworm (Plate XVIII. fig.2).

The majority of these stages (Plate XIII. fig.2 to Plate XVI, fig.3) have been taken from 45 day roots, but the later ones were obtained from 52 day roots (Plate XVII, fig.1) or from the soil in the vicinity of 60 day roots (Plate XVII, fig.2).

Development of the Female. The development of the female is more direct than that of the male. Unlike the latter it continues to feed. A new cuticle forms underneath that of the mature second-stage larva but as the larval body does not shrink as it does in the case of the male, the presence of the two closely apposed cuticles can be determined only in favourable cases (Plate XX. fig.1. Plate XXI. fig.2). The new cuticle is thick and striated.

The earliest female stage that has been recognised is that depicted in Plate XIX. fig.1. It shows the striated cuticle and the long genital tube which is growing down towards the posterior end of the body where the rudiment of the vagina is just being formed from the body wall. The vaginal rudiment is somewhat in front of the anal opening but is not truly ventral in position.

In later stages the gonad moves bodily towards the posterior end of the larva (Plate XIX, figs. 2 & 3), and there unites with the now fully formed vagina in such a way that a paired genital organ is produced (Plate XX, figs.1 & 2).

Meanwhile the larva has been altering in size and shape, but not in length. From the long cylindrical form depicted in Plate XIX, fig.1, it passes gradually through the stages drawn in Plate XIX, figs. 2 & 3, and Plate XX, fig.2, to the flat leaf-shaped forms shown in Plate XX, fig.3, and

Plate XXI, fig. 1.

The growth which results in this peculiarly flattened larva and which is due to an increase in size of both the intestine and body wall, takes place mainly in a dorsal or a dorso-ventral direction, thus giving the appearance of a laterally compressed organism. Further increase in size is due mainly to swelling of the intestine and to the growth of the ovarian tubes. But growth is no longer confined to a dorso-ventral direction. Rather does it tend to be lateral giving first a club shaped cyst (Plate XXI. fig.2) which with further distention becomes spherical (Plate XXI. fig.3).

The club shaped cysts are readily found in 48-52 day roots while the later spherical stages occur in 51 day roots onward.

While these changes in external form are taking place, the vagina shifts round and becomes ventral in position but not quite terminal, and makes communication with the exterior through a vulva in the inner thick cuticle (Plate XXI. fig.2). At the same time, as a result of the swelling of the cyst, the cortical tissue of the root ruptures and exposes the posterior end of the organism and its vulval region (Plate VII. fig.1). The female is now sexually mature and is believed to be fertilised by the male which will be found in the soil from the 60th day onward.

After fertilisation, the ovarian tubes grow rapidly. They absorb the food stored in the walls of the body and the intestine and soon occupy all the available space within the cyst. Growth of the cyst is still maintained, however, with the result that the whole body emerges from the root but still remains attached to it by its head end (Plate VII. fig.2). The newly emerged cysts are white in colour, but soon they change to yellow - about 70 days - then to yellow-brown and finally become dark brown - about 80 days.

Meanwhile the eggs within the cysts have been developing. In their early stages they are merely egg-cells without external covering (Plate VIII. E.c.) Later they acquire chitinous shells - about 70 days - (Plate XXI. fig.3; Plate XXIII. fig.1) within which the larvae reach maturity about the 80th day. (Plate XXII; Plate XXIII. fig.2).

The rate of development under ordinary field condition in which the temperature is distinctly lower (see Table I.) is slowed down considerably, e.g. in the West of Scotland, the brown cysts begin to appear both on early and late varieties of potatoes about the 100th day after planting.

Peculiarities in Life History of Potato eelworm. While in the main, the life history of the potato strain of H. schachtii resembles that of the beet strain as worked out by Strubell (61) and Chatin (6), yet there are certain marked differences as for example -

(a) the time required for the completion of the life cycle, and

(b) the development of the male.

(a) The potato strain is unlike the beet strain in that it requires one whole season for the production of a mature cyst whereas in the beet strain the life-cycle can be completed in about 30 days at a soil temperature of 25°C. (Rensch (44)). The white cysts of the former never contain mature eggs and therefore are but an early stage in the development of the brown cyst. In the beet strain, on the other hand, the white cysts are summer cysts and contain fully formed larvae (3).

(b) The lengthening of the young male of the potato strain within the second larval cuticle takes place prior to the formation of the spear and spicules, whereas for the male of the beet strain, Strubell (61) has shown that the spear and spicules are acquired before the body proceeds to lengthen. Chatin's (6) illustrations however, do not correspond with those of Strubell in this respect. He indicates that the young male has increased considerably in length before the spicules make their appearance just as we have shown to occur in the potato strain.

There are apparently differences also in the biology of the mature cysts. Rensch (45) states that when fully formed cysts of the sugar beet strain are placed in water at a suitable temperature the larvae begin to hatch out, the rate of hatching being most rapid at the optimum temperature of 25°C. *Our attempts to hatch out larvae from brown cysts of the potato strain have met with no success either at this or at any other temperature when in distilled water.* The cysts invariably become invaded by fungi, mainly of the genus *Isaria* and the fungal hyphae protrude through the open cyst neck in great abundance. Baunacke (3) records that in the beet strain it is from the vulval canal that the hyphae protrude. Even when the fungal attack has been avoided to a great extent, by incubating the cysts in moist sand, no hatching has resulted; and even if the cysts are broken and the larvae actually set free from the eggs before incubation larval movement can be observed only occasionally and at its best is sluggish.

It appears therefore that temperature, along with a certain amount of moisture, is not sufficient to induce the larvae to hatch from the cysts or even to activate those already freed from the eggs. Reinmuth (43) arriving at a similar conclusion, states that temperature difference is not the only factor which controls the period which the larvae spend in the cysts and that the cyst wall must be removed before the larvae will become active.

When potato setts were planted in the same soil from which the cysts used in the above experiments were extracted it was found that after a period of 14 days, at a temperature of 18°C, the rootlets of the potato plants were invaded by larvae. It would appear therefore that the brood in the cysts was brought to life by excretions from the actively growing potato roots, assuming always that free larvae of H. schachtii do not occur in the soil during winter. That these excretions do cause the larvae to hatch out under ordinary soil conditions is no doubt correct; but some other factor or factors seem to play an essential part. Potatoes grown at room temperature in sterile sand inoculated with unbroken cysts remained uninfected at the end of 3 months, and no free larvae could be demonstrated in the sand.

Further when cysts were incubated at room temperature in the washings collected from potato roots growing in sterile sand in a large filter funnel - and presumably containing the root excretions - few larvae hatched out. Even when incubated at the same temperature in the extract from macerated potato roots, the cysts failed to yield up their brood.

These experiments indicate that root excretions in themselves are not sufficient to bring about hatching, as has been shown to occur in the case of the beet strain by Rensch (45) and by Baunacke (3). The minerals present in the soil solution may be an important additional factor but this requires further investigation.

CYST CONTENTS OF SOILS

It has already been noted that the female eelworm after fertilisation becomes converted into a lifeless cyst, which encloses and protects the eggs and developing larvae. These cysts may be of two forms - (1) White, (2) Brown. The brown cysts are mature resting cysts and they are found in proportionately greater number as the season advances. In early summer the great majority of the cysts are white, whereas in October the reverse is the case, the majority being brown.

The cysts remain loosely attached to the rootlets of the plant during the growing period but on death of the plant find their way into the soil and remain dormant until the next potato crop is planted.

An examination of the soils where potatoes are grown in close sequence in our area was made with a view to determining their cyst contents. The method adopted is as follows:- A number of bores of the soil in the field (to a depth of 9") is taken by means of a soil sampler to give a representative sample of the field or area under test. The soil after being air dried is crushed and passed through a 2 mm. sieve in order to remove stones etc. From this sieved soil 10 ccs. is taken and placed in a graduated ½ litre flask. The flask is partially filled with water and shaken vigorously for a few minutes. It is then filled almost up to the 500 ccs mark with water and allowed to stand, when the cysts along with pieces of organic matter float to the top. Finally the flask is filled up to the very lip with water and the cysts decanted on to a filter paper.[x] When pouring, the flask is given a sharp turn to prevent the cysts clinging to the surface. The filter paper is then spread out and the number of cysts counted. Generally ten determinations were made from each sample, but when necessary additional counts were made.

TABLE III./

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[x] The whole operation should be done without undue delay as the cysts may sink to the bottom if the air bubbles on their surface and in their interior become displaced. The cysts, however, do not readily sink in water. 116 cysts were floated in water and examined at regular intervals. In four days 11 cysts (9%), and in five days 43, (37%), had sunk to the bottom of the flask.

TABLE III. gives the counts for some of the Ayrshire soils.

TABLE III.

Sample No.	District	No. of cysts per 10cc. in the top 9" of soil	
19	Girvan	141.5	
24	Girvan	136.9	
18	Girvan	120.4	
13	Girvan	106.9	
43	Troon	96.0	
7	Maidens	80.7	
32	Girvan	80.8	
12	Turnberry	66.6	Strong infection
30	Girvan	65.6	
25	Girvan	59.0	
39	West Kilbride	51.4	
38	do.	31.6	
40	Ardrossan	28.6	
1	Maidens	22.5	
31	Girvan	21.8	
6A	Turnberry	15.9)	
6	Do.	11.2)	Medium Infection
36	Seamill	9.3)	
41	Do.	7.0)	
33	Girvan	3.0)	Weak Infection
34	Do.	3.0)	
35	Ballantrae	1.0)	

These figures are alarming when it is remembered that each fresh mature cyst contains on an average 300 eggs and larvae and that 10 cc. of soil weighs 11.5 grammes on an average. 1 lb. of soil (No.19) would contain 5591 cysts and a potential 1,677,300 eelworms.

Rensch (44) has grouped the beet soils in Germany as showing (1) Strong Infestation (2) Medium Infestation, (3) Weak Infestation. Strongly infested soils are those in which he obtained in 75 grammes of soil 120 cysts of which at least 15% are complete; Medium infested soils contain 60-120 cysts in 75 grammes of soil of which 15% are complete, while Weak infested soils, have less than 60 cysts in 75 grammes of soil of which 15% are complete. Rensch (44) states that as a result of examining soils from all parts of Germany those classified as having strong infestation gave a very marked reduction in yield of beet because the roots had been attacked by the eelworm.

It is apparent from Table III. that the degree of infestation in Ayrshire soils is very much greater than that obtaining in Germany. Soil No.19 would contain 923 cysts per 75 grammes and of the 22 soils examined, 15 would fall into Group I, 3 into Group II, and 4 into Group III,

It is very important when making cyst counts of soils to bear in mind that the cysts do not yield up all their larvae at once. On the contrary the larvae continue to hatch out from the cysts for several years with the result that the eggs present in the interior of a cyst become fewer and fewer with age. Fuchs (12) has shown that in the case of the beet strain of Heterodera schachtii a three year old cyst contains on an average 40-50 eggs. In the case of four year old cysts 60% of the cysts contain 30-45 eggs, 25% are empty, 9% have less than 30, and 6% more than 45 eggs. In five year old cysts the majority (55%)are empty while 35% have from 10-20 eggs and the remainder (10%) have egg counts outside these limits.

From this it is obvious that a mere cyst count is of little value in cases of long standing infection, since many of the cysts will be either empty or contain relatively few eggs. In all our estimations, the cysts comply with Rensch's standard that at least 15% should be complete.

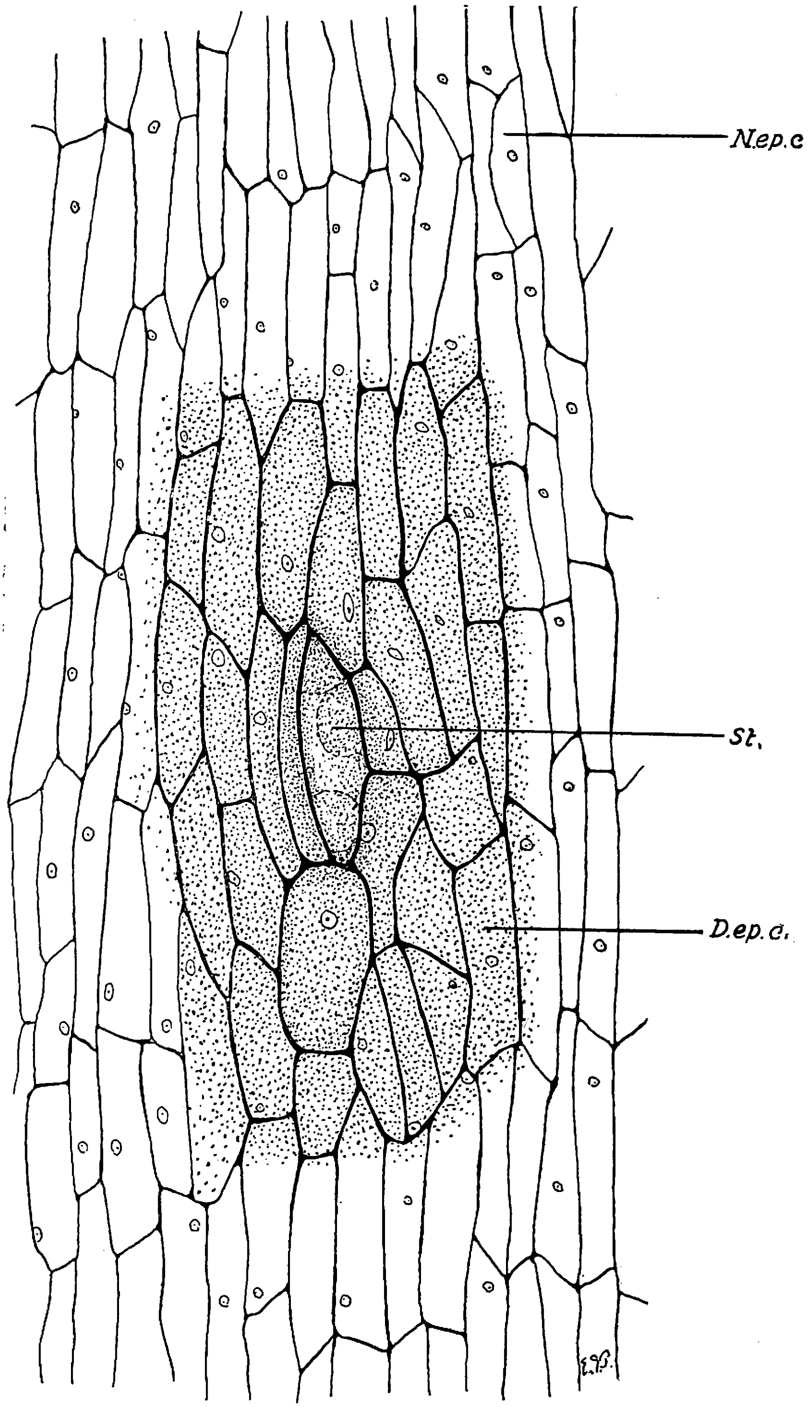
The effect of Soil Reaction on the severity of the Disease.

From a preliminary estimation of the acidity of a number of infested soils by the electrical conductivity method, it was thought that a relationship existed between the reaction of the soil and the severity of the disease. In some of the fields examined patches could be located where the crop was extremely poor and the eelworms easily detected on the roots, surrounded by an area where the plants were apparently healthy, and relatively few eelworms could be noted. In others the whole fields were equally bad. Soil samples were taken from the diseased and healthy areas and their reaction determined electrometrically by the quinhydrone electrode with the following results.

TABLE IV.

	pH of Healthy Area	pH of Diseased Area	Remarks on the severity of the Disease
Farm No.1.	4.95	5.97	Very badly affected.
2.		5.49	Badly affected.
3.		5.13	" "
4.	4.45	5.07	Moderate crop.
5.	4.15	4.37	Good crop (only few cysts noted).

From thispreliminary examination it seemed that the disease was more severe on soils which had a pH value of over 5, and that on the more acid soils, those with a pH under 5, plants were not affected to any appreciable extent. This point was however investigated and samples were drawn from as many centres as possible where the disease was present. From the results obtained it was found that no definite relationship existed between the severity of the



STOMA FROM POTATO STEM INVADED BY BACTERIA. (x360.)

D. ep. c. = Diseased epidermal cell; N. ep. c. = Normal epidermal cell; St. = Stoma.

disease and the reaction of the soil although there was a certain amount of evidence to show that soils with a pH of less than 4.2 and soils with a pH of over 5 tended to suffer more when attacked by H. schachtii than those between 4.2 and 5. There were, however, exceptions to this and it is more likely that the reaction of the soil influence the growth of the potato and not the activities of the eelworm. It is fairly well known that the potato prefers an acid soil but we are not aware that any attempt has been made to determine the optimum pH value of the soil for this crop. Most of the Ayrshire early potato soils, however, have a pH varying from 4.2-5.5. Where the pH is in the region of 6, lime has been recently applied.

Potatoes grown on soils with a pH of less than 4, fail more or less completely. The stomata on the leaves and haulms apparently become functionless and in their dead and dying condition are invaded by bacteria. The bacteria, when isolated were found to be non-pathogenic to a normal plant. In order to test this point more fully a soil with a pH of 3.8 was taken and potatoes grown on it. The crop was almost a complete failure. In the early stages of growth the haulms appeared normal but by the end of July the plants made no headway. An examination showed that all the stomata were affected (Plate X.). When potatoes were grown on the same field and where 30 cwts lime per acre had been applied, they were normal and gave a good crop. The pH of the limed section was 5.5.

Peters (42) working on the potato soils in Lincolnshire of which the pH value varied from 6 to 7 (as determined by the colorimetric method) tentatively concludes that there is a definite relationship between the pH values of the soil and cyst concentration. Cysts were more numerous in soils with a pH of 6 and fewer in soils with a pH of 6.7. Morgan and Peters (35) suggest an optimum acidity of pH 6.15 for the eelworm.

We cannot find any evidence on the Ayrshire soils to support these conclusions. Cysts were as numerous on the very acid as they were on the less acid soils.

TABLE V./

TABLE V.

Table showing the pH and the Cyst Counts of Typical Ayrshire Early Potato Soils

pH.	Cyst Count per 10 ccs. soil
6.26	59.0
6.12	3.0
6.07	106.9
5.92	65.6
5.90	120.4
5.80	80.8
5.73	31.6
5.66	136.9
5.61	5.0
5.50	51.4
5.21	21.8
5.05	28.6
4.93	141.5
4.91	1.0
4.55	80.7
4.48	7.0
4.48	15.9
4.48	66.6
4.38	11.2
4.34	22.5
4.26	9.3

Smith (51) in his examination of Potato soils infested by Heterodera Schachtii in Lancashire and Cheshire also finds that in the case of sandy soils there is no correlation between the pH of the soil and its cyst content, although he believes a significant negative correlation to exist in the case of peaty soils.

Peters (42) further believes that the prevalence of Heterodera schachtii can be checked by liming. In Ayrshire, applications of lime which have raised the pH value of the soil to over 6 have not improved the crop yield on heavily infested soils nor brought about any marked reduction in the cyst contents of the soils.

Samples 13, and 19 were taken from one field, the first being from patches where the disease was very bad three years previously, and where lime had been applied. Observations on this field during years 1927 and 1928 showed that there was no increase in crop on the limed portions. The unlimed (Sample 19) still out-yielded the limed sections (Sample 13).

POWER of ADAPTATION of H. SCHACHTII

The eelworm Heterodera schachtii is polyphagous in nature and has been noted by Markinowski (31) and by Baunacke (3) as a parasite upon a very large number of cultivated plants and weeds. However there can be no doubt that even in its unspecialised form it shows a preference for certain crops.

The plants listed by Rensch (44) as being strongly attacked are Sugar beet, mangolds, spinach, cabbage, lettuce. Less strongly attacked crops include oats, barley, wheat, and peas, slightly attacked, beans and leguminous plants while not attacked normally, are rye, potato, poppy, flax, onion, carrot, carroway. Of weeds the commonest species are almost invariably strongly attacked. However this grouping should not be taken as absolute.

Where a suitable host plant is cultivated too often in a rotation as is the case with sugar beet in Germany or early potatoes in certain parts of Great Britain, the eelworm in spite of its original polyphagous nature can become specially adapted to that particular crop and develop a power of attacking it more strongly. The extreme case - when the worm is restricted for nourishment to one host plant - results in the development of a highly specialised strain which may differ in certain biological and morphological characteristics, from the original unadapted strain, and this is undoubtedly what has occurred in the case of the potato - a crop which the eelworm could originally attack only with difficulty. Further when polyphagous the damage done to any of its many hosts is almost negligible but when monophagous the injury may be so serious as to prevent the economic cultivation of the crop upon which it has specialised.

When the eelworm has achieved this stage of specialisation it prefers the new host to the old, and indeed it is often only with difficulty that it can be induced to re-attack the species of plant which it originally infected. Such for example has occurred in our own experiments when we attempted to get the potato strain of *H. schachtii* to re-attack the beet from which it supposedly originated. The transference in this case completely failed in the first year, but in the second year, 50% of the beets were noted as suffering slightly from eelworm attack.

Tischler (65) again, finding *Heterodera schachtii* attacking *Circaea lutetiana* managed to transfer the infection in course of time to *C. intermedia* only when the former species was not available as a host plant.

This power of specialisation which nematodes possess can be further illustrated by referring to the eelworm *Tylenchus dipsaci* which causes serious losses to bulb growers in Holland. Slogteren (49) a Dutch investigator, cites the case of the Hyacinth and Narcissus. When Hyacinth bulbs were grown in the same pot as Narcissus bulbs in soil infected with the Hyacinth strain of *T. dipsaci*, the Hyacinth bulbs were alone attacked. On the other hand when the experiment was repeated with soil infested with the Narcissus strain of *T. dipsaci,* the Hyacinth bulbs were not attacked. Originally *Tylenchus dipsaci* could attack with equal facility both Hyacinth and Narcissus bulbs.

The more a strain of the parasite has accustomed itself to a definite host, so much the more quickly and more surely will the eelworm follow the directive stimulus given off in the root excretions of this plant. The hatching eelworms will, therefore, be able to reach their host plant more rapidly. As the plant is suitable for them their development on it will be hastened, and by further nutritive physiological adaptations to the host, the successive generations will follow each other more quickly and the numbers increase accordingly. Rensch (44) states that while originally the eelworms could produce only one brood per summer, a generation can be produced in about 4 weeks' time under specially suitable conditions such as obtain with an adapted host and a high soil temperature.

In the case of the strain specialised on potatoes, however, we have definitely established that there is only one generation in the growing season, either on early or late varieties. In fact when the life history of the potato eelworm is taken into account it appears to be impossible to have more than one generation especially during the growing season of the early potato crop. The time during which the early potato crop is allowed to grow varies from 90 to 120 days, which is no more than sufficient for the completion of the life cycle of the eelworm under field conditions - a period of from 80 to 100 days being necessary. (See p.40). This accords with the observations of Duffield (9), who dealing with Heterodera schachtii on hops, states that there is only one generation per year.

It seems quite possible that previous workers on the life history of Heterodera schachtii have failed to recognise that the cysts may continue to set free larvae during the whole growing season, and that consequently, on an infected plant, larvae in all stages of development may be found. One, therefore, gets on an invaded plant a continual succession of larvae throughout the summer but not a succession of generations.

VARIATIONS in MORPHOLOGY DUE to ADAPTATION

In the process of adaptation to a particular host, size and body form may be modified.

The following Table (Table VI.) shows the measurements in millimetres of the cysts taken from the Ayrshire early potato soils. For comparison other authors' figures are given.

TABLE/

TABLE VI.

TABLE SHOWING MEASUREMENTS of CYSTS of H.schachtii from VARIOUS SOURCES

Source	Max. Cyst size in mm.	Min. Cyst size in mm.	Mean Cyst length in mm.	Mean Cyst breadth in mm.	Av.length / Av.breadth	No. of determinations
Sample 24. Potatoes. Girvan.	.865x.578	.293x.186	.465	.367	1.27	530
Sample 39. " W.Kilbride.	.705x.755	.306x.199	.489	.372	1.31	404
Sample 34. " Troon......	.948x.789	.346x.199	.565	.438	1.29	412
Triffitt. "Lincolnshire.	.995x.80	.132x.109	.576	.405	1.37	-
" " Hertfordshire (1927)	.477x.382	.05 x.036	.317	.245	1.299	-
" " " (1929)	.91 x.69	-x-	-	-	-	-
Wollenweber "	-x-	-x-	.835	.543	1.54	-
Triffitt, Beet.Halle Salle..	.85 x.63	.56 x.25	.73	.43	1.69	-
Strubell "	1.3 x -	.80 x -	-	-	-	-
Markinowski "	.88 x -	.39 x -	-	-	1.55	17
" Oats............	.78 x -	.48 x -	-	-	1.93	10
Triffitt "	.85 x.63	.53 x.35	.71	.50	1.44	-
" Hops............	.695x.571	.333x.295	.534	.402	1.36	50
" Mangolds........	1.1 x.68	.43 x.20	.84	.51	1.62	-

The measurements of the potato strain of H.schachtii do not accord closely with those given for the beet strain. The mature female in the form of a cyst is throughout smaller than that of H.schachtii proper and furthermore it is much more rounded and has not the characteristic lemon shape which the typical schachtii has. If we refrain from taking into consideration the neck of the larger sized cysts the ratio of length to breadth is 1.012 to 1, i.e. practically spherical. (See Plate XXII).

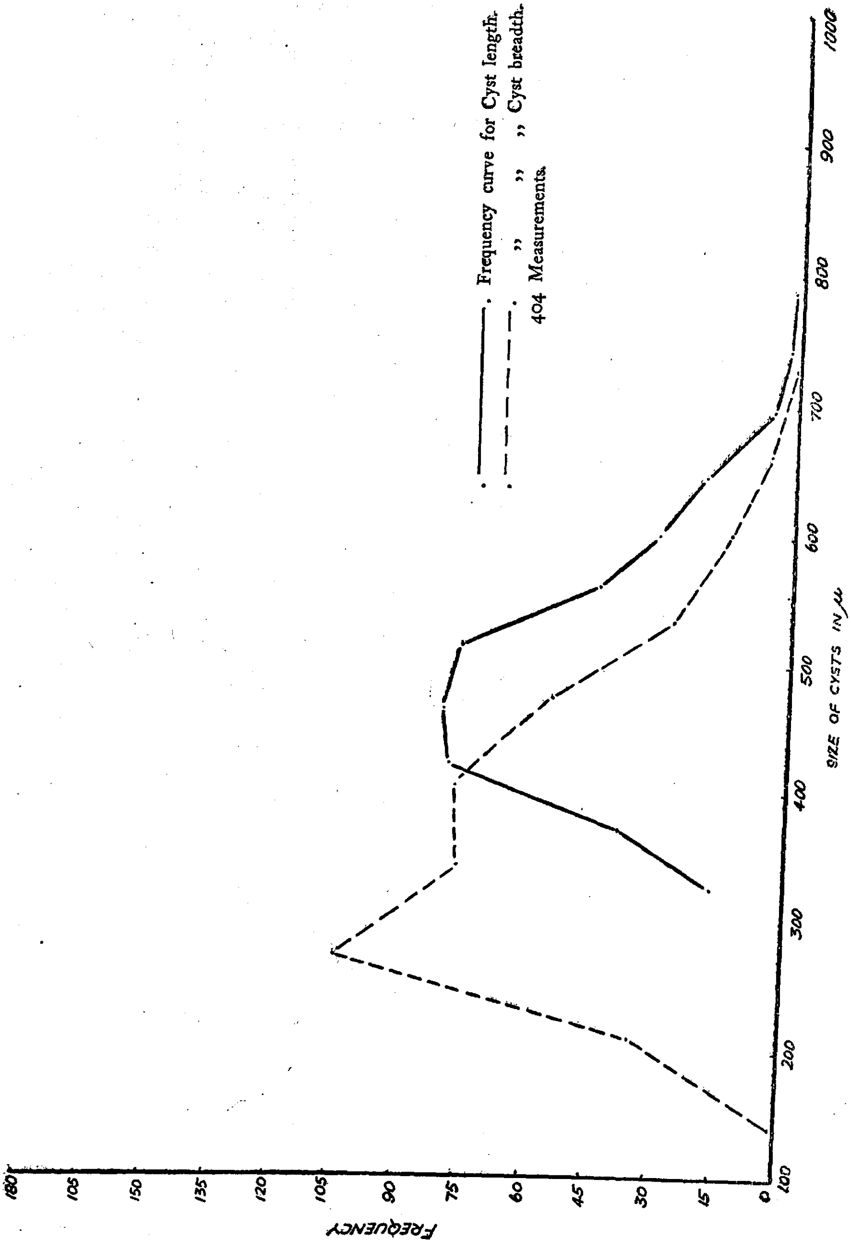
Baunacke (3) records these rounded cysts as occurring on beet but states that these represent well developed and well nourished individuals. In view of recent work this does not seem feasible. It seems to us quite possible that in the sugar beet fields several strains of H. schachtii occur. The strain which forms the rounded cysts must have been parasitic either upon the potato or some closely allied solaneous weed, e.g. Solanum nigrum, (see p.3). Baunacke could only have seen the rounded cysts in the soil and not on the beet plant.

It is interesting to note that Zimmermann (77) and Triffitt (68) in their publications also figure the potato strain as forming typical rounded cysts.

In addition to the difference in shape of the cyst, there are other more significant modifications. In the beet strain there is a well marked vulval canal whereas in the rounded potato strain the minute vulval canal can be observed in the cyst wall only with difficulty (see Plate XXI. fig.3.)

The extreme tip of the neck of the cyst shows an aperture of considerable size due to the disintegration of the thin wall immediately surrounding the buccal cavity, (Plate XXII), and it is through this aperture that the larvae usually escape although on occasion they may emerge from the vulval opening.

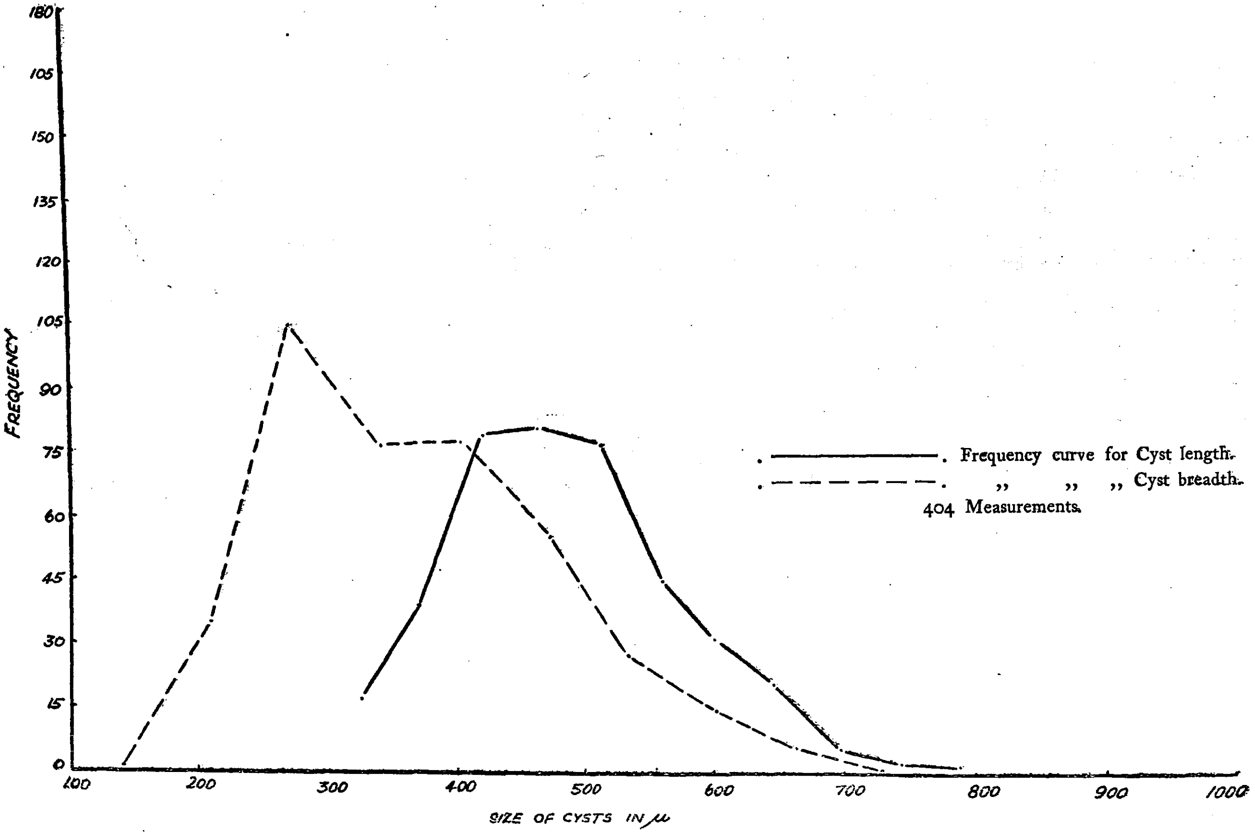
The cysts measured in samples 24 and 39 were obtained from early potato soils in Girvan and West Kilbride districts respectively, those in sample 43 were from the Troon district after late potatoes (see Table VI). In all cases, however, the nature of the soil was very similar - that of a sandy loam (see p. 7). It is apparent that the dimensions of the cysts nourished on early potatoes are much less than for those nourished on late potatoes. Further the measurements of the cysts from the Girvan soils are appreciably smaller than those from the West Kilbride soils. It is to be noted that in the Girvan district the potatoes are lifted about 2 weeks earlier than in the West Kilbride area. The time of planting of the setts, however, is approximately the same.



FREQUENCY CURVE FOR CYSTS OF SAMPLE 39.

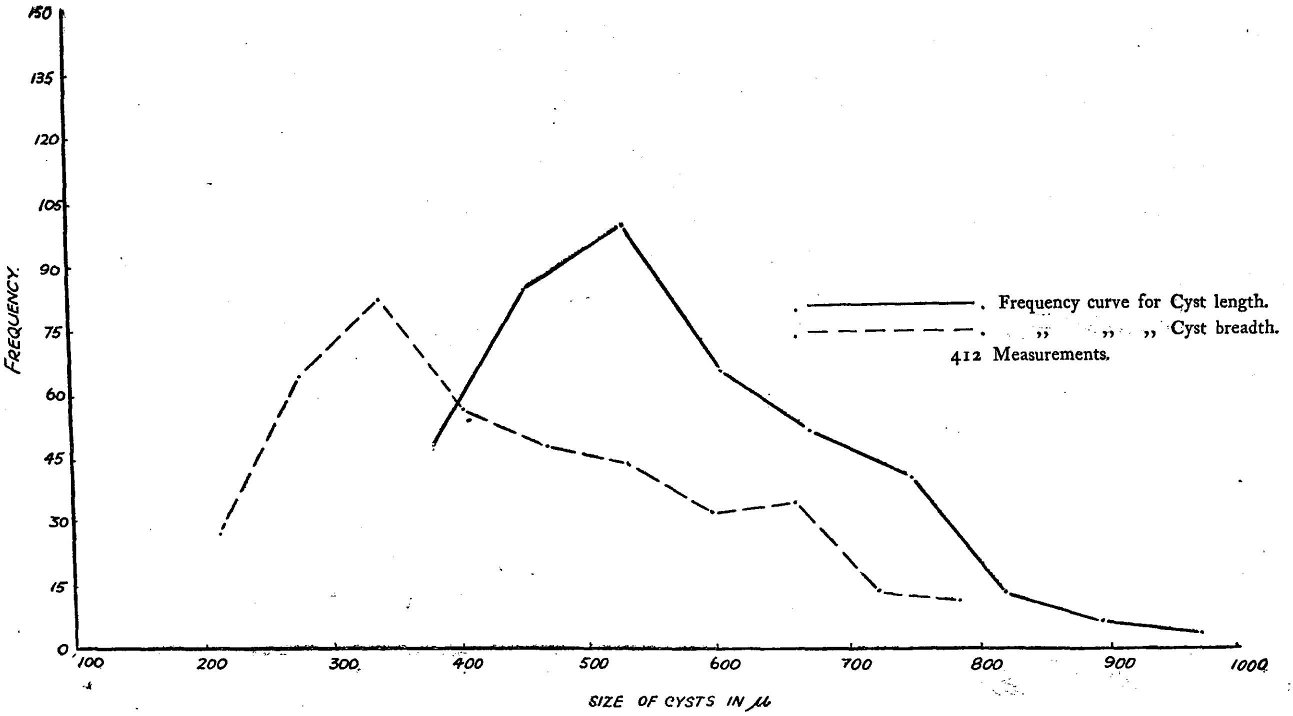
Diagram I

phasise the great variation in cyst size and the fact that



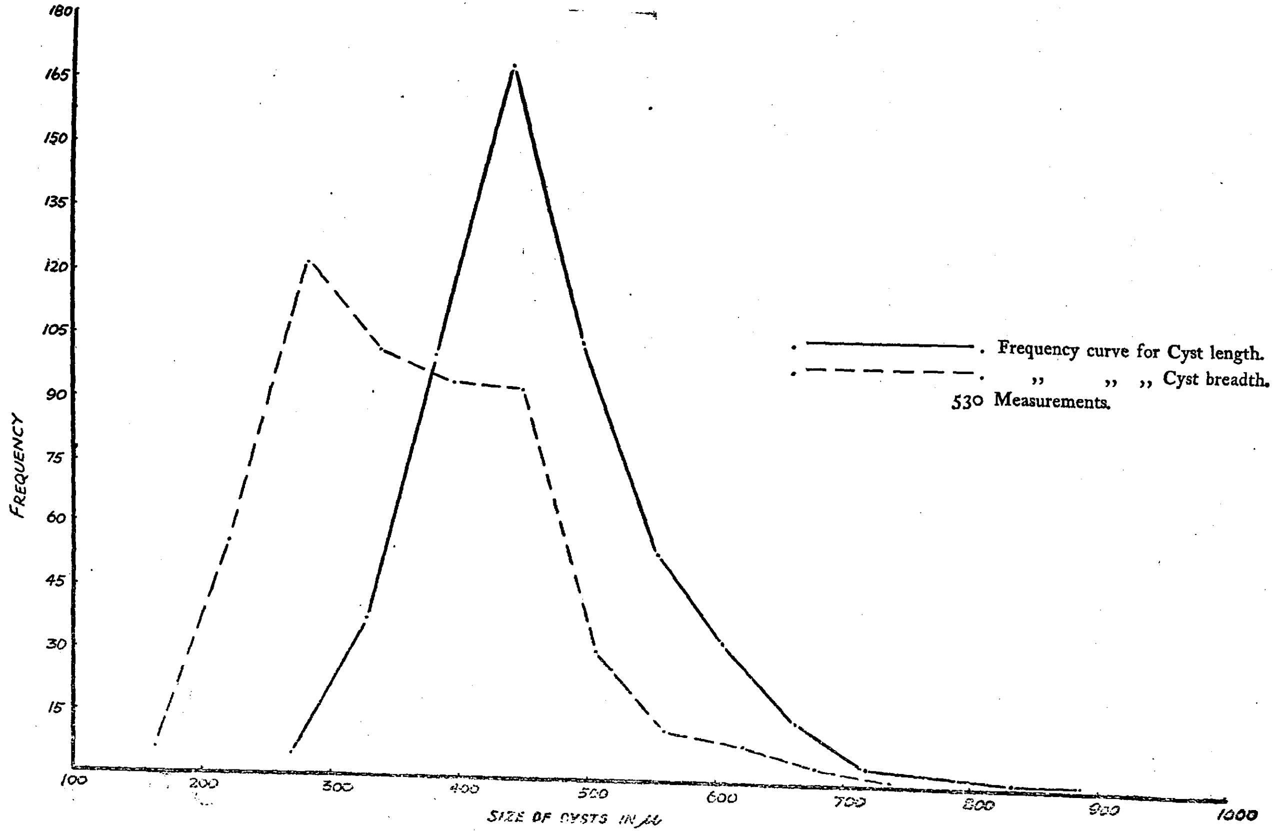
FREQUENCY CURVE FOR CYSTS OF SAMPLE 39.

Diagram I



FREQUENCY CURVE FOR CYSTS OF SAMPLE 43.

Diagram II



FREQUENCY CURVE FOR CYSTS OF SAMPLE 24.

Diagram III

From this it would appear that the average size of the cyst is intimately connected with the length of time that the female is allowed to develop upon the plant. Triffitt (69) on the contrary, states that the size of the cyst is dependent upon soil conditions or other external factors.

The measurements recorded by Triffitt (66) for cysts taken from the Lincolnshire soils are similar to those given by us. Especially does this apply to those separated from the Troon soils. They vary, however, very markedly from those of the Hertfordshire soils but even there the proportion of length to breadth is in close agreement. Triffit (69) in a later publication states that many of the small sized cysts present in the Hertfordshire soils are transparent and void of eggs. We are of opinion that these should be regarded as females interrupted in the early stages of development, and are therefore not true cysts. The measurement which she gives for the minimum size of cysts containing eggs (.31 x .17 mm) corresponds with those recorded in the Girvan and West Kilbride soils.

Frequency curves (Diagrams I, II and III) have been plotted for both the length and the breadth of the cysts from the three districts and while these resemble in the main the frequency curve of a single variant, yet they serve to emphasise the great variation in cyst size and the fact that our measurements although very numerous (404, 412, 530) are not sufficient to eliminate apparent irregularities in the curves. Such irregularities however, are not sufficiently marked to warrant the opinion that we are dealing with other than a single normal variable.

Length of Neck of Cyst. The length of the neck of the cyst was determined in a number of cases. The results are given in Table VII. together with those obtained by other workers.

TABLE VII.

Table showing measurements of Length of Neck of Cysts from various sources.

Source	Av. length of neck in mms.	Av.length of Cyst. / Av.length of Cyst	No. of determinations
Sample 43. Potatoes. Ayrshire.	.1405	4.321	48
Triffitt, Do. Lincolnshire	.107	5.7	-
" Beet. Halle Salle..	.086	8.6	-
Markinowski, "	.09	7.0	17
" Oats.............	.12	5.38	10
Triffitt, " Sweden......	.084	8.8	-
" Hop, ...	.07	8.47	-
" Mangold..........	.077	9.91	-

Size of Eggs. The size of the eggs in the interior of the cysts from the three districts was determined. The figures are given in Table VIII.

TABLE VIII.

Table showing measurements of eggs of H. schachtii.

Sample		No.of eggs measured	Av.size of eggs in mms.
24. Girvan.	Cyst No.1.	15	.098x.044
	Cyst No.2.	15	.097x.045
39. W.Kilbride.	Cyst No.1.	20	.090x.040
	Cyst No.2.	20	.097x.043
43. Troon.	Cyst No.1.	20	.099x.043
	Cyst No.2.	10	.096x.049
		100	.096x.040

The eggs from the various cysts are fairly constant in size. The average of all measured in .096x.044 mm.

Table IX. gives the measurements of eggs of H. schachtii recorded by other workers.

TABLE IX.

Table showing measurement of eggs of H. schachtii from various sources.

Source	Av.size in mms.	No. of determinations
Triffitt, Potato. Lincolnshire.	.088x.041	45
" " Lancashire...	.110x.055	-
Wollenweber, "	.097x.040	-
Strubell, Beet.................	.080x.040	-
Chatin, "	.080x.040	-
Triffitt, "	.088x.040	-
" "	.114x.045	-
" Oats.................	.080x.028	-
" "	.106x.041	-
" Hop.................	.089x.042	-
" Mangold.............	.112x.046	-

The average of length to breadth of the eggs is 2.146 which compares very closely with Triffitt's figure of 2.194. In shape the eggs are always slightly concave on one side. (Plate XXIII. figs. 1 & 2.).

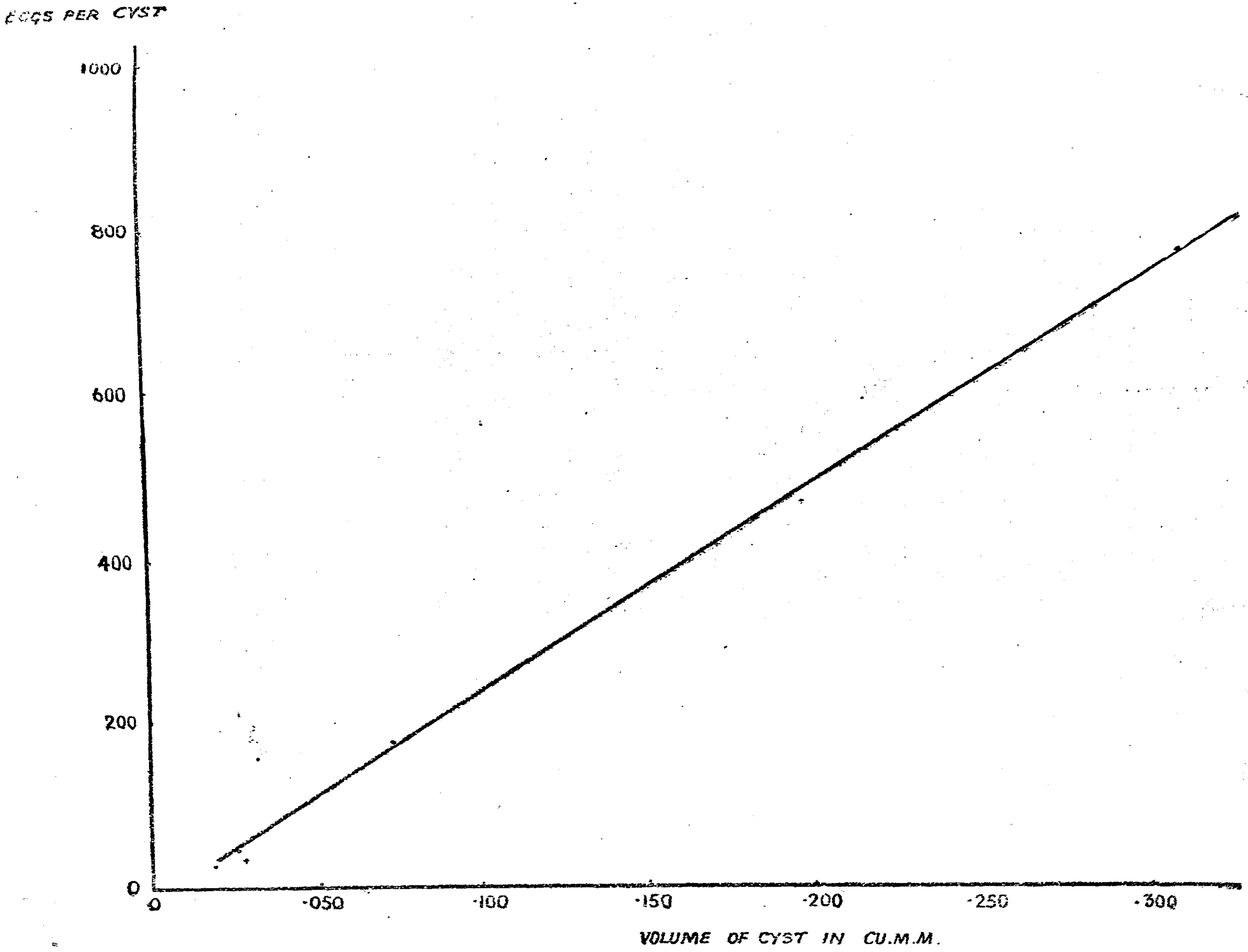
Size of Larvae. The measurements of larvae, taken from the interior of cysts are given in Table X. and for comparison those of other authors.

TABLE X.

Table showing measurements of Larvae of *H.schachtii* from various sources.

Source	Av.length in mms.	Av.breadth in mms.	Av.spear length in mms.	No.of measurements
Sample 24. Potatoes, Ayrshire.	.449	.019	.021	17
Sample 43. " "	.428	.019	.021	33
Triffitt, " Lincolnshire..	.458	.026	.011-.027	-
" " Lancashire	.460	-	.023	-
Wollenweber "	.372	.018	.016-.019	-
Strubell, Beet...........	.36	.160*	.023	-
Chatin, "	.35	.150*	.022	-
Triffitt, "	.29	-	.025	-
" "	.457	-	.024	-
" Oats...........	.47	-	.022	-
" "	.289	-	.027	-
" Hops...........	.39	-	.023	-
" Mangolds.......	.50	-	.026	-

* These figures recorded by Strubell (61) and by Chatin (6) are obviously wrong. From the drawings which they give in their publications, it is evident that they should read .016 and .015 mm. respectively.



CURVE INDICATING RELATION BETWEEN VOLUME OF CYST AND EGG CONTENT.

Diagram IV

No. of eggs per cyst. The actual number of eggs found by us in fresh fully developed cysts varied from 22 to 780. Typical measurements of cysts with their egg contents are recorded in Table XI.

TABLE XI.

Table showing the measurements of typical cysts together with the number of eggs and their average size.

Cyst size mms.	No. of eggs per cyst	Average size of egg mms.
.412 x .239	22	.096 x .047
.466 x .279	32	.091 x .044
.400 x .319	45	.098 x .044
.572 x .466	180	.097 x .043
.758 x .678	476	.097 x .045
.878 x .798	780	.099 x .045

In diagram IV. the volume of these cysts is plotted against their egg contents.[x] The resulting straight line, indicates that the volume of the cyst, is directly proportional to the number of eggs it contains. Most of the cysts contain between 250-300 eggs.

In Table XII the measurements of the mature male are given, and for comparison those recorded by other authors. The figures given by Triffitt (66) for the potato strain of H.schachtii are not in very close agreement with ours,

[x] In calculating the volume the diameter is assumed to be $\frac{\text{length + breadth}}{2}$

especially those taken from Lincolnshire soils.

TABLE XII.

Table showing measurement of mature male of Heterodera schachtii from various sources.

Source	Length in mms. Max.	Min.	Average.	Length / Breadth	Stylet in mms.	Spicule in mms.
Ayrshire (Potato)	1.144	.745	.905	28	.027	.032
Triffitt (Potato, Lincoln.)	.704	.591	.641	31	.035	.042
" (Potato, Hertford)	1.23	.91	1.13	28	.026	.039
" (Beet)....	.777	.727	.728	34	.035	.04
Strubell (Beet)....	1.000	-	.8-.9	32	.03	.033
Chatin (Beet)....	-	-	.8	-	.029	.033
Markinowski (Beet).	1.37		.8-.9	48	-	-
Triffitt (Oats)....	.818	-	.786	33	.031	.038
" (" Sweden)	1.48	1.24	1.36	39	.028	.036
" (Hop).....	.962	.71	.85	29	.027	.032
" (Mangold).	1.59	1.22	1.42	43	.029	.034

A review of the figures given in Tables VI. to XII. emphasises the great variation in size of the cysts, males larvae etc. of Heterodera schachtii on different hosts. These variations occur within such wide limits that the boundaries cannot be drawn sharply enough to justify the formation of new species or varieties. Wollenweber (75), however, on a basis of size differences prefers to regard the potato strain as a distinct species and proposes to call it H.rostochiensis.

Even on the same host plant, remarkable differences in size of the parasite occur. For example Triffitt (Table XII) gives the average length of the male of the potato strain in one series as .641 mm. and in another as 1.13 mm. Again, her figures, quoted in Table X. show the average length of the larvae of the beet strain as .29 mm. in one instance and as .457 mm. in another. It appears therefore, that variation in size of the parasite on any one host may be as great as that on different hosts.

The only constant morphological feature in which the potato strain differs from the beet strain is that the cyst is almost spherical and contains no obvious vulval aperture. But even this is not sufficient to differentiate a new species since Goffart (14) has shown that this characteristic is only temporary and that when the potato strain is induced to attack sugar beet the cyst becomes indistinguishable from that of the ordinary beet strain. Thus there is no necessity to go even as far as Zimmermann (77) who designates the potato strain H.schachtii forma solani. The correct view is probably to accept such adapted nematodes, even although they show some slight morphological differences, as merely strains of the original Heterodera schachtii.

Depth of Infection of soils.

The greatest number of cysts are found in the top nine inches of soil and the numbers rapidly decrease from there downwards. In the case of the Ayrshire early potato soils, the subsoil is in the nature of a coarse gravel and few of the soils extend downwards to a greater depth than 1 foot. It is, therefore, to be expected that the number of cysts in the soil or subsoil below 9" will not be large. Table XIII shows the numbers found:-

TABLE XIII.

Number of cysts per 10 c.c. soil at various depths.

Sample	0"-9"	10-18"	19-27"	28-36"
19	141.5	24.9	2.4	-
13	106.9	36.3	15	-
7	80.7	.4	.5	.1
25	59	1.5	-	-
1	22.5	4.0	.4	-

Baunacke (3) and others point out that the cysts present in the upper 9" of soil are those ready for immediate attack, while those in the deeper layers are held in reserve and are the true carriers of lasting infection. Therefore a soil should be regarded as heavily infested the deeper down the cysts go. It is interesting in this connection to compare the two soils 13 and 19. While in the surface soil, there are more cysts in sample 19 than in 13, the cysts are more numerous in the deeper layers in sample 13. Accordingly soil 13 is much more potentially dangerous than soil 19.

So far as we have been able to ascertain no cysts occur beyond a depth of 36". Thorne and Giddings (63) record the cysts of Heterodera schachtii (beet strain) as occurring at a maximum depth of 30 inches while Baunacke (3) gives 31.5 inches as the limiting depth at which cysts occur.

The question of the maximum depth at which the cysts are found is of great importance when we come to consider the question of methods of controlling or eradicating the eelworms. While it might be feasible to consider methods of sterilising the surface soil either by chemical agents or by heat, yet it would be impracticable especially from an economic standpoint to sterilise the soil by any method to a depth of 36".

SPREAD OF THE DISEASE

In the field the disease spreads from an infected patch slowly but surely. In order to determine the rate of spread a level piece of ground was chosen and a plot inoculated in the centre with soil containing eelworm cysts. Potatoes were grown on the plot every year and accurate records were taken of the rate of spread. This was determined by examining the roots of potato plants for cysts and noting the distance from the point of inoculation at which infected plants occurred. The rate of spread varied somewhat from year to year but on an average it was found to be 8-10 feet per annum.

The cysts when dried readily float in water, (appearing like small silver balls), and consequently on a sloping piece of ground cysts may be carried downwards in the surface water and a much higher figure be recorded than that given in our experiments.

In the field the original infection of the ground may be due to cysts being carried in drainage water or in streams or by the wind. Again cysts may be transported by man or by animals or by implements used in cultivation; but the most common source of infection is undoubtedly due to cysts being introduced on the seed potatoes ~~(see p.~~).

On one of the many samples of seed potatoes examined, the average number of cysts per sett was as high as 15. The method adopted to determine the "cyst content" of the sett was to wash the individual tubers thoroughly in water and then to decant the supernatant liquid on to a filter paper. The number of cysts on each paper was then counted.

In these experiments we were dealing with potatoes which had been grown on light sandy soils and had on that account little earth adhering to their surface. In the case of potatoes grown on heavy soils and lifted when the soil is wet, the number of cysts per tuber would be very much greater depending on the amount of adhering earth.

There can be no doubt, therefore, that the carrying of cysts on the seed tubers is the common method by which soils become infected, and, if we assume that setts bearing 15 cysts be planted in 27" drills and spaced one foot apart we introduce approximately 300,000 cysts per acre and these may on hatching give rise to 90,000,000 larvae - a very heavy initial inoculation.

We cannot urge too strongly the necessity for controlling the spread of this disease by preventing the use of "infected" seed potatoes. It must, however, be remarked that although the use of such seed will undoubtedly infect all soils with the potato eelworm, yet its presence will only influence the yield to a very small extent during the year in which the tubers are planted. The adoption of a rotation will minimise its further effect and possibly prevent it doing damage to any serious extent. On the other hand in gardens, allotments, or in districts where potatoes are grown more or less without a break, the initial infection introduced in the seed will increase to such an extent that potato growing will become unprofitable in the course of a few years. In our opinion it is important to protect such growers from unwittingly introducing infection on their seed.

CONTROL

Individual Resistance. We have already remarked that in nematode nests where the greater number of the plants are badly attacked by Heterodera, some few plants occur which are strong and vigorous, andwhich seem to offer a considerable amount of resistance to invasion. (see p 17). It may be that some plants impart in their secretions a greater stimulus to the brood in the cysts than others and at the same time attract the greater number of the hatching larvae. On the other hand the unaffected plants may show some physiological peculiarity such as a deficiency in some food constituent essential for the normal development of the larvae.

We have saved the produce from these healthy plants in the hope that a strain of a particular variety could be obtained which would show, at least for some time, resistance to Heterodera attack. We could scarcely imagine that such resistance would be permanent because by necessity the eelworm would sooner or later adapt itself to the conditions obtaining in these plants. Resistance of this nature is undoubtedly due to some physiological factor and as such can only be temporary. While some of the strains which we have isolated are promising, it is obvious that it will take some time before sufficient seed can be obtained to carry out experiments on a field scale.

Varietal Resistance. We are much more hopeful, however, of securing a strain or a variety which would be immune because of peculiar morphological conditions obtaining in the roots.

Dr A.C. Arzberger (1) working on Cowpeas, finds that two varieties are comparatively resistant to nematode attack. He shows that the corky layers of the roots of these varieties have thicker and more suberised cell walls and fewer broken areas than the other less resistant varieties. Further the starch containing cells are more remote from the periphery of the root and the starch is not so abundant as in the cortex of susceptible varieties. Here we have a case of morphological immunity and the possibility is opened up for the breeding of potato varieties which will prove immune to eelworm attack.

Nillsson Ehle (41) finds that resistance to Heterodera attack in barley is a dominant character. Varieties of barley fall into two categories, viz:- resistant or nearly resistant (Chevallier type) and susceptible (Princess type). No intermediate degrees and values are found between the two extreme types. This would seem to show that resistance to Heterodera invasion is due to a single genetic factor. However the problem of breeding resistant varieties is rendered more difficult as the most resistant types are the least productive.

In order to test if any variety of potato has a well marked power of resisting Heterodera attack, 24 varieties of potatoes were planted in drills on badly infested ground. We tentatively group them as follows:-

Most Resistant.	Crusader, King George, Rhoderick Dhu, King Edward, Kerr's Pink, Great Scot.
Less Resistant.	Majestic, Arran Chief, Arran Consul, Lochar, Scottish Farmer, Eclipse, Herald.
Still less Resistant.	Abundance, Epicure, Golden Wonder, Duke of York.
Least Resistant.	Glencoe, Bishop, Arran Victory, Royal Kidney, Field Marshal, Katie Glover, Catriona, Ally, Immune Ashleaf, Doon Star.

This experiment has been repeated for three years, the varieties being for the most part the same. It is perhaps worthy of mention that of all the early varieties tested, Eclipse and Herald appeared to be the most resistant.

Chemical Control Measures.

For the past fifty years attempts have been made by many of the most capable investigators, e.g. Kühn (23, 24,25,26), Hollrung (19,20), Muller and Molz (36,37,38), Fuchs (12), Strubell (61), Stift (57), and Baunacke (3), to eradicate Heterodera schachtii from the sugar beet soils in Germany but no one has yet devised any effective control by the use of chemical compounds.

In this connection we might quote Kühn (23) who states that "To devise an expedient by use of which it may be possible in agricultural practice to exterminate directly the nematodes in the field is, without any question about it, to be renounced. One may indeed succeed by one or other substance to kill the nematodes, but only with quantities of this substance which would cause an exorbitantly high expenditure". Later Hollrung (20) came to the same conclusion. Baunacke (3) too as a result of his researches, states that direct methods of killing the nematodes is impossible. Goffart (13) finds by using Calcium Cyanide, one of the most effective soil sterilants, that an expenditure of £300 per acre would be entailed to control the potato eelworm. Thorne (64) gives the quantity of Calcium Cyanide that must be applied to control the beet eelworm as 1600 lbs per acre.

There can be no doubt, therefore, that H. schachtii is extremely resistant to chemicals, due, in part, at least, to the fact that the cuticle of the eelworms and the wall of the cyst are almost impenetrable to substances in solution.

From our experiments with soil disinfectants we may state that of all the chemical compounds which we used, we had most success with Calcium Cyanide at the rate of 6 cwts per acre and Uspulun at the rate of 1½ cwts. per acre; but even with these substances the yield of potatoes was only very slightly increased and the cyst content of the soil, was not greatly reduced.

Edwards, (10), however, reporting on experiments in Lincolnshire, on ground infected with Heterodera schachtii and Corticium solani shows that he gets a great increase in yield of potatoes from the application of drained creosote salts at the rate of 8½ cwts per acre. He does not mention, however, that, as a result of using this substance, there was any marked reduction in the cyst count of the soil. Further, in the field where the experiment was carried out, a definite rotation was practised, - Potatoes, Oats, Wheat, Clover and Seeds, Clover and Seeds, Potatoes, Cabbage, Peas, Potatoes, - and as we have already pointed out, it is only when a crop like sugar beet or potatoes is grown in close sequence that any serious damage accrues from eelworm attack, - in other words only when the strain becomes highly specialised. For that reason we are inclined to believe that the effect of the application of drained creosote salts was not due to its influence on the eelworms but to some beneficial influence it had upon the soil.

As regards other control measures against Heterodera, Kühn, (23) and Fuchs (12) have shown that sterilisation of the soil by burning is useless. Jordan (21), and Karpinsky (22) have obtained negative results from control measures based on soil cultivation such as deep ploughing, shallow ploughing etc. At best soil sterilisation and cultivation methods affect only the top 9" of the soil.

Starvation is of no avail. Bare fallowing for 8 years - Fuchs (12) - and for six years - Hellriegel (16) - has proved useless.

According to Baunacke (3) flooding of a meadow for six years only served to spread the infection.

The larvae and the eggs are extremely resistant to cold. The eggs can be frozen for 6 days in ice blocks at - 9°C and still develop when thawed out, while repeated freezing at a temperature of - 6°C did not injure the larvae (3).

So much have workers been impressed with the futility of attempting to control <u>Heterodera schachtii</u> by purely empirical methods that they have abandoned chemical and physical measures and turned to those with a biological aspect.

Biological measures of Control

(a) Inoculation with fungi. Attempts have been made to inoculate the cysts with various fungi but without much success (31). The cysts themselves seem to harbour fungi in relatively large numbers which undoubtedly attack the eggs and developing larvae and destroy them. Probably under soil conditions the action of these fungi is developed to its fullest and one cannot hope to increase to any marked extent the mortaility among the cysts by inoculating the soil with micro-organisms.

(b) The effect of chicory. In the literature dealing with the control of the eelworm on sugar beet it is frequently stated that the roots of chicory and onions are not attacked by Heterodera schachtii (beet strain) (20,28,36,38,73) and consequently we find it recorded that chicory is often grown on 'beet-sick' soils in Germany. It is stated by Rensch (45) that the roots of chicory impart through their secretions a strong stimulus to the larvae in the cysts to hatch out but that the root contains some 'bitter stuff' which makes it unsuitable as a host for the hatched larvae. However Plumeche, quoted by Baunacke (3) states that the roots of chicory can even be invaded by the beet eelworm, and it would therefore appear that a strain of Heterodera can in course of time adapt itself to any host however unsuitable that host may originally have been for its growth and development.

It was, however, considered probable that there might be some possibility of reducing the cyst content of soils by growing chicory and consequently an experiment was laid down on a badly infested soil in Ayrshire. The seed was sown in drills on the 15th May 1929 and a sufficient number of samples of the soil taken to arrive at an accurate estimation of its cyst content. The crop was raised on the 17th September and the soil was again sampled on that date. As was to be expected no larvae or cysts were found on the roots at any time during the summer.

Table XIV. shows the cyst counts in the soil under chicory and on the section of the field where no chicory was grown.

TABLE XIV.

	No. of cysts per 10 ccs. soil	
Date of Sampling	Ground not under Chicory	Ground under Chicory
May 15th	87.4	78
Sept 9th	87.2	69.2

By growing a crop of chicory on a heavily infested soil a reduction of the cyst content has apparently been brought about - in this case by 11%. As the soils in the two sections were sampled with great care this reduction is probably significant. Further if the theory advanced by Rensch (45) is correct that chicory stimulates the larvae to hatch from the cysts, there may be a marked reduction in the number of eggs or larvae in the cysts that remain in the soil, irrespective of any reduction in the cyst count of the soil. The whole field is now under potatoes (1930) and it will be interesting to see if the growing of chicory has had any marked influence on the crop yield.

(c) The effect of Mustard. We were induced to try the effect of a crop of white mustard (Sinapis alba) in eradicating eelworms from the soils since we learned that at one time some farmers grew this crop after early potatoes with advantage. The mustard seed was sown broadcast on July 10th 1929, on the harrowed surface at the rate of 20 lbs per acre and the cyst content of the field determined at that date and again on January 13th, 1930 after the crop had been ploughed in. The field selected for the experiment was one in which the potatoes in 1929 were badly affected with eelworm.

There was no appreciable reduction in the cyst content due to the growing of mustard.

Triffitt (67) has recently obtained some very remarkable results from experiments devised to test the influence of this plant on the normal development of H.schachtii (potato strain).

From a series of pot cultures carried out by her, she states that very few cysts were found present on potato plants growing on infested soil where mustard was growing in the pots along with the potatoes or where it had been grown in the pots previous to planting the tubers. On the other hand in the control pots, - those in which no mustard was growing or had been previously grown, - there was a relatively large number of cysts present on the roots of the potatoes.

The author (67) is inclined to believe that in this plant and especially in its roots some substance is present which has a detrimental effect on the nematodes at some stage in their life-history, and that the substance is liberated from the living roots and also from the whole plant on decomposition.

It would be remarkable if mustard should have any such effect on Heterodera schachtii as Marcinowski (31) and Kühn (23) both state that Mustard is extremely sensitive to attack by H. schachtii (sugar beet strain). It would therefore appear from the work of these authors that there cannot be any substance present in the plant which can kill out either the free larvae or the larvae in the cysts.

(d) Activation of the brood within the cysts.

The most recent methods adopted for combating Heterodera schachtii take advantage of the fact that the cysts (sugar beet strain) in the presence of root secretions of a suitable host plant are induced to liberate their larvae. Baunacke (3) showed that the washings of sugar beet roots containing, it is assumed, the root secretions in solution, stimulated the larvae to hatch from the cysts. Rensch (45) repeated the experiment and confirmed this finding. Taking advantage of this, Rensch devised a solution chemically similar to the secretion of sugar beet roots and applied it to the soil when the ground was bare of crop. He hoped thereby so to impregnate the soil that all the cysts would be induced to hatch out their brood. In the absence of a host plant as occurs when the ground is not under crop in autumn and winter, the larvae would die of starvation since they, on hatching, contain no store of reserve food and further they would be exposed to very unfavourable conditions. Rensch hoped that by adopting this procedure he would eradicate the eelworms from the soil or at least greatly reduce their numbers.

Further in spring after the seed of the host (beet) was sown, he again sprayed the soil with another solution, chemically similar to that secreted by beet seedlings, in the hope that the hatched larvae, being unable to receive a direct stimulus from the roots of their host plant owing to the whole soil being uniformly impregnated, would wander aimlessly about in the soil in their endeavour to locate their host. (Baunacke(3) and others have definitely shown that the larvae are directed to the roots of the host plants by a positive chemiotactic stimulus). Unfortunately this ingenious method was not successful when carried out in a field scale chiefly on account of the cost.

However, there can be no doubt that the control methods of the future must be based on similar biological principles.

Reinmuth (43) has recently published a paper on the control of Heterodera schachtii on potatoes, by a method, similar in principle, to that of Rensch described above. Briefly his procedure is as follows:- (1) As soon as the potato crop is lifted the green shaws are cut up into small pieces and incorporated in the soil. (2) Then the ground is immediately seeded with either Sweet Clover (Melilotus albus) or Lupins (Lupinus luteus) and a catch crop grown, which is ploughed in early in September. (3) In September the ground is top-dressed with stinking animal oil at the rate of 6 cwts per acre. For convenience of application, the oil is mixed with sawdust.

No explanation is given of the effects which these crops have on the eelworm but we would imagine that they would stimulate the hatching of the larvae from the cysts - it may be that practically all crops do stimulate hatching. Further it is not made clear by Reinmuth whether the animal oil has the same effect on the cyst or whether it acts as a sterilant. He seems to indicate, however, that at first it brings about hatching of the larvae from the cyst and then acts on the hatched larvae as a "lethal" agent. The result of the treatment of the land in this way brings about a great decrease in the cyst content of the soil and a marked increase in the potato crop in the following year.

Experiments based on Reinmuth's method are being at present carried out by us.

(e) The adoption of a rotation.

We have already indicated that the potato strain of Heterodera schachtii only causes marked injury when potatoes are grown year after year on the same soil and consequently if a rotation be adopted the damage done will be very small. Unfortunately in Ayrshire the adoption of a rotation is more or less impossible as these light sandy soils are practically useless for ordinary cropping purposes. The mere adoption of a rotation, however, without the keeping down of weeds, would be useless as the potato strain would quickly adapt itself to the prevalent weeds and the ground would probably continue to remain as heavily infested as before. A departure, even for a short time, from a definite rotation, would result in a state of affairs as bad as ever since the eelworms present in the soil would soon specialise on whichever crop is grown in close sequence. On the other hand if a fairly wide rotation is adopted and no perennial crops grown, such as clovers, grasses, etc. a definite reduction in eelworm infestation will be brought about. A relatively small number of the eelworms will survive, which must of necessity be polyphagous.

In this connection Zimmermann (77) records very good results from withholding potatoes from highly infested ground for three years. On some of the Ayrshire soils marked results are obtained even by withholding potatoes from the field for one year.

The Effect of early lifting.

In Ayrshire the lifting of the crop begins early in June, i.e. when the cysts have just appeared externally on the roots and are still white or yellow in colour. One would expect on that account that there would be a great mortality among the larvae in the cysts especially as the shaws are left on the surface of the ground until they wither. We have observed that the larvae and cysts on the young roots die under the action of direct sunlight almost immediately. Marcinowski (31) states that the encysted females and males protected by root tissue are more resistant to drying but that they would probably die on young roots brought to the surface and exposed to wind and sun for a few hours. The larvae on the other hand are killed, according to Strubell (61) by being exposed to drying in air for one hour.

Rain, however, counteracts the beneficial effect of drying and it would be safer and altogether more satisfactory to burn all shaws and roots immediately after lifting. This would undoubtedly decrease the number of cysts in the soil since the cysts are loosely attached to the roots when freshly lifted and on drying they fall off and find their way into the soil.

We have carried out no experiments to show the beneficial influence of this practice on the crop in subsequent years but we are inclined to believe that it would not be as striking as would be expected, as during the actual lifting and the subsequent shaking of the roots to dislodge the potatoes, very many of the cysts would be set free into the soil. Much however would depend upon the growth of the shaw at the time of lifting. If the potatoes are lifted very early this practice would be of considerable benefit as the most of the females would not have emerged from the root, but on the other hand, if the majority of the females were visible externally as would be the case with late raising, the effect would not be appreciable. We have data which go to support this. On one field part of the crop was lifted early in July in 1928 while the remainder was not raised until August.

In 1929 there was a marked difference in the health of the two sections. The crop on the section raised late in 1928 was obviously much poorer. On making a cyst count of the soils in the two sections the following results were obtained.

	Cyst Count per 10 c.c.soil (Average of 10 estimations)
(1) Raised early in 1928....	.3
(2) Raised late in 1928.....	8

To us it would appear that the most beneficial practice would be to plant infested ground with "chats" immediately after the early potato crop is lifted. In order to save expense the chats could simply be broadcast and ploughed in. When the plants have reached a fair stage of development and before any cysts are visible - about 50 days - they could be grubbed up, and gathered immediately into one large heap where they might rot. This operation should be done on a dry sunny, and preferably, windy day. The whole aim would be to uproot the potatoes before the females are fertilised as there can be no doubt that the females once fertilised can develop their eggs and larvae without being attached to a host plant. This has been demonstrated by Wilfarth (72). He watched detached rootlets containing females from autumn to spring in a moist sand-turf mixture and found in spring that a large

Fig. 1

Fig. 1.—Dung applied in drill at time of planting of potatoes.

Fig. 2

Fig. 2.—No dung applied but liberal application of artificial manures given. Note margin of dunged plot on left.

On both areas dung had been applied in the two preceding years.

number contained larvae. This has been confirmed by Marcinowski (31).

The above method of control is in reality a modification of the trap-plant method of Kühn (26). The principle of this method is to sow a quick growing susceptible crop on the infested soil and to uproot it after it has been invaded by the eelworm larvae, but before the females have become sexually mature. Rape was the trap-plant used by Kühn and with it he was able to grow three successive crops in one summer. Fuchs (12) carried out similar experiments, but preferred to use White Mustard as the trap plant.

That this procedure can be futile under certain conditions was demonstrated by the experiments of Baunacke (3) in Saxony and Brandenburg, in which he conclusively proved that these plants were never attacked by the specialised sugar beet eelworm. Any highly specialized strain e.g. the potato eelworm will only attack its own particular host plant. It follows therefore, that if any combating method, based on Kühn's procedure is to be successful, then the trap plant used must be that on which the strain has specialized.

The Effect of applying the farmyard manure in the drill.

The most promising preventive measure we have tried is to apply a heavy dressing (20 tons per acre) of farmyard manure in the drill at the time of planting of the crop[x]

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[x] The farmyard manure is normally applied on the flat before ploughing. See page 6

(Plate XI). It seems that the adoption of this practice prevents the eelworms attacking the potato plants until they are fairly well established. Ultimately, however, they are invaded but as already mentioned when potatoes are attacked late in the growing season, the damage is not nearly as great as when they are attacked in the early stages of growth. It may be that in the presence of an abundant supply of organic matter the larvae act as saprophytes for some time and subsequently invade the roots. Strubell (61) states that the larvae of Heterodera schachtii can live to sex maturity in humus rich soil without a host plant. Further Berliner and Busch (4) state that the eelworm can develop on rotting root remains under certain conditions.

On the other hand the beneficial influence of the application of farmyard manure in the drill may be due to the encouragement of the development of females for Molz (32) has shown that heavy nitrogenous manuring increases the proportion of females to males. It has also been shown (61, 3) that H. schachtii is monogamous and therefore this disturbance of the sex ratio would result in only a proportion of the females becoming fertilised thus causing a decrease in the number of cysts. It is questionable, however, if this would be lasting. Hertwig (17) in a very comprehensive handling of the sexual question states that "The power of living substance to react in a male or female direction is a biological fundamental, which for the lasting of life, is just as immutable as the power of growth and reproduction". If this be accepted, then there can be no doubt that any alteration in the proportion of males to females can only be temporary and that nature will quickly reassert itself and restore the correct ratios between the sexes.

From our observations on fields in Ayrshire where this method of applying the farmyard manure is adopted we can vouch for its beneficial and lasting effect. Fields manured in this way have produced good crops over a period of years, whereas on those manured on the flat in the autumn the usual procedure - the yield and the health of the crop is distinctly lowered in the following season.

EXPLANATION of PLATES XII to XXIII

a.	Anus
B.w.	Body wall
B.y.m.	Body of young male
Cut.	Cuticle
Cut.fem.	Cuticle of young female
E.sh.	Shell of egg
Ex.p.	Excretory pore
1st L.Cut.	Cuticle of 1st Stage larva
G.o.	Genital organ
G.r.	Genital rudiment
I.egg	Immature egg
Int.	Intestine
Lum.int.	Lumen of intestine
M.cut.	Cuticle of male
N.r.	Nerve ring
Oes.	Oesophagus
Oes. b.	Oesophageal bulb
Ov.t.	Ovarian tube
Rec.	Rectum
R.oes.b.	Ridiment of oesophageal bulb
R.sp.	Rudimentary spear
R.spic.	Rudimentary spicules
R.vag.	Rudiment of vagina
Sal.gl.	Salivary gland
2nd L.Cut.	Cuticle of 2nd Stage larva
2nd L.rec.	Wall of rectum of 2nd stage larva
2nd L.sp.	Spear of 2nd stage larva
Sp.	Spear
T.	Testis
T.l.	Tail of larva
Vag.	Vagina
Vul.	Vulva
Y.m.cut.	Cuticle of young female

PLATE XII. Development of first stage larva.

fig.1.	Early first stage larva.	28 days (X180)
fig.2.	Late first stage larva	31 days (X180)
fig.3.	First stage larva undergoing moult	31 days (X180)

PLATE XIII Development of second stage larva.

fig.1.	Early second stage larva.	35 days (X180)
fig.2.	Late " " "	45 days (X180)

PLATES XIV. to XVIII. Consecutive stages in development of male from the mature second stage larva.

PLATE XIV.

fig.1.	Second stage larva commencing to moult.	45 days (X180)
fig.2.	Slightly later stage	45 days (X180)
fig.3.	Still later stage in which the body of the young male, beginning to lengthen, folds upon itself. The internal organs have become indistinct.	45 days (X180)

PLATE XV.

figs.1&2.	Further stages showing increase in length of body of young male. In fig.2 there is the first suggestion of coiling within the 2nd larval cuticle.	45 days (X180)
fig.3.	With further increase in length, the testis becomes visible again.	45 days (X180)

PLATE XVI.

fig.1.	Further stage showing spear and spicules in a rudimentary condition.	45 days (X180)
fig.2&3.	Later stage in which the oesophageal bulb and oesophagus becomes visible. The specimen in fig.3 has been dissected out of the 2nd larval cuticle.	45 days (X180)

PLATE XVII.

fig.1.	Mature male within 2nd larval cuticle.	52 days (X180)
fig.2.	" " as it occurs in the soil.	60 days (X180)

PLATE XVIII.

fig.1.	Mature male enclosed within 2nd larval cuticle inside the rootlet.	60 days (X90)
fig.2.	Mature male which has burst out of the 2nd larval cuticle but is still inside the rootlet.	60 days (X90)

PLATES XIX to XXII. Consecutive stages in development of female from the mature second stage larva.

PLATE XIX.

Fig. 1.	Earliest stage in development of female, showing thick cuticle, long genital tube and rudiment of vagina.	45 days	(X180)
fig. 2.	Later stage of same.	45 days	(X180)
fig. 3.	Still later stage in which the genital organ has moved toward and is now fusing with the vagina.	45 days	(X180)

PLATE XX.

fig. 1.	The genital organ has now fused with the vagina and has become paired. The female cuticle can be seen lying underneath the 2nd larval cuticle.	45 days	(X180)
figs.2&3.	Later stages of same in which increase in size is taking place in a dorso ventral direction.	45 days	(X180)

PLATE XXI.

fig. 1.	Flat leaf shaped stage.	45 days	(X180)
fig. 2.	Club shaped form of cyst derived by lateral growth from the leaf shaped stage. The vagina has now shifted ventrally and has made connection with the vulva. The ovarian tubes are greatly increased in length. The 2nd larval cuticle is again seen to be distinct from the female cuticle.	52 days	(X180)
fig. 3.	White spherical cyst containing immature eggs.	65 days	(X90)

PLATE XXII.	Mature brown cyst containing eggs with larvae.	80 days	(X90)

PLATE XXIII. Eggs from white and brown cysts.

fig. 1.	Egg from white cyst.	(X690)
fig. 2.	Egg from brown cyst.	(X690)

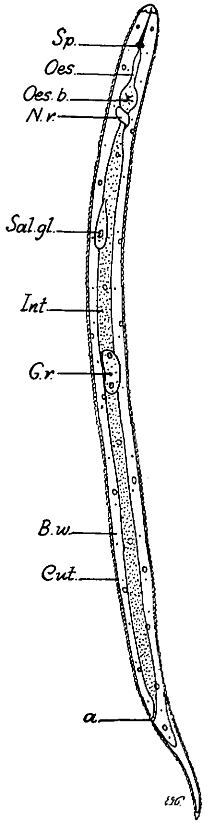


Fig. 1

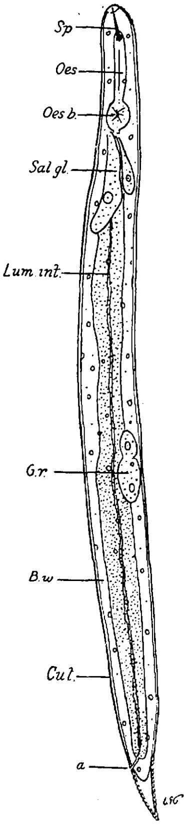


Fig. 2

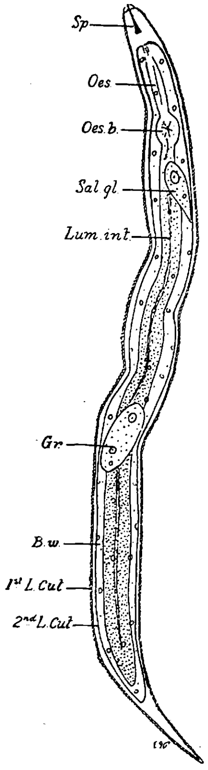


Fig. 3

PLATE XIII

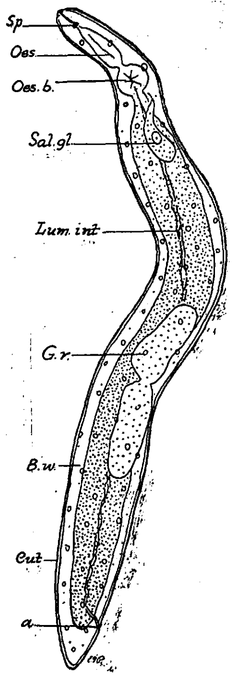


Fig. 1

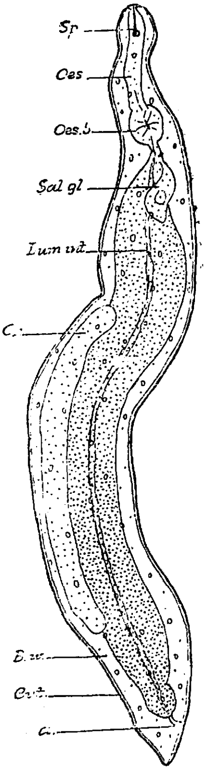


Fig. 2

PLATE XIV

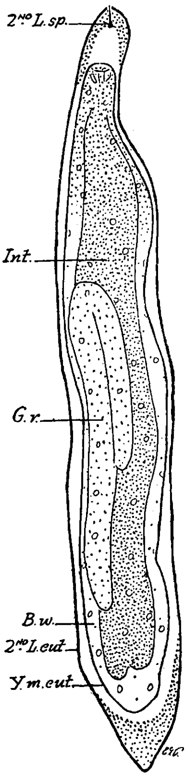


Fig. 1

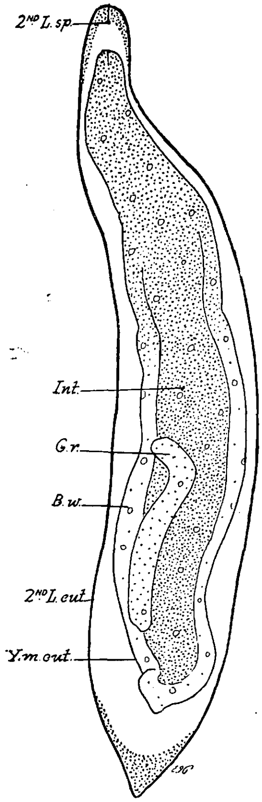


Fig. 2

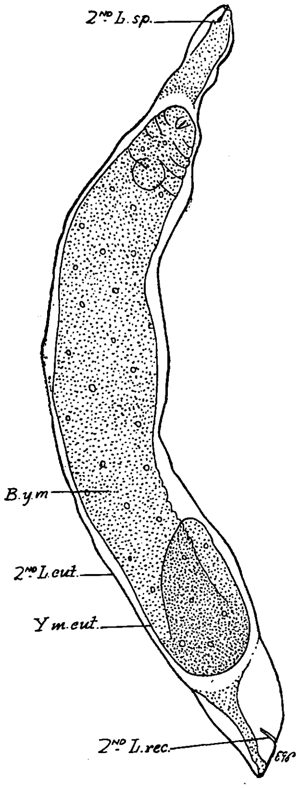


Fig. 3

PLATE XV

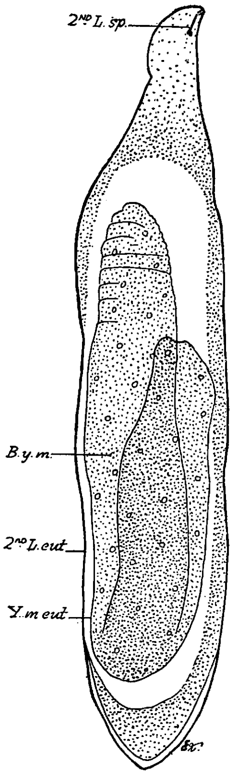


Fig. 1

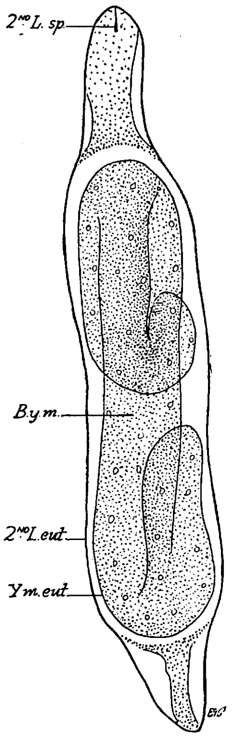


Fig. 2

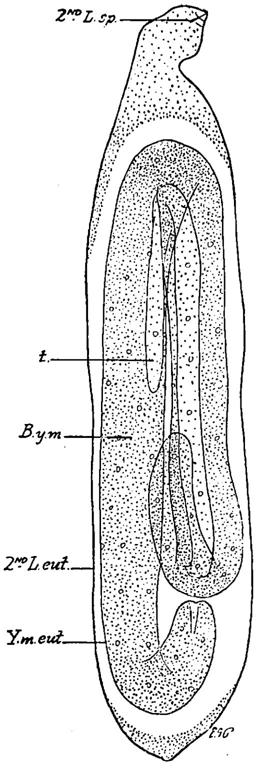


Fig 3

PLATE XVI

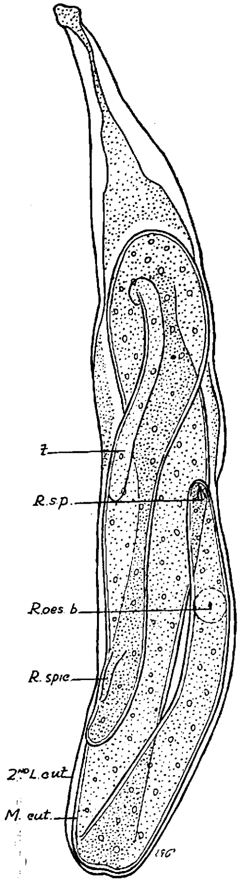


Fig. 1

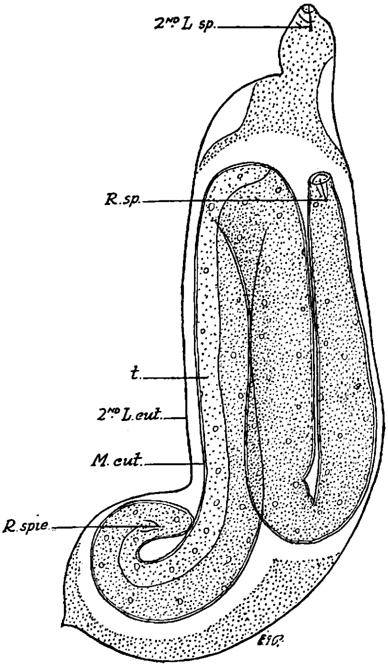


Fig. 2

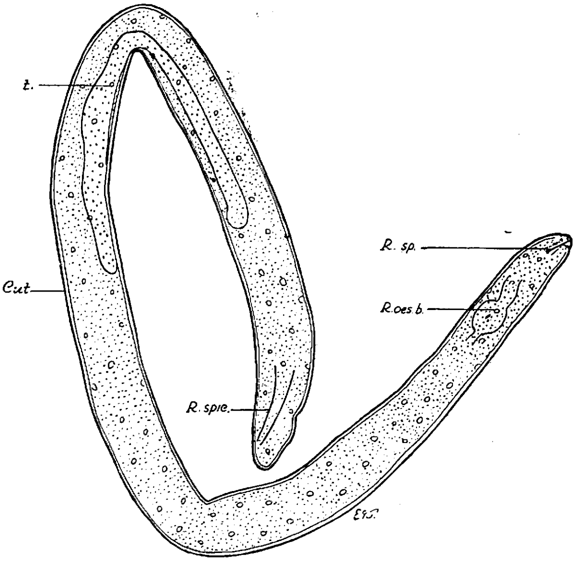


Fig. 3

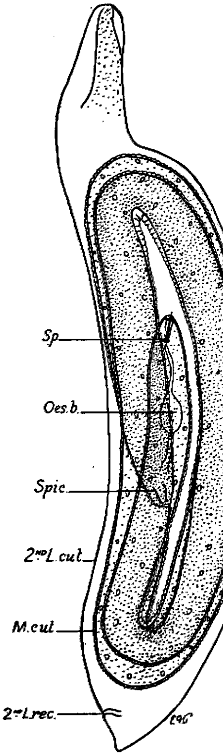

Sp
Oes.b.
Spic.
2nd L.cut.
M.cut.
2nd L.rec.

Fig. 1

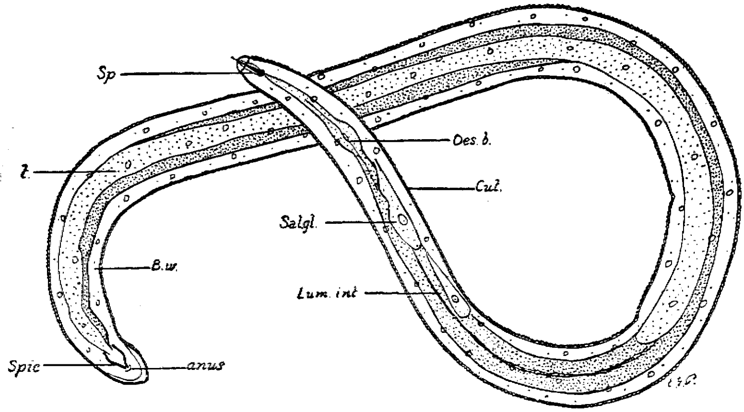
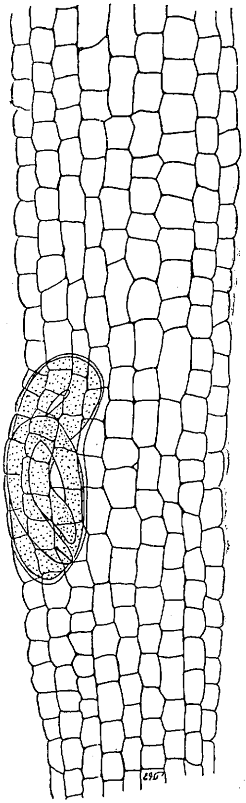

Sp
l.
B.w.
Spic
anus
Oes. b.
Cut.
Salgl.
Lum. int

Fig. 2

PLATE XVIII



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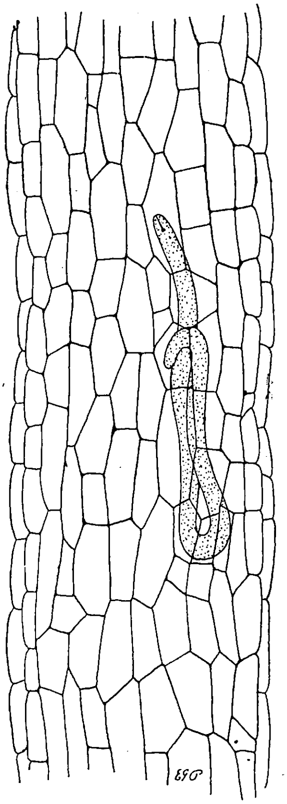

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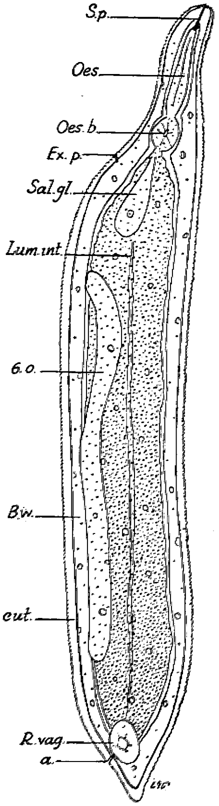

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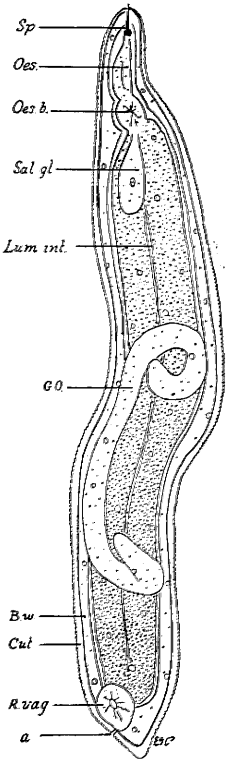

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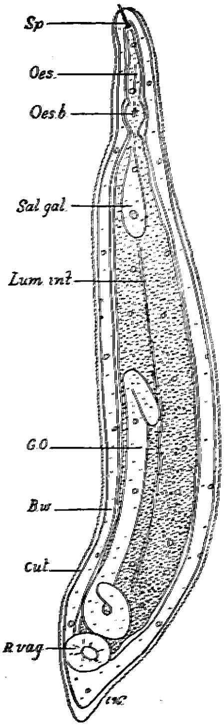

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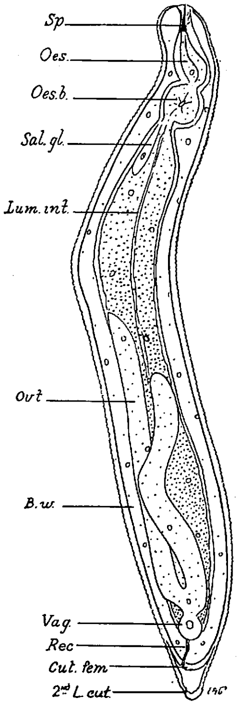

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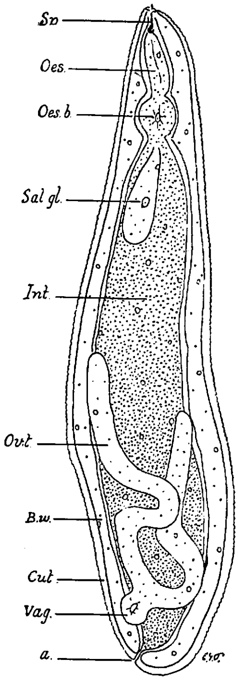

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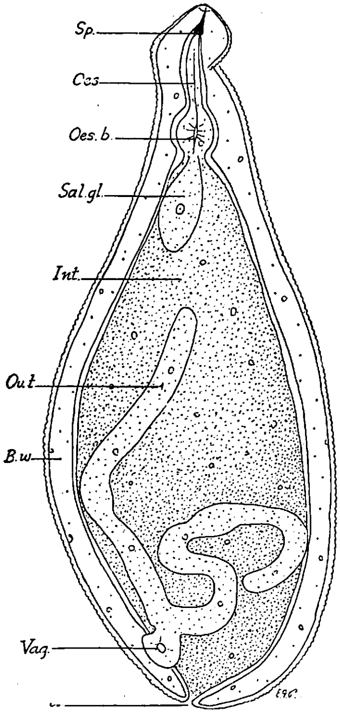

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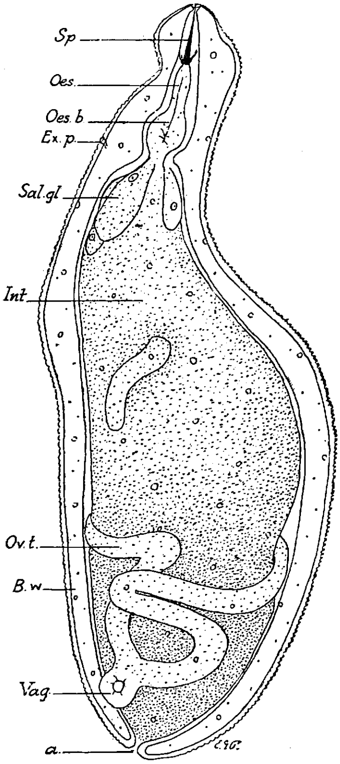

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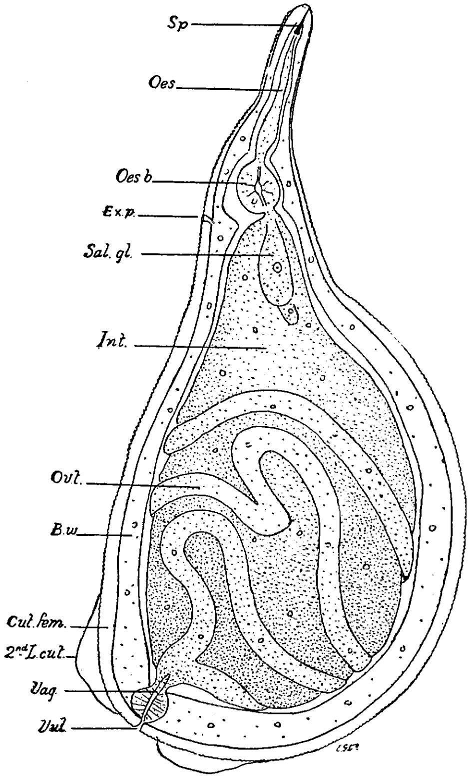

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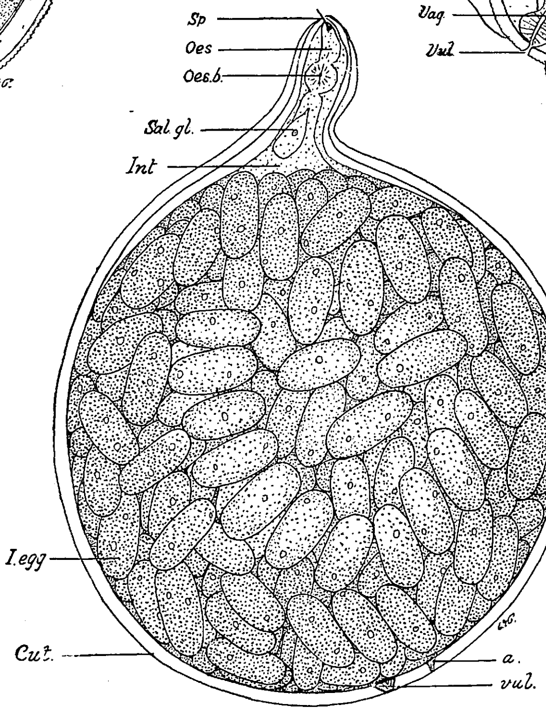

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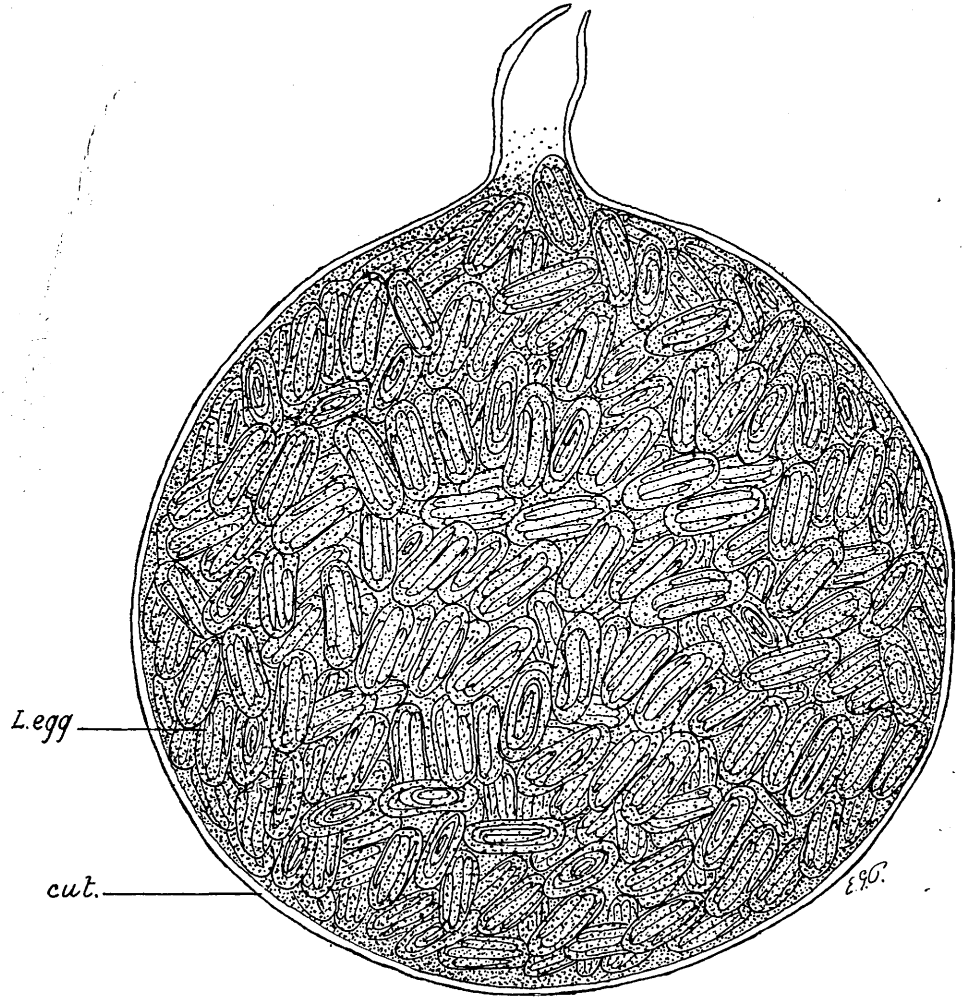
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PLATE XXIII

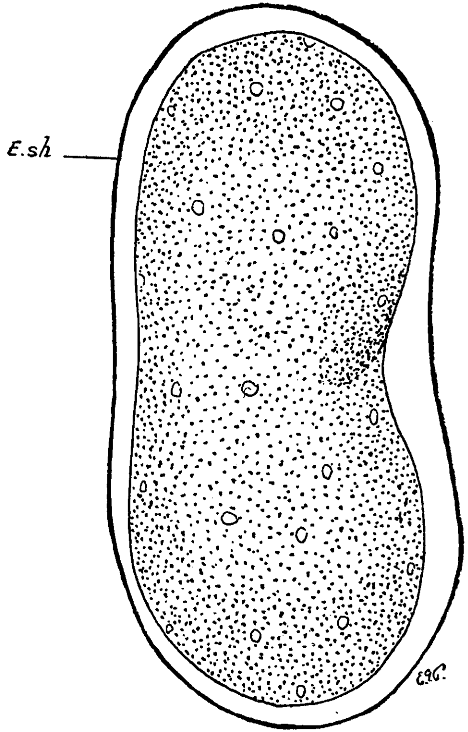

Fig. 1

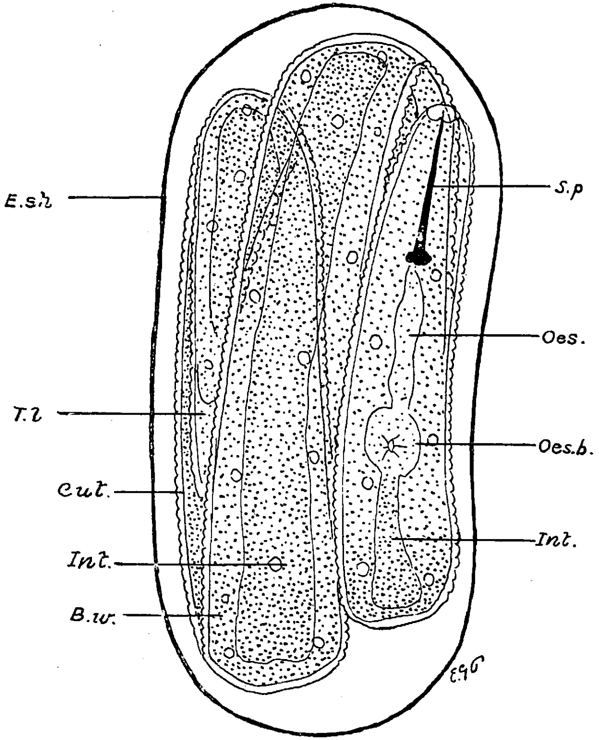

Fig. 2

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FIELD EXPERIMENTS

BY

REGINALD ARTHUR BERRY, PH.D., F.I.C.
AND
DANIEL GRANT O'BRIEN, M.A., B.Sc.

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FIELD EXPERIMENTS.

By REGINALD ARTHUR BERRY, Ph.D., F.I.C.,
Professor of Agricultural Chemistry,
and DANIEL GRANT O'BRIEN, M.A., B.Sc.,
Formerly of the Chemistry Department, now Lecturer in Agriculture,
West of Scotland College of Agriculture, Glasgow.

(With One Text-figure.)

Since 1899, field trials have been carried out at the Experiment Station, Kilmarnock, of the West of Scotland College of Agriculture and in the counties in the administrative area of the college. Their object was (1) to compare the yield of different varieties of oats and turnips, and (2) to determine the best system of manuring different crops grown under varying conditions of soil and climate. Single plot tests were invariably employed and in the interpretation of the results experimental errors were not taken into account as at that time (1902–1909) very incomplete data were available for that purpose. The results were published in college bulletins[1]. As the figures contained therein are often quoted it seemed highly desirable that the extent of the experimental errors in trials of this kind should be available. With this object in view an examination of the results has been undertaken which forms the subject matter of this short paper. Since the Oat Trials provide the largest amount of data, attention was directed principally to this crop.

Oat Tests at Different Centres.

As many as 12 different varieties were sometimes used in a test and from 20 to 35 tests were conducted annually at centres distributed over the counties in the West of Scotland. It has already been pointed out that single plots were invariably employed, a procedure which allowed of the inclusion of the maximum number of varieties with least amount of space and which involved least cost. The seed corn supplied was from the same bulk sample.

The scheme of the experiment was as follows. A member of the college staff selected and laid off the ground and superintended the

---

[1] Report on Experiments on the Comparative Merits of Varieties of Oats by R. P. Wright, Second, Third and Ninth Annual Reports, West of Scotland College of Agriculture.

seeding of the plots. College officers inspected the plots at intervals during the course of the experiment. To prevent undue attention of birds during the ripening of the crop, the plots were located in a field of oats. Several standard varieties were included in each test to act as a basis for comparison. The size (1/20th acre) and shape of plot, the weight of seed corn, and the method of harvesting, threshing and weighing of the produce were the same at each centre for each year. The varieties were cut as they ripened and the weight of grain and straw along with other relevant data obtained for each plot.

The statistical method of examination followed was the same as that adopted by Wood and Stratton[1] in their valuable paper on "Errors in Agricultural Experiments" published in a previous issue of this Journal. Frequency curves were first constructed, the number of centres being plotted on the vertical and the yields of grain in lbs. on the horizontal axis. This was done for the three varieties, Potato, Sandy and Banner and the curves are shown in Fig. 1 below.

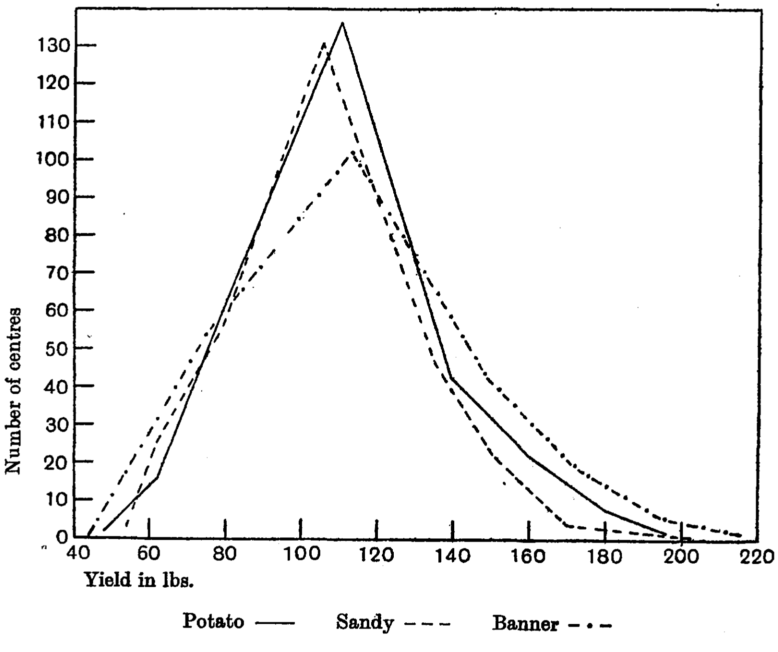

Fig. 1.

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[1] Wood, T. B. and Stratton, F. G. M. "The interpretation of experimental results." *Journ. Agric. Sci.* **3**, Part 4.

The three curves appear to conform fairly closely to a normal frequency curve. From this we may assume that we are dealing with a normal variable, and that the figures are suitable for averaging and for the calculation of the probable error. The slight divergence from the normal on the left of each of the three curves, represents the centres with the lowest yields. The cause of this is, no doubt, due to the almost complete elimination of the poorest types of soil in the selection of centres.

The probable error of the average total grain yield for five varieties for each year calculated from the difference of each plot from the average yield of grain was next calculated. The figures obtained are summarised in Table I below. In order to shorten the table as much as possible, the annual figures for one variety only, namely, the Potato oat is included.

Table I.

		Total yield of grain in lbs. per plot				
	Number of centres	Average over all centres	Lowest at any one centre	Highest at any one centre	P.e. of av. in lbs.	Percentage p.e. of av. in lbs.
Potato 1902	35	120	78	183	19·4	16·2
„ 1903	21	104	52	146	18·9	18·2
„ 1904	29	103	60	193	24·0	23·3
„ 1905	32	118	70	179	17·3	14·7
„ 1906	25	110	59	162	19·1	17·4
„ 1907	21	108	63	170	19·2	17·7
„ 1908	30	114	58	192	19·5	17·1
„ 1909	31	136	75	198	19·1	14·0
„ 1902–1909	224	114	52	198	20·6	19·2
Sandy 1902–1909	224	110	58	202	19·5	16·8
Banner 1902–1909	193	117	49	218	24·4	20·8
Mounted Police 1906–1909	107	124	62	227	23·5	19·0
Wide Awake 1906–1909	107	128	57	262	25·6	20·0

Comparing the yields of the old Scotch varieties, namely, Potato and Sandy over a long period and grown under the soil and climatic conditions prevailing in the West of Scotland, it is found that the Potato oat gives an average yield of grain amounting to about 4 per cent. more than Sandy. New varieties such as were in cultivation at that time, *e.g.* Banner, gave a yield amounting to about 6 per cent., Mounted Police 13 per cent. and Wide Awake 16 per cent. above that of Sandy. The last two named new varieties were not introduced into the trials until 1906, and the number of centres yielding figures were 107 compared with over 200 in the other cases. It is probable that if these varieties, namely Wide Awake and Mounted Police had been under test for the full period, their superiority over the older types would approximate to that of Banner.

It is noticeable from the table that the newer varieties show a greater range of variation in the yields of grain compared with the older varieties. The former give higher maximum yields compared with the latter. The elimination of the poorest types of soil in the selection of centres would adversely affect comparisons between the yields of old and of new varieties in so far as the older varieties would do better than the new varieties under poor soil and climatic conditions.

It is a well-established fact that the old Scotch varieties of oats are hardier and can better endure the greater extremes of soil and climate than some of the new varieties. For this reason and from the remarks made above respecting differences in yields, a variation of the experimental error due to variety was to be expected. Examination of the figures in Table I confirms this. The average probable error for the old varieties works out at about 18 per cent. and for the new varieties at about 20 per cent. of the total yield of grain.

Table II below gives the average yield of grain, the probable and the percentage error for Potato oat in each of the counties during the period 1902–1909.

Table II.

County	Number of centres	Average yield of total grain per plot	Average grain yields in lbs.	
			Probable error	Percentage error
		lbs.		
Ayrshire	46	120	19·35	16·13
Argyllshire	27	121	19·61	16·21
Dumfriesshire	30	108	19·40	17·96
Dumbartonshire	26	103	19·05	18·50
Lanarkshire	36	107	20·67	19·32
Stirlingshire	30	118	20·59	17·45

According to the above table the error for each county is similar to that for the combined counties. This means that the climatic and soil conditions met with in any of the counties in question would appear to be as diverse as that obtained in the whole of the West of Scotland, in so far as they affect the Potato oat.

From the foregoing figures the probable error on single plot trials for the old Scotch varieties of oat is about 18 per cent. and for the new varieties about 20 per cent. of the yield of grain. Adopting the recognised method of calculation, this means that when experimenting in any county in the West of Scotland with two varieties of oats which are only likely to show a difference in their grain yields amounting to about 5 per cent., it is necessary to have 214 centres with no duplication of plots at any centre in order to endeavour to obtain a conclusive result from a single year's trials, and 53 centres where the varieties under test differ in yielding power by 10 per cent.

Taking Potato oat as the standard type, it is seldom found that any other variety will surpass it in yielding power by more than 10 per cent. under the varying conditions of soil and climate that obtain in the West of Scotland. Banner surpasses it in yield of grain by from 2 to 3 per cent.

Converting the yield of grain per plot into yield per acre it is interesting to note that the following figures are obtained for the varieties in question.

Sandy	52 bushels per acre
Potato ...	54 ,, ,,
Banner ...	56 ,, ,,
Mounted Police	59 ,, ,,
Wide Awake ...	61 ,, ,,

OAT TESTS AT THE SAME CENTRE.

Using duplicate plots, Wood and Stratton found the probable error to be 5 per cent. of the yield of grain, whilst Hall and Mercer[1] showed that with 1/40th acre plots repeated five times the error was reduced to 2 per cent. It is possible, however, that the probable error is not the same for each crop. Allowance must be made for individual characteristics of crops in so far as they are affected by methods of sowing, cultivation, harvesting, immunity or otherwise to fungoid and insect pests, shedding of grain, etc. The cumulative effects of these will operate in varying degrees according as they apply to each crop. The following figures provide some data on this point. The method of calculation employed to arrive at the figures is as follows. The mean yield of duplicate plots is obtained and the difference of each plot from the mean is expressed as a percentage of the mean and the error then calculated in the usual way.

1/40th acre plots.

	Number of duplicate plots	Probable error of duplicate plots %
Oats 1910–11 grain	55	6·0
,, ,, straw	55	4·2
Hay ,,	28	1·2
Potatoes	19	3·4

Although the experimental data from which the above calculations are made are too few to arrive at a real comparison of the errors for different crops, nevertheless they are sufficient to indicate that differences in the probable errors do exist.

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[1] Mercer, W. B. and Hall, A. D. "The experimental error of Field Trials." *Journ. Agric. Sci.* 4, p. 2.

SUMMARY.

Single plot trials owing to the magnitude of the experimental errors, are practically useless as a test for comparing the yields of grain of one variety of oat with that of another. At best the results are only applicable to the particular experiment in question. For the old Scotch varieties of oat the probable error on trials of this kind amounts to about 18 per cent. of the yield of grain and to about 20 per cent. for the new varieties. Adopting single plot trials an 18 per cent. error means that when determining the superiority in the yield of grain of one variety over that of another and when the difference is not likely to be more than about 5 per cent. it would be necessary to have 214 centres with no duplication of plots at any centre in order to endeavour to obtain a conclusive result from a single year's trials. When the difference is likely to be about 10 per cent. it would be necessary to have 53 centres.

Data are supplied showing that the experimental error for different crops also for the grain and straw of the same crop is not the same. It is greatest for the grain and lowest for the hay crop.

This communication was included in a former paper by the authors published in a previous issue of this Journal, but at the suggestion of the editors, it was withdrawn for publication as a separate paper.

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RESEARCH BULLETIN No. 1

THE WEST OF SCOTLAND AGRICULTURAL COLLEGE

DEPARTMENT OF PLANT HUSBANDRY

The Endotrophic Mycorrhiza of Strawberries

and its Significance

BY

D. G. O'BRIEN, M.A., B.Sc., B.Sc.(Agric.)

AND

E. J. M'NAUGHTON, B.Sc.(Hons.), B.Sc.(Agric.)

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THE WEST OF SCOTLAND AGRICULTURAL COLLEGE.

STRAWBERRY DISEASE INVESTIGATION.

The prevalence of this disease was brought to the notice of the Governors in 1923 by the fruit growers of Lanarkshire. Following an inquiry the Horticultural Department of the College reported that the disease was spreading not only in Lanarkshire but in other districts in Scotland; that enormous losses were being sustained by fruit growers; and that unless the ravages of the disease could be stopped the attempt to grow strawberries must be abandoned.

Application for a grant to aid in investigating the problem was made by the Research Committee of the College to the Development Commissioners, through the Board of Agriculture, and eventually in 1926 financial assistance was obtained. A series of field experiments was arranged under the control of the Horticultural Department. The mycological work was assigned to the Plant Pathological Department of the College.

This bulletin records the result of the researches pursued by the latter department. A report by the Horticultural Department will be issued in due course.

W. G. R. PATERSON,
Principal.

SUMMARY.

The present paper contains the results of a mycological investigation, undertaken in the years 1926 and 1927 by the authors, into a serious disease of strawberries in the Clyde Valley of Scotland, known locally as "The Lanarkshire Strawberry Disease." The investigation was first confined to the disease as it occurs in Lanarkshire, but later was extended to include strawberry-growing districts throughout Great Britain, as evidence was forthcoming to show that the symptoms throughout the country had various points in common.

In view of the complex nature and widespread occurrence of the trouble, it should be understood that much research work still remains to be done before the problem is finally solved: meantime, on the evidence presented in this paper, the authors have come to the following conclusions as to the cause and nature of the disease:—

1. The disease of strawberries, best defined as "root weakness," is a general one.
2. Diseased plants are characterised by a paucity of absorbing rootlets. The other symptoms of the disease are but signs of starvation consequent upon this.
3. The only constant organism found in the living roots of unhealthy plants is an endotrophic mycorrhizal fungus of the type bearing arbuscules and vésicules.
4. This organism invades chiefly the fine absorbing roots of the strawberry plant.
5. At or about flowering time of the strawberry plant, fine fibrous roots are produced in great amount and, coincident with this, the maximum infestation occurs. The disease is most destructive at this critical stage.
6. Starch and other materials are removed from the root tissues by the action of the arbuscules, and there is no evidence of any return of starch to cells when once depleted of their contents. The vitality of the roots is therefore lowered.
7. The arbuscules are never completely digested by the host cells, so that the fungus benefits at the expense of the plant.
8. At the points where strong infestation occurs, the finer rootlets are ruptured and drop off into the soil. To this we ascribe the poverty of absorbing roots noted on diseased strawberry plants.

9. We regard this endotrophic mycorrhizal fungus as a parasite, and believe it to be the fundamental cause of the disease.

10. The disease tends to be slow-acting and chronic in its nature, but the fungus is capable of bringing about death of the plant if infection is severe.

11. The disease assumes really serious proportions when aggravated by conditions inimical to the growth of the strawberry plant. But, according as the mycorrhizal attack is severe or slight, and as conditions are unfavourable or favourable for plant growth, so is the ultimate damage greater or less.

12. The so-called "Lanarkshire Strawberry Disease" represents this trouble in its most serious form.

13. The endotrophic mycorrhizal fungus paves the way for the entry of secondary fungi and bacteria which, under certain conditions, may invade the weakened root tissues and intensify the disease.

14. The root fragments, which are broken off from diseased plants, serve to infect the surrounding soil.

15. Young runners from affected plants are free from disease until they strike root in the soil, when their roots become infected.

16. The disease is transmitted by infected runners.

17. Some evidence is produced to show that the fungus is not specific to the strawberry, but may invade other plants such as grasses and clovers. Infection of the strawberry crop may be traced to such sources.

18. Control measures are outlined.

INTRODUCTION.

Within recent times it has become increasingly evident that the general health of cultivated strawberries is not all that could be desired, judging from the numerous reports received from growers in practically all parts of the country. The complaint is chiefly to the effect that there has been a considerable, in some cases a very serious, reduction in yield, compared with the yields obtained in former times. So serious indeed is this state of affairs that, in certain areas, cultivation of the strawberry as a crop has ceased to be an economic proposition where once it was a flourishing industry. This is particularly the case in the Clyde Valley of Scotland, where the trouble is probably more acute than in any other part of the kingdom.

With a view to obtaining more precise information as to the cause and nature of the trouble, competent workers in many strawberry-growing districts have taken up the problem, and several papers have been published dealing with various aspects of the life-history of the strawberry plant, both in health and in disease. So far, the reports from the various centres on the latter subject show little or no agreement either as to cause or symptoms of the trouble.

In the spring of 1926, an investigation was commenced by the West of Scotland Agricultural College into the disease prevailing in their area, the mycological aspect of the question being assigned to us. The results of this work are embodied in the present paper.

An examination of plants from the main strawberry-growing districts made us realise that the most characteristic symptom of the strawberry disease was one usually spoken of as " root weakness ". There was considerable evidence to show that this condition was widespread throughout the country, although it varies in intensity in different districts and on different soils, being particularly severe in the Clyde Valley area.

Where " root weakness " was present, primary roots were characterised by their lack of lateral branches and finer fibres. The browning of the primary roots, regarded by some observers as a characteristic symptom of the disease, we found to be more or less normal, and to be caused by exfoliation of the cortex due to the formation of a zone of cork cells close to the stele of the root in the later stages of growth. This observation accords with that of Mann and Ball (27). Although this discoloration is more pronounced and more extensive on plants growing under definitely bad conditions, as in very wet soils, it must still be regarded as normal to these conditions, and not as an effect of the general disease so disastrous to the strawberry crop.

The paucity of finer root fibres on unhealthy plants could not be accounted for by the normal failure of the plant to provide these under wet conditions. We found the same poverty of absorbing roots, but to a lesser degree, on affected plants which had been grown under the best soil conditions. In all these cases, making due allowance for a normal amount of non-production in plants grown under wet conditions, there was an obvious lack of finer absorbing roots. From vestiges of finer fibres found attached to the main roots and root branches, it was clear that these had been produced but, at some later date, had dropped off owing to some definite but hitherto undetermined cause.

Other symptoms of the disease were merely the usual signs of starvation arising from the loss of the fine absorbing root parts. Thus, the affected plant had a stunted appearance, small sickly leaves, and showed a marked inability to produce and ripen fruit. Plants could be obtained in all stages of decline according as the loss of rootlets was relatively slight or severe. Death of the whole plant even might ensue. There is, therefore, no line of demarcation between health and disease; the question is one of degree.

Most workers on strawberry troubles are agreed as to these general characteristics. Wardlaw (41), for example, appropriately describes the disease as it occurs in the Clyde Valley as follows:—" The observer is struck by the absence in a diseased plant of those fine roots and rootlets which play the chief part in the actual absorption of mineral salts and water from the soil. It is concluded that the destruction wrought on the fine absorbing roots is responsible for the various signs of starvation perceptible in the green aerial part of the plant, and for its final decline and death, in fact for all the symptoms usually associated with the 'Lanarkshire Strawberry Disease.' "

In certain strawberry-growing districts where the malady occurred in a relatively mild form, the diseased plants were scattered throughout the field; on the other hand, where the disease was serious, the affected plants occurred in definite patches of irregular shape and area, although diseased plants were found sporadically distributed throughout the field. Often whole fields were devastated. This condition was most marked in the Clyde Valley area, but a similar state of affairs, in varying degrees of intensity, has been reported from Southampton, the Cheddar and the Tamar Valleys, Cambridgeshire (Wisbech), Cheshire and Worcestershire. Generally speaking the diseased patches marked the wettest parts in the field, more especially where the soil was in the nature of a heavy clay; but patches were even found to occur on light, well-aerated and well-drained soils. To account for these appearances, since no evidence was obtained by other workers, or by himself, as to the presence of any definite causal organism, Wardlaw (41) ascribed the source of the trouble to bad soil conditions, which weaken the plant and so render it liable to invasion by various soil fungi, such as *Pythium* and *Fusarium*, which may ultimately parasitise and kill the plants. The lack of fine absorbing roots, to which undoubtedly the disease is due, was traced by Wardlaw in the case of heavy soils to bad drainage, lack of humus and lime, and other factors commonly associated with soils of this type. On the other hand, where he found the disease to occur on soils that were comparatively free and open, he alternatively diagnosed the trouble as due to an impoverished condition of the soil which brings about a general lack of vigour consequent on starvation.

We cannot concur with this view that the trouble is primarily caused by bad soil conditions, since there was abundant evidence to show that the disease might be serious even on good soils, liberally manured and well-drained. Apart from disease, it is recognised that strawberry-growing cannot be successfully practised on soils that are waterlogged in the winter and spring months. Such soils do not normally exist to any extent in the Clyde Valley, although many fields in this district tend to be of a wet nature.

Among other causes to which this disease has been attributed are drought, wetness, winter injury, too high a temperature, too low a temperature, lack of cultivation, root injury due to cultivation, too fertile and too infertile a soil, exhaustion by too long a growing season, degeneration, virus, specific fungi, bacteria, insects and eelworms. Doubtless injuries have been traced to all of these, especially as the strawberry is a delicate plant sensitive to environmental influences, but none of the suggested causes could satisfactorily explain the widespread nature of the trouble.

With the conception that the disease was a general one, occurring in varying degrees of seriousness, we commenced our investigations.

Preliminary examination revealed the fact that no organism capable of causing serious injury was to be found on any part of the plant other than the roots, and accordingly it was to the roots that we ultimately confined our attention. During the course of our work, freshly-collected plants were examined at regular intervals throughout the year; these plants were obtained from all parts of the Clyde Valley, care being taken to select, examine and compare plants grown on all types of soils and under varying conditions.

Numerous plate cultures were made from the roots of unhealthy plants, and various organisms isolated. No one fungus could be isolated with any degree of constancy sufficient to warrant its being regarded as the sole cause of the disease. The fungi commonly cultured out from the roots of unhealthy plants could usually be relegated to the genera *Pythium*, *Fusarium*, *Cephalosporium* or *Rhizoctonia*. Most of the fungi belonging to these groups are regarded as weak parasites incapable of attacking the healthy plant. Further, on microscopic examination, we found no evidence of invasion of healthy roots by any of these organisms, although they were often to be noted in dead and dying tissues. The possibility of the trouble being due to attack by special pathogenic strains of such fungi was also precluded.

These, and fungi of a similar nature, have been frequently isolated by other workers, but in no case has it been shown that any of them played a primary part in the causation of the strawberry disease.

We were accordingly forced to the conclusion that these fungi were secondary, and could not account for the widespread trouble as it appears in the field.

It is a well-established fact that many of these organisms occur commonly on dead and dying roots, and certain observers record the presence of such fungi on apparently healthy roots, from which they have been isolated and identified in pure culture. The usual sterilisation methods, of course, were adopted previous to plating out. We would point out that, in most healthy roots, there are normally present small pits and depressions in the nature of blemishes of the external surface of the root; these are caused by injuries, sometimes by mechanical, sometimes by insect energy. Sections cut through such areas showed that the cells in the neighbourhood of the wounded part were in a dead and dying condition, and often inhabited by ordinary fungal saprophytes and weak parasites incapable of penetrating into the undamaged tissues. The routine methods of sterilisation, while effectively sterilising the exterior of the root, fail to reach fungi harboured in the small pits and depressions; thus the observer may erroneously be led to the conclusion that the organism which has been isolated from apparently healthy and sterilised roots is a true parasite. Such small pits are very common on the roots of the strawberry plant. The strawberry plant has about 50 per cent. of its root system in the top two inches, and over 90 per cent. in the upper six inches of soil (3). For this reason it is very subject to minor root injuries, in addition to the more evident damages consequent on the ordinary cultivation processes. Moreover, the fine fibrous roots are particularly delicate; the cortex being well developed, and showing little or no evidence of cutinisation in its peripheral layer. Apart from the liability of the roots to injury, the cortex of the finer roots is of an ephemeral nature, being exfoliated when secondary thickening occurs. The functionless and dying tissue of the exfoliated cortex offers an admirable medium for the growth of soil saprophytes, and however efficient superficial sterilisation may be at this stage, the worker cannot fail to isolate such organisms as constitute what is, after all, the normal soil flora.

If these fungi are dismissed as being secondary in their origin and effect, and more definite evidence of parasitism is sought, two alternatives may be suggested as to the nature of a possible fungal organism: (*a*) the fungus might be incapable of growth in pure culture; a pure parasite may not necessarily grow on a saprophytic medium, and many of our most virulent plant pathogens cannot be artificially cultured, *e.g.*, *Ustilagineae*, *Uredineae*, many *Ascomycetes** etc.; (*b*) the fungus might culture out only with difficulty, and be of such slow growth as to be readily swamped by the secondary organisms which almost invariably occur on the plates. The culturing method, although useful in many cases, does not always yield positive results; and as we, like others, had been unable to isolate any organism of constant occurrence, we were compelled to examine diseased plants in other ways, and to abandon for the time being the plate culture method.

The simple procedure we adopted was the microscopic examination of cut sections of infected plants. Certain parts of the root were carefully selected, and hand sections made of the fresh material. This rapid method was found to be more useful for our purpose than the microtome method, because of the very large number of plants that passed through our hands.

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* The spores of the *Ustilagineae* and the *Uredineae* will, of course, germinate on artificial media, but the *full* life cycle cannot be carried through outside the host.

At the outset, our attention was directed to the primary roots. Microscopic examination of these showed no evidence of invasion by any parasitic micro-organism. We were therefore led to study the finer roots through all stages of their development, and also to examine the detached fibres found in the soil in which the mother plants had been grown.

This systematic work has established the fact that a definite fungus is constantly present in the living tissues of the finer rootlets of all plants which show traces of the disease.

Extending the area of our observations beyond the Clyde Valley, we obtained typical diseased plants from elsewhere in Scotland and from the chief strawberry-growing districts in England. *The specific fungus was found to be present wherever strawberries were grown, occurring to an extent which varied according to soil and climatic conditions, method of growing practised, and variety. It was identified by us as an endotrophic mycorrhizal fungus of a constant type. A detailed study of this fungus, and particularly its inter-relation with its host, the strawberry plant, made us conclude that this endotrophic mycorrhizal fungus is the primary and underlying cause of the general disease affecting strawberries throughout Great Britain.*

THE ENDOTROPHIC MYCORRHIZAL FUNGUS OF THE STRAWBERRY PLANT.

Specificity.—The fungus conforms in all respects to the general type of mycorrhiza, which is characterised by bearing organs known as "arbuscules," and "vésicules." The various members of this group have no specific names, and are usually referred to in terms of the host plant on which they are found. This fungus will, in the following pages, be designated the "Endotrophic Mycorrhiza of the Strawberry Plant" or, briefly, "the Strawberry Endophyte." In all probability this organism is not specific to the strawberry, but, like the special type to which it belongs, can attack other plants. It has been recorded that perennials, such as grasses and clovers, are specially suitable as host plants for such mycorrhizas; but annuals, such as cereals, are also open to attack.

The characteristics of endophytes of the type bearing arbuscules and vésicules have been admirably described by Gallaud (19), and others. No description of the strawberry endophyte is to be found in literature, yet it closely resembles the *Arum maculatum* type of Gallaud. Since our conclusions as to the nature of this fungus on the strawberry may be of some importance to the grower and to the scientist, and further, as much of the literature is difficult of access to the average reader, we have thought it advisable to give a full account of our observations and researches on the endophyte as it occurs on the strawberry plant.

PREVIOUS REFERENCES TO THE STRAWBERRY ENDOPHYTE.

Reference to literature showed that the strawberry endophyte had not been made the subject of special study, although its presence has been noted by Gallaud (19), Peyronel (36), Jones (23), and White (42).

SELECTION OF MATERIAL AND MICROSCOPIC METHODS.

Selection of Material.—We have not found the endophyte on the primary roots of established plants, although we have noted its presence on the developing first roots of young runners. It was only in the fine fibrous roots that the fungus could be located with certainty. Selection of the part to be examined we found to be a matter of considerable difficulty, since the fungus mycelium is not continuous throughout the roots of infected plants, but has a localised and irregular distribution. Thus it is possible to select pieces of a badly infected root which reveal no trace of the fungus. The difficulty of selection is further increased by the fact that there is no visible evidence of the fungus externally. With a little practice, however, one is able to distinguish infected parts of the roots with a fair degree of certainty. We found this to be specially true in the early summer months when the fine roots formed at that time became infected. In consequence of the unequal distribution of the endophyte, the fibrous roots at this time become somewhat contorted and twisted, being more or less swollen at irregular intervals along their length, and varying in diameter from place to place. The swollen parts, which mark the points of abundant infection, tend to be brittle.

These symptoms were not by any means obvious, and are evident only to the careful and practised observer, enabling him by a rapid inspection to make a first choice of the most likely pieces for section cutting. Nevertheless, the roots have an abnormal appearance in these respects, although the symptoms are vague and not absolute. Later in the year, rapid elongation of the rootlets obscures these symptoms, and it is much more difficult to select infected portions with accuracy. By this time, many of the older infected rootlets have dropped off into the soil, leaving the primary roots almost completely devoid of absorbing fibres.

Microscopic Methods.—In fresh preparations, a certain amount of practice is necessary to observe even the main features of the fungus. This is due, in part, to the thinness of the hyaline wall of the invading mycelium. The arbuscules also, in spite of their size and characteristic appearance, easily escape observation in unstained preparations. Preliminary examinations of fresh tissue, after some experience of the endophyte has been gained, are nevertheless very helpful in locating the fungus and in selecting parts for further observation.

For the study of the distribution of the endophyte in the roots, we found that sections were most conveniently stained by immersion in Acid Fuchsin for about half a minute, with subsequent washing in 92 per cent. alcohol. This stain was prepared according to the following standard method:—

Acid Fuchsin, ...	1 gm.
Picric Acid (sat. sol. in 92 per cent. alcohol), ...	100 ccs.

Staining in this way was at once speedy and efficient, and the action was even more marked in fresh unfixed tissues; in this state, the endophyte absorbs the stain more quickly than the host tissues, assuming a deep red colour and giving good differentiation of the fungus from the host. The latter stains only very faintly.

An alternative method recommended by Gallaud (19) and extensively adopted by other workers is as follows:—Sections from fresh roots are directly immersed in a saturated solution of Cotton Blue in Lactic Acid for 12 to 24 hours. After destaining in water and alcohol, most of the colour is eliminated, but the fungus, nuclei, lignified and cutinised cell membranes, retain the blue stain. The sections are then graded through alcohol and mounted in Canada Balsam. If more permanent preparations are desired, it is better, after washing with water, to steep the sections for several minutes in a 5 per cent. solution of Tannin. The Fuchsin method, although more crude, is to be commended for its rapidity, especially where a large number of roots have to be immediately examined.

For careful work, we followed the methods described by Gallaud and others. Pieces selected for cytological examination should be fixed immediately on collecting in Flemming's solution, Picro-Formol* or 75 per cent. alcohol. Of these, Flemming's solution† is the least suitable, as the fat present in the arbuscules is stained black. Sections are then cut and stained with Meyer's Hæmalum, Haidenhain's Iron Hæmatoxylin, or Diamant Fuchsin. The nuclei show up well by this method. If desired, Eosin or Light Green may be used as counter-stains.

LIFE-HISTORY.

Method of Entrance and Mycelial Characteristics.—Little or nothing is known of the external life of endotrophic mycorrhizas. It has been generally assumed that, on the ultimate death of the host plant, they lead a saprophytic existence in the soil. If this were so, one should be able to culture out fungi of this type on artificial media; but there is no conclusive evidence in literature that any endotrophic mycorrhiza bearing arbuscules and vésicules has ever been isolated.

On several occasions we were fortunate enough to get sections which clearly showed the entry of the fungus into the rootlets from the outside (Plate I). At each point of infection, entrance to the host tissues is effected by a single hypha, the fungus being stimulated and attracted to the roots by root secretions. There is no trace of the "cellules de passage" mentioned by Janse (22), the "Durchlasszellen" of Burgeff (12); nor does penetration ever occur through the root hairs. Penetration, we have always noted to be direct, the fungus entering the exodermis itself at no specialised point. The thin outer walls of this layer are uncutinised, or only slightly cutinised, and offer practically no resistance to the path of the fungus.

Once the fungus has entered the roots, its connection with the extra-radical mycelium is lost. There is no possibility, therefore, of any interchange of nutritive materials between the mycelium within the host tissues and the outside. Such fragments of the external mycelium as can be observed are of a brownish colour, more or less void of contents and with cutinised walls. In the first layer of cells of the invaded tissue, the internal mycelium was often brown, after which it becomes thin-walled and hyaline, and has highly granular contents.

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*Picro-Formol is made up as follows :—

Formalin, 40 per cent., ...	30 ccs.
Picric Acid (sat. sol. in water), ...	20 ccs.
Glacial Acetic Acid, ...	5 ccs.

†One advantage of the use of Flemming's solution for fixation is that it blackens the infected places more quickly and more deeply than the uninfected regions, so aiding in selection of material for sectioning.

The cortex of the strawberry root consists usually of seven or eight layers of cells. In the first three or four layers, the cells are polygonal in transverse section and fit together closely, so that there are no intercellular spaces. This might be said to constitute the outer cortex of the root. In the remaining portion, or inner cortex, on the contrary, the cells are more or less cylindrical, and numerous large intercellular spaces are present, triangular or quadrangular according as they are formed by the boundary walls of three or four cells.

The entering mycelium radially traverses the cells of the outer cortex without let or hindrance, boring its way through any cell walls that come in its path, and giving off one or more branches. In the outer cortex, the mycelium is definitely intracellular. On reaching the inner cortex, the mycelium makes use of the intercellular spaces for its further progression and becomes thereafter almost exclusively intercellular. The inner cortex is the zone of longitudinal infection—the intercellular spaces offering ideal conduits for the passage of the advancing hyphae. Longitudinal infection does not usually extend beyond a total distance of 5 to 6 mms. from the original point of infection. If, as commonly happens, many points of infection occur on one rootlet, the resulting areas of infection may overlap; so that, in badly diseased roots, infection may be continued over a considerable distance. Points of infection may be grouped, and it is common to find isolated and localised areas of intense infection. As Gallaud (19) describes in *Arum maculatum*, and as we find in the strawberry, it is at such centres that the roots are more swollen than usual and exceptionally brittle. This variation in the distribution of mycelium on the rootlets explains why section after section may be examined in which no fungus can be found, and that too, on a rootlet which, in other portions of its length, may show intense infection.

In addition to variation in points of infection and longitudinal spread, the mycelium branches profusely, and extends inwards, until its progress is arrested by the endodermis, the greatly thickened walls of which offer a sound barrier to its passage. Lateral extension, however, may continue until the stele is completely surrounded.

The diameter of the entering mycelium tends to decrease as it advances towards the inner cortex; but with the formation of arbuscules in this zone, the mycelium becomes more robust, and branches irregularly and more freely, so that as many as five hyphal branches may occupy a single large intercellular space. In the smaller intercellular spaces, the hyphae mould themselves closely to the cell walls, filling in all interstices and assuming the shape of the intercellular spaces themselves. In general, the mycelium is irregularly cylindrical, varying in diameter from 6 to 14μ.

While, in the outer cortex, the cell walls are directly penetrated by the entering hyphae, this method cannot be regarded as general. In the inner cortex, the advancing hypha crosses usually from one intercellular space to another by boring its way through the middle lamella. If, as often happens.

hyphae run parallel in two adjacent intercellular spaces, traversing branches may connect these together. This anastomosis is of frequent occurrence, and bridge connections are commonly established where the parallel hyphae are moulded closely on both sides to the angles formed by the ends of abutting cells. The crossing here is easily accomplished, since there is only a relatively small amount of radial wall between the hyphae.

Septa are seldom found, and are not constant in any part, being quite irregular in distribution. We have never found them in young, actively-growing hyphae, and it would seem that they are only formed to separate living from dead and abandoned parts.

We sometimes observed portions of mycelium which did not appear to be dead, since they were well supplied with contents. These had, however, thicker walls than usual, a yellowish colour, and a more highly refractive appearance. Such portions were found to occur only in older tissues, especially where vésicule formation was abundant. It is possible that they represent the encysted condition of mycelium described by Magrou (26).

No intensive study of the cytological details of the fungus has been made, but it was noted that the mycelium was multinucleate.

The Arbuscules.—On reaching the inner cortex, the mycelium forms the characteristic structures known as " arbuscules " (Plate I; and Plate II, fig. 2). As already stated, the arbuscules, in spite of their size and well-marked peculiarities, may readily escape observation. Their somewhat regular distribution in the innermost layers of the cortex, and the fact that, in unstained preparations, they show up as indistinct greyish, granular masses, more or less completely filling the cells, may lead the observer to suppose that here he is concerned with the normal contents of cells richly laden with reserve materials. Yet it is somewhat strange, that many of the early workers on mycorrhiza failed to recognise the presence of organs so distinctive. Certainly Schlicht, 1888, and Janse, 1897, observed them, but did not consider them to be important. It was not until 1905 that their full significance was definitely established by Gallaud (19), who gives an excellent account of their structure and function. Arbuscules are commonly present on the finer strawberry roots, and are at once rendered distinct by any ordinary staining method.

Where arbuscules are about to be formed, short lateral branches are produced at intervals on all sides from the hyphae in the intercellular spaces. These penetrate the cells at right angles and, once within, branch profusely in an irregularly dichotomous fashion into finer and still finer ramifications (Plate IV, fig. 3). Only the coarser of these branches are evident, the very fine ramifications being so intimately associated with the protoplasm of the cell, and so interlaced, that they appear as a bunch-like mass borne at the end of a short trunk (Plate III, fig. 1). To these organs, on account of their tree-like appearance, Gallaud gave the name " arbuscules." The finer details of the arbuscules can only be distinguished under very high magnification and with careful technique. Arbuscules are invariably terminal, and borne on these short lateral branches. They are always intracellular and, where formed, terminate the growth of the hyphae. The mycelium from which the branches arise, however, is capable of continued growth in the intercellular spaces. The intensive ramification of the arbuscules, giving an enormous surface area in proportion to bulk, their intimate contact with the cell protoplasm, and the extreme fineness of their cell walls, enable the arbuscules to act as highly efficient suctorial organs. Arbuscules, therefore, are to be regarded as true haustoria designed to absorb nutritive material from the host cells. True arbuscules only are met with in endotrophic mycorrhizas.

Starch and other substances, are removed from the host cells by the arbuscules. It would seem that a diastatic enzyme, at least, is produced by the fungus, since there is a pronounced disappearance of starch from the invaded cells. Rarely can any starch be detected in cells bearing arbuscules (Plate VI). Actual invasion, however, does not seem to be essential to this process, and cells are found to be depleted of their contents where they border on intercellular spaces bearing mycelia. In this connection, the almost perfect moulding of the hyphae to the cell walls permits a ready diffusion of enzymes into the host cells, and the subsequent withdrawal of the dissolved materials by the fungus. The zone of influence appears to extend beyond even the actual area of infection, as dissolution of starch can be observed several cells in advance of the leading mycelium. These characters are well shown on Plate VI.

According to the species of host plant, and the type of endotrophic mycorrhiza present, the arbuscules have a long or a short life. Sooner or later the arbuscules undergo a transformation. The first evidence of this is shown by the assumption on the part of the arbuscules of a rounded or more compact appearance (Plate III, fig. 2). The individual arbuscule loses its characteristic flocculence, becomes granular, and contracts considerably, while the hypha which bears it becomes empty, and is often cut off by a transverse wall from the living mycelium. Finally, the arbuscule takes on a somewhat lobed appearance and at this stage it may be regarded as dead (Plate III, fig. 3). These bodies constitute what Janse (22) termed "sporangioles," and Petri (34), "prosporoidi." Gallaud (19) preferred the former designation, but showed them to be what they really are, partially transformed arbuscules.

The transformation of the arbuscules to this stage takes place as we have outlined, and it is generally agreed that there is a reaction on the part of the plant which is directed against the invading organism. This reaction represents the mechanism of defence. Opinions vary with regard to the precise method by which the transformation is brought about; but for convenience in subsequent remarks we shall use the word "digestion" to characterise this process.

As far as we have observed, the arbuscules are never digested further than the sporangiole stage in the roots of the strawberry plant. Various observers have shown, in other plants, a more or less complete disappearance of the arbuscules from the cells. M'Lennan (32), for example, working on the root mycorrhiza of *Lolium temulentum*, showed that the lobed masses of the sporangioles ultimately burst, liberating in the host cell a supply of fat or oil, which she claims the plant uses to its advantage.

Some workers on other types of mycorrhizas mention the recovery of the invaded cells after the disappearance of the arbuscules and the mycelium. Magnus (25), on *Neottia*, notes that after the formation of sporangioles the invaded cells return to their normal life, having killed and digested the fungus. He indicates that, coincidently, there is a return of starch to the cells. Shibata (40), confirmed by Petri (34), has shown in *Podocarpus* and *Psilotum*, that after the fungus has been digested, the cells resume their normal activities. *With the strawberry plant and its endophyte, on the other hand, we have never observed any return of starch to cells when once depleted of their contents.* This could not occur in the strawberry, since sporangioles and the host cells die at this stage. It is important to note that the nuclear changes observed by Magnus (25) to take place in the host cells, and thought by him to be signs of increasing activity accompanying the digestive process, were regarded by Bernard as degenerative changes. According to Bernard (4), in the struggle between the parasite and the invaded cell both adversaries finally succumb :—" Dans la lutte entre le parasite et la cellule qu'il envahit, les deux adversaires ont fini par succomber."

The Vésicules.—According to Gallaud (19), the "vésicules," so named by Janse (22), constitute a normal organ of this type of endophyte.

Vésicules arise usually as terminal swellings on hyphae in the intercellular spaces, although in some plants they may be both intra- and intercellular (Plate IV, fig. 2). The tip of a hypha about to form a vésicule swells out at the end and becomes filled with a dense homogeneous mass of multinucleate protoplasm. Later it becomes vacuolated and filled with reserve materials. especially oil (Plate IV, fig. 4a). When mature, the vésicules are usually oval or rounded in shape, and vary greatly in size (Plate IV, fig. 1). In the strawberry plant they measure commonly 80 to 130μ by 40 to 70μ. They are provided with a double wall : a thick external one, somewhat brown in colour, and a thin inner membrane (Plate IV, fig. 4,a). Often the vésicule becomes separated by a septum from the hypha on which it is borne.

The mature vésicule is generally regarded as a resting body, but it may be only of a temporary nature, as a vésicule may quickly recommence growth by the production of a hypha which, in its turn, soon swells out at its end to form a second vésicule. This may take place before the first-formed vésicule has been cut off from the living mycelium, in which case both vésicules may have contents. Frequently, when the primary vésicule has been cut off by a transverse wall from the hypha on which it is borne, the second one is formed at the expense of the first which becomes emptied of its contents (Plate IV, fig. 1). Owing to the shape of the intercellular space, the hypha arising from the primary germinating vésicule is almost invariably produced at the rounded distal end. The process may continue, and the second vésicule give rise to a third, and so on; so that a vésicule may appear to be intercalary in origin (Plate V, fig. 1). A chain of vésicules may thus be formed, the individuals of which may be closely grouped, or spaced (Plate V, fig. 2). Vésicules, as a rule, only arise after the fungus has reached its maximum development, at which time the mycelium is well supplied with nutrient materials. They therefore become more numerous as the season advances. The vésicules vary in abundance in different host plants, being common on some, and very rare on others. On the strawberry plant, so far as our observations extended, we found vésicules to be comparatively rare in Scotland and relatively frequent in England. Climatic conditions, especially rainfall, seem to have some influence upon their development.

We have evidence to show that the production of vésicules varies also with variety of strawberry. We found them particularly abundant on the variety "Duke" grown in England.

Vésicules are generally regarded as resting cysts which fall into the soil with the decaying roots, and it is held that, when suitable conditions arise, they can germinate and infect new plants. No one, however, has been able to observe germination of the vésicules outside the plant. Since they proliferate so freely inside the plant tissues, they certainly function as temporary storage organs; but it is probable that those with thicker walls can serve to tide the fungus over the winter. Peyronel (35) believes that the vésicule is a form of sporangium, and records the presence of spores in vésicules of the endophyte of wheat.

AFFINITIES.

With regard to the affinities of this fungus, there is no reliable evidence, morphological or cytological, which might enable one to refer either this, or any similar mycorrhiza, to a definite fungal group. Bernard (4, 5, 6) has been successful in obtaining pure cultures of the orchid type of mycorrhiza, and these he has placed in the genus *Rhizoctonia*. Constantin and Dufour (14), Ramsbottom (37), Peyronel (36), and Huber (21) confirmed and extended Bernard's work. Burgeff (11) also isolated the same fungi as Bernard, but gave them the generic name *Orcheomyces*. Rayner (38) isolated a *Phoma* species from *Calluna vulgaris*. It may be accepted as definitely established that endophytes of the Orchid, and some few special types, *e.g.*, *Phoma*, can easily be grown on pure culture on the usual media. With regard to the type bearing arbuscules and vésicules, all attempts to grow them artificially have been unsuccessful.

There are, however, certain characters which suggest the possible affinities of these organisms. According to Janse, Petri and Gallaud, the two types of mycorrhizas, the Orchid type and the type with arbuscules and vésicules, belong to one group. The endophytes of Orchids, Peyronel states, have no affinity with the true endophytes, but belong without doubt to the *Basidiomycetes* and to the *Mycomycetes* (36).

Some evidence as to the taxonomic position of the present type of mycorrhiza may be gathered from a consideration of the mycelial characteristics, the arbuscules and the vésicules.

Considering the mycelium, the irregular distribution of the septa, and their somewhat rare occurrence, gives little aid in the classification of the fungus. Septation appears to occur only for the purpose of isolating the protoplasm of living hyphae from dead regions. Gallaud (19) considers this analogous to the false septa of Mucors, and regards the arbuscules as being similar to the "crampons" of the *Mucorineae*, though the latter differ in mode of formation. Nothing can be gleaned as to the possible affinities from the mode of branching of the mycelium, which is quite irregular.

Peyronel (35) says that the Mucors, especially the Mortierellas, have a mycelium which shows striking analogies to the mycelium of this type of endophyte; the sporangia, he states, are altogether different.

It is to the vésicules that we must turn for guidance. If, Payronel (35) says, sex organs were present in this type of endophyte in addition to the vésicules, the fungus would be placed among the *Oomycetes* in the proteoid family of *Saprolegineae*, or *Peronosporiaceae sensu lato*, beside *Pythium*, *Blepharospora* and *Phytophthora*. Bruchmann (9) thinks that the endophyte of *Lycopodium annotinum* is a *Pythium*, and regards the vésicules as oogonia. Goebel (20), on *Lycopodium inundatum*, believes them to be chlamydospores (Dauerconidien) of a *Pythium*. Bernatsky (8) says that the vésicules of *Psilotum* are sporangia arrested in their development, and identified them with the sporangia obtained by him from a *Hyphomyces* isolated from *Psilotum*. Peyronel (35) records the occurrence of spores from vésicules obtained from wheat and maize, and expresses the conviction that vésicules represent true sporangia. He only hesitates to classify these fungi among the *Phycomycetes* because of their strong resemblance to the genus *Endogone*;* the sporangia of *Endogone macrocarpa* being remarkably like the vésicules produced by many mycorrhizas.

Peyronel (35) thinks that the endophytic mycorrhiza of this type and the Endogones originate from a primitive type of very variable fungi from which are derived two divergent series, the *Phycomycetes* and the *Mycomycetes*.

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*The genus *Endogone* is a group of doubtful affinity placed differently with the *Ascomycetes* (*Gasterales* and *Tuberales*); with the *Oomycetes* by Baccarini (1), and in a group intermediate between the *Oomycetes* and the *Zygomycetes* by Bucholtz (10) more recent authorities regard them as true *Zygomycetes*.

THE STRAWBERRY ENDOPHYTE AS A PARASITE.

From the foregoing statements it will be apparent that *we regard this endotrophic mycorrhiza as a definite parasite, and believe it to be the fundamental cause of the trouble known as "root weakness" in strawberries,* at present so widespread in this and in other countries. We have come to this conclusion on the evidence we now adduce.

HISTORICAL EVIDENCE.—MYCORRHIZAS IN GENERAL.

The term "mycorrhiza," to many mycologists and students of plant disease, has come to imply simply a state of symbiosis or mutualism. That this is so, is largely due to the influence of Frank, who, as far back as 1885, first postulated this theory to explain the relationship that exists between such fungi and their host plants. Frank's work, while very valuable, has received undue prominence; with the result that, as Rayner (39) says, "The scanty space assigned to Mycorrhiza in botanical text-books is occupied chiefly with the results of his observations and with speculations upon their significance." Since Frank's time, our knowledge of this subject has been greatly extended, and his conceptions have been materially modified. The whole situation is aptly summed up by Rayner (38): "It is evident, from a consideration of Mycorrhiza in general, that it is still impossible to frame a definition that will include all known cases. The theories of earlier observers implied a strict symbiosis with reciprocal advantages of an obvious kind. Later workers tend rather to regard the relationship as primarily one of parasitism on the part of the fungus, tolerated and often turned to account by the plant, or even becoming indispensable to it."

The whole theory of symbiosis or mutualism, with regard to mycorrhiza, rests on the assumption that the invading organism, whatever benefits it gained from its host plant in the early stages of its life-history, is finally and completely digested by the action of the host cells upon it, and the digested products utilised by the plant. In true symbiosis there is a living together to mutual advantage; the fungus benefiting by the carbohydrates and other materials extracted from the host cells, while the plant, by the ultimate digestion of the fungus it nourished, gains in return all that the fungus originally extracted from it, plus such food material as the latter was able to obtain on its own account.

Cases of true symbiosis between the plant and its mycorrhiza undoubtedly do occur, notably in certain chlorophyll-free plants where the presence of the endophyte is even indispensable to the host. In the ordinary case of ectotrophic mycorrhiza again, many examples can be cited to show that the host plant benefits by the metabolic exchange which takes place. This can be readily understood, since the fungus has not only an internal, but an external mycelium, by virtue of which it may be able to obtain from its surroundings, and to pass on to its host, various mineral substances valuable to the plant.

Where endotrophic mycorrhiza of the type bearing arbuscules and vésicules is concerned, the amount by which the host plant benefits depends upon the extent to which digestion of the endophyte takes place, and upon the inter-relations of host and fungus. As we shall see from a review of the opinion of the leading modern authorities on this subject, it is possible to find in different plants all degrees of infection, varying from pure symbiosis to pure parasitism.

According to Magrou (26), only the arbuscules may be destroyed by digestion, and the mycelium live on and continue to infect other cells. The mycelium again, may form encysted filaments which are still more resistant and which help to perpetuate the fungus. The same observer classifies the digestive action as being either, (1) rapid and energetic, and so protecting the plant against invasion; (2) late in commencing, and partial and slow, so that digestion does not stop the progress of the fungus, which becomes established; and finally, (3) there may be no reaction at all on the part of the plant, in which case infection is mortal.

Endotrophic mycorrhizas are usually confined to the cortex of roots, but Magrou shows the possibility of their infecting the lignified vascular bundles and so causing the death of the host plant.*

Melin (33), working on both ectotrophic and endotrophic mycorrhizas, classifies them according as they are active, less active and inactive; with, in each class, forms of low, medium and relatively high virulence. At the present day it is recognised that there is no sharp distinction between ectotrophic and endotrophic mycorrhizas.

Bernard (7) described the state of symbiosis in these words:—" L'état de symbiose, est en quelque sorte un état de maladie grave et prolongée intermediare entre l'état des plantes atteintes d'une maladie rapidement mortelle et celui des plantes qui jouissent d'une immunité complète." The classical researches of Bernard have done more to illuminate this obscure subject than those of any other worker and, in view of his many and great contributions, his conclusions cannot be lightly passed over. To him the mycorrhizas of Orchids were parasites which attacked the young plant on germination, persisting in the adult throughout life as a chronic condition of disease (4). The presence of mycorrhiza in root tissues represented to him a pathological condition (7).

According to MacDougall (30), some of the endotrophic mycorrhizas on maple trees were beneficial to their hosts, while others acted as internal parasites of the roots. The ectotrophic mycorrhiza of the majority of trees he treats as cases of parasitic attack on the roots, though probably not very harmful (31).

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*In the strawberry plant, we cannot show that the central cylinder is ever invaded by its endophyte; but in the stele of dead rootlets examined during the winter and spring months, in material obtained from the Clyde Valley, we have often found very abundant oval, cyst-like bodies with thick brownish walls, closely resembling vésicules, attached to dead and degenerated hyphae. These bodies are somewhat smaller than the vésicules found in the cortex, measuring only 40 to 60 μ by 20 to 30 μ.

Koki Masui (29) agrees with MacDougall that the relation is one of parasitism, and regards the mycorrhiza of the conifer *Abies firma* as a parasite which acted on the host in such a way as to reduce the rate of growth, and which sometimes had fatal results.

In the case of mycorrhiza on trees, of which most information is available, infection is regarded as definitely parasitic by Gibelli, 1883; Henschel, 1887; Sarauw, 1893; Möller, 1903; Pastana, 1907; Ducomet, 1909; Fuchs, 1911; Weyland, 1912; MacDougall, 1922; and Koki Masui, 1926. On the other hand, Frank, 1892; Stahl, 1900; Müller, 1903; Tubeuf, 1903; Elenkin, 1907, represent the case for symbiosis. There is, therefore, no preponderance of opinion on one side or the other. In all probability the true state of affairs is to be found in the view taken by Melin (33) :—" With regard to the maintenance of a balance of power between the mycorrhizal fungi and their hosts, it is clear . . . that the development of normal mycorrhiza is bound up with and directly depends upon, the physiological state existing in each of the two symbionts, the health and vigour of the seedlings on the one hand, and the conditions of the mycelium on the other each playing an important role . . . Given an unhealthy condition of the latter (*i.e.*, the roots) and mycelium of high 'virulence' the relation may degenerate into one-sided parasitism with the fungus in command of the situation. Assuming healthy root condition, the development of normal mycorrhiza depends upon these physiological qualities in the mycelium which constitute 'virulence' (39)."

It will be seen that, in all types of mycorrhizal infection, cases of parasitism have been definitely established. The tendency of the more modern workers is to bring these fungi within the domain of the Plant Pathologist.

MYCOLOGICAL EVIDENCE.

On considering the mycological evidence bearing on the strawberry endophyte, it is manifest that all the known facts go to support the view that this mycorrhizal organism is of a parasitic nature.

The general occurrence of this mycorrhiza on cultivated strawberries has already been noted (p. 9). It is found in the living tissues of the finer roots of its host plant. The cortex of the invaded plant is depleted of its contents, proof of this being found in the demonstrable disappearance of starch from cells within the zone of influence of the fungus. The arbuscules, which act as haustorial organs, do not appear to be digested beyond the sporangiole stage, by which time the parasitised cells are undoubtedly dead. There is no return of starch to cells once depleted of their contents; and the constant drain on the resources of the plants by the withdrawal of nutrient material seriously inhibits growth, especially of the roots.

It has been shown by Gallaud (19), *for Arum maculatum,* and by us on the strawberry plant, that the *swollen portions of the root, marking points of abundant infection, were of a brittle nature. At such points the root fibres* *ruptured and dropped off into the soil* :—" Enfin la turgescence est plus grande et les racines se rompent plus facilement aux endroits où les champignons sont plus nombreux ". When the rupture takes place at some distance from the apex, a considerable length of root may be lost. If, as often happens, a strongly infected portion occurs but a short distance from the root tip, the break will take place at this point. The starved rootlets may continue to produce short lateral branches which in their turn may be attacked. The roots of infected plants, therefore, have a somewhat irregular appearance, being in some portions profusely covered with stunted fibres, and in others practically bare. Infected plants are thus deprived to a greater or less extent of their finer root fibres. *To this we ascribe the poverty of absorbing roots noted by all workers on strawberry plants suffering from root weakness.*

The strawberry endophyte would take its place in Melin's scheme (p. 20) as being active and of high virulence, while digestive reaction on the part of the plant must be referred to the second category of Magrou's scheme (p. 19), since digestion in the strawberry is late in commencing and partial and slow in its action. Considering the balance of power between fungus and host, it is clear that the relation tends to be one of parasitism.

If strongly infected in the early stages of growth, plants are liable to succumb; but, once established, the danger is not so great, and plants may linger on in a more or less chronic state of disease until they too finally die.

The unhealthy condition of the roots due to the presence of this mycorrhizal organism may be further aggravated by adverse environmental conditions. Thus, *according as the mycorrhizal attack is relatively severe or slight, and as conditions are unfavourable or favourable to the growth of the strawberry plant, so is the ultimate damage greater or less.* Given severe infestation, and decidedly bad conditions when the finer absorbing roots are being produced, the damage wrought may be so severe as to cause devastation of whole fields. All degrees of parasitism may occur, each capable of explanation in the light of local soil and climatic conditions. Undue wetness or dryness, too heavy or too infertile a soil, high acidity or alkalinity, bad cultivation, too high or too low a temperature, or any soil or climatic factor tending to reduce the vigour of the plant, will serve to aggravate the trouble, especially when it favours the development of the mycorrhiza. Peyronel (36) mentions that in soils constantly impregnated with water, due to heavy rainfall, the mycelium absorbs nutritive elements in great abundance and the hyphae last longer in a more perfect condition and attain great dimensions. In drier soils the mycelium is generally, but not always, less developed. Where wet conditions prevail, therefore, one would expect a more pronounced invasion of the tissues than would be the case in drier conditions; while the plant, owing to the persistence of the mycelium in an active state, would suffer more. This would account for the occurrence of patches of intense infection in the wetter parts of the field.

We have already referred to the comparative constancy with which fungi of various kinds may be cultured out from the roots of strawberry plants suffering from root weakness. We concluded that these organisms were secondary in their origin and effect. *The only constant organism found by us in the living tissues of unhealthy plants was the endotrophic mycorrhiza which we have just described.* In rootlets which had been attacked by this fungus, however, we frequently noted the presence in the weakened tissues of organisms which were undoubtedly of a secondary nature. Where the endophyte was absent and the roots uninjured, or where the rootlets were but recently invaded, we never found these fungi on plants which otherwise lived in a healthy environment. Certain environmental conditions, such as wetness, favour not only the development of the mycorrhiza, but promote at the same time an excessive development of soil fungi which may parasitise the declining plants. Melin (33) notes that conifers infected with mycorrhiza may be subsequently invaded by secondary fungi under certain soil conditions. Peyronel (36) finds that in addition to the endotrophic mycorrhiza discovered in the plants studied by him, other fungi were common in the dying or dead infected roots. These belonged principally to the genera *Pythium*, *Fusarium*, *Didymopsis*, and *Rhizomyxa*. Other workers record also *Mortierella*, *Mucor*, *Trichoderma*, *Cephalosporium*, and *Alternaria* in similar circumstances. Constantin (13) mentions the invasion of the roots of sugar cane by bacteria and parasitic fungi following attack by mycorrhiza. Most frequently isolated by us were *Cephalosporium*, *Pythium*, *Fusarium*, and *Rhizoctonia*. *The fact that this endotrophic mycorrhiza paves the way for the entry of secondary fungi and bacteria explains why so many observers have from time to time cultured out such organisms and erroneously attributed the disease to them.*

Since our attempts to obtain the endophyte in pure culture were not successful, we were unable to conduct direct inoculation experiments and so prove the pathogenicity of this fungus. Several preliminary experiments, while not conclusive on this point, yielded interesting results.

After a simple experiment showing that infection did not pass to the runners through the stolon, young strawberry runners were allowed to strike root in pots of sterilised soil. Some of these were inoculated in the autumn of the past year (1927) with root tips from diseased plants. Control pots were kept. The root portions selected for inoculation were chosen with great care, those only being taken which showed the slight swelling indicating the presence of mycorrhiza, but which were unblemished externally. The selected portions were then sectioned and examined microscopically. Those which showed the presence of the mycorrhiza in a young and active condition, and no sign of any secondary organism, were used for inoculation. These portions were then buried about two inches deep in the soil surrounding the young runner in the experimental pot. It is too early as yet to reach any definite conclusion on the results of this experiment, as the symptoms of the trouble do not appear until spring at the earliest; but when some of the plants were examined in December, 1927, those that had been inoculated showed a marked deficiency of absorbing roots when compared with the control plants. Further, the rootlets of the plants from the inoculated pots were found, on microscopic examination, to have become infected with the endophyte; whereas those of the control pots were free. There was no evidence of any secondary fungi in either case.

Infected runners taken from the field and potted up were noted to recover and to become almost normal when grown in the greenhouse. Under such ideal conditions, root development, for some time at least, more than keeps pace with root destruction by the mycorrhiza; but these plants too decline as the mycorrhiza eventually gains the upper hand and, usually in about one year's time, the plants again show decided symptoms of disease.

FIELD EVIDENCE.

From a consideration of the life history of the strawberry plant along with that of its endophyte, many obscure points can be explained away.

Late in March, or early in April, the strawberry plant begins active growth, and for the next three months, coincident with the production of foliage, fine fibrous roots are developed on the primary roots formed in the previous autumn. The strawberry endophyte invades these absorbing roots as they are produced. *The critical period for the plant is from May to June, when flowering takes place and subsequently fruit formation, since at this stage the maximum strain is put upon the resources of the plant. By this time mycorrhizic invasion is at its height, and the plant begins to lose its rootlets in the manner we have described.* As would be expected, plants at this period become semi-starved, owing to the loss of their absorbing roots, and are unable to produce and ripen fruit to their full capacity. Where the loss of rootlets is severe, plants may be quite barren of fruit and die of starvation.

Ball and Mann (3) state that "From the middle of March to the middle of May there is a gradual decrease in the weight of the roots. Since the appearance of numerous new roots of the fine 'feeding' type was recorded over this period this result at first sight seems surprising." If the reader refers to the extract from their Table, reproduced below, he will find that if the weight given for the 28th May is excluded as being exceptional, then the decrease may be held to continue till the end of July, after which there is a rapid increase in root formation.

TOTAL DRY WEIGHT OF THE ROOTS OF TEN PLANTS (*in grams?*).

Date.	Weight.
March 16th,	15·31
April 20th,	13·9
May 11th,	13·03
May 28th,	15·41
June 26th,	13·79
July 20th,	19·39
August 10th,	29·08

We are not in a position to know whether the plants selected by Mann and Ball in their experiments were free from mycorrhizic invasion. In all probability they were not, although certainly they could have been only mildly attacked. Presuming that this latter is the case, *the gradual decrease in root weight found by them to occur at the time of new root formation is not surprising. By this stage, many of the new and invaded roots would have dropped off into the soil.*

The increase in root weight, noted as commencing in July, corresponds to a period of extensive root growth during which new primary roots and abundant lateral roots are formed. At this period, mycorrhizic invasion cannot keep pace with the rapid growth of the roots, and infected plants surviving recover to a large extent. Jones (23), working on a mycorrhizal fungus on the roots of legumes, mentions that on clover in midsummer, great numbers of rootlets in the upper foot of soil die, leaving the plants with a greatly reduced root system. Alfalfa also was found to suffer heavily, and had a "surprisingly meagre number of living root ends possessing root hairs which can absorb water in the upper eight inches of soil." Root development came on again in late summer and the infecting fungus was left behind, root growth progressing more rapidly than the fungus invasion.

Ball and Mann further state (3) that "during the early stages of growth up to the opening of flowers, there is a very marked disappearance of food materials, especially starch, from certain tissues of the roots." This disappearance of starch, while perhaps due in part to its dissolution and translocation in the form of soluble sugars to growing regions, may very probably owe its removal also to the action of mycorrhiza.

The studies on the influence of some cultural practices on the normal development of the strawberry plant, undertaken by Ball and Mann (3), yield some interesting results, especially in regard to the practice of root trimming. The procedure adopted was to cut off one-third to one-half of the runner roots before planting out. The resulting plants showed a greater abundance of stronger primary roots better furnished with laterals. "The bulk of the root system was equal to, if not greater than, that of a normal plant whose crown was of the same size—hence the volume of new roots was greater, the increase being both in laterals and in new primaries with their laterals." A year later, the plants were in no way superior in vigour to those the roots of which had not been trimmed. This practice of root trimming has been extensively carried out in the Clyde Valley and elsewhere with good results. To us the explanation is clear. The primary roots of young runners, as well as the finer fibres, are usually infested by mycorrhiza, particularly in the region of their tips. It follows, therefore, that *when roots are trimmed the greater part of the infection is removed with almost immediate benefit to the plants when set out in the field.* A limited amount of infection is almost certain to be left on the plants with the result that, a year later, owing to fresh invasion, the good effects of the process are lost.

The results obtained by Ball and Mann on the effects of early and late planting are easily explained by the mycorrhizal hypothesis. According to these authors (2), "the longer the runners remain *in situ* the less vigorous will be the resulting plants." "Progressively smaller crops were obtained from the later dates of planting, but the most remarkable difference was between the runners planted in August and September, the former cropping over three times more heavily than the latter." But, at the same time, "runners planted in late October were in no way superior to runners set out in the spring when plants of the two series were compared in May. Furthermore, the plants of both series were not sufficiently vigorous to carry a crop." Commenting on their findings, these authors attribute the results obtained to: (1) the crowded condition of the parent plants, and the runners in the strawberry bed retarding the growth of the latter; and (2) the damage to the developing root system when the plants are eventually removed; the resulting injury being greater, the longer the runners remain in the old bed. This explanation seems to be inadequate, especially as, according to them, root trimming which deprives the plant of one-third to one-half of its roots exercises an immediately beneficial effect! We have found mycorrhiza to be generally present on strawberries and to have an injurious effect on them, most marked in the runner stage of development. *The longer the runners remain in the neighbourhood of their parents, the longer they are exposed to infection and the more heavily will they be invaded. Thus, runners planted out late cannot be expected to give rise to vigorous plants. By early planting, however, the runners are removed earlier from their parents, are less infected, and will produce much healthier plants.* It is obvious that young runners taken away in August will be almost free from infection; whereas those removed in September will have become strongly infected, as the mycelium at that time is in a particularly vigorous condition and favoured by the warm, moist autumn soil. By the month of October, colder conditions prevailing, the mycorrhiza ceases to be active and remains more or less dormant until spring. Runners planted out in Octobers, therefore, would not be in any way superior to those that remained *in situ* and were planted out in spring.

It should be noted that, in the Clyde Valley, runners are almost invariably planted out in the spring, and hence have been exposed to infection from the parent plants for the maximum period. When planted out, therefore, the runners are already strongly infected, and the disease in consequence generally makes itself evident in one year old plants. In England, where autumn planting is practised, runners are more mildly infected, and the disease is not generally apparent until the second, or even third year, according to time of planting and degree of initial infection.

The seriousness of the disease depends not only upon the time of planting, but also upon the system of planting adopted. The method of planting out strawberries in the field differs markedly in Scotland and in England. In Scotland, the bed system is almost exclusively adopted; whereas, in England, the runners are set out in drills. *There is no doubt that the Scottish practice of planting out runners in beds is one other factor accounting for the greater severity of the disease in that country.* Under this system, runners are planted closer together in the rows than they are in England; there is, therefore, greater opportunity for infection of young runners from their parents. All cultivation in the bed, again, is purely superficial and done by hand—the paths only being horse-hoed—with the result that the mycorrhiza is left practically undisturbed and under ideal conditions for its development. In England, on the other hand, where the drill system is practised, the plants are more widely spaced and the soil is kept constantly stirred by horse-hoeing. On this account, conditions are less favourable for the growth and development of the mycorrhiza. Peyronel (36) finds mycorrhizic infection is most abundant where there exists tangled masses of roots, especially in such places as are never disturbed by cultivation. *The strawberry mycorrhiza, therefore, would probably reach its maximum development under sward conditions. This would explain the well-known fact that strawberries frequently fail when planted out on ground newly broken from old pastures which perhaps have never been under strawberries before.*

It does not follow that because the endophyte is parasitic on strawberries that it will act similarly on other plants. Bernard (5, 6, 7) has shown in the Orchid type of mycorrhiza that there are varying degrees of specificity between fungus and host, and instances definite cases of this. Thus, seeds of *Phalaenopsis* were inoculated with pure cultures of the mycorrhizas obtained from *Cattleya, Phalaenopsis* and *Odontoglossum* respectively. The strain from *Cattleya* parasitised and killed the seed. There was no reaction by the host cells. That from *Phalaenopsis* itself produced seedling infection, but infection was controlled by the digestive action of the embryo. The mycorrhiza from *Odontoglossum*, on the other hand, infected the seedling, but the digestive reaction on the part of the plant was excessive. Burgeff (11) and Knudson (24) confirmed and enlarged certain aspects of this work.

Although the work was carried out with the Orchid type of mycorrhiza, it is possible that a similar specificity might obtain in the case of the endophytes bearing arbuscules and vésicules. The strawberry endophyte, therefore, might either be truly specific, or non-specific. If the latter is the case, then there opens up the interesting problem of a variation in virulence of the mycorrhiza on the strawberry plant itself according to the source from which it was derived.

CONTROL.

In the present state of our knowledge, it is difficult to indicate effective control measures for this trouble. Remembering that the disease is ever present and is greatly aggravated when environmental conditions are unfavourable to the strawberry plant, it is evident that any control measure must first aim at modifying the influence of any adverse local factors.

The more important of these factors have already been enumerated. Anything which will promote rapid and good root development will serve to hold the disease in check and to prevent its assuming a serious aspect.

As regards manuring, it is well known that phosphates encourage the development of roots, especially the fine absorbing fibres; on that account phosphates should form the chief constituent of any manurial dressing applied to strawberries. On the other hand, nitrogenous manures, which favour the development of foliage, and which in excess tend to lower the vitality of the plant, should be used with caution. In this connection, the manuring of strawberries with farmyard manure, while essential for successful growth of the crop, should not be overdone, and the quantity applied regulated chiefly by the type of soil.

Good cultivation is essential both before and after planting of runners, since it reduces the amount of mycorrhiza in the soil and at the same time encourages the development of the roots.

Wet conditions favour the development of the mycorrhiza, and have a weakening influence on the plant itself. Drainage, therefore, should be efficient in all cases. The growing of the plants in drills improves the soil in this respect and undoubtedly is preferable to the bed system. The crop can be horse-hoed more effectively in the critical early stages and this, in addition to improving conditions for root growth, keeps down weeds which might harbour the mycorrhizal organism.

When planting out runners, certain definite precautions should be observed. Runners for planting out should be obtained only from districts where they are formed relatively early, and where the soil is of a free, open nature such as promotes a vigorous root growth. The older the plant, the greater will be the amount of infection around it, and the greater will be the danger of the runners being heavily invaded by the fungus. For this reason, runners should always be selected from young mother plants, and early planting should be adopted wherever possible.

Root trimming should be carried out, especially in districts where late or spring planting is necessitated, for reasons already stated.

It is not advisable to try to secure a crop in the first year. If deflowering is practised at this time, the economy to the plant will result in more prolific root formation, with subsequent benefit in the second year. During this period, any plants showing pronounced symptoms of the disease should be carefully removed and destroyed by burning.

The close cropping of strawberries, so widely adopted, serves to increase the amount of mycorrhiza in the soil; so that fields which have been under this crop for a long period of years become heavily infected. Where possible, a system of rotation should be practised. The best fields for strawberry cultivation are those which have been constantly under the plough, and which have borne different crops in the preceding years. It is a mistake to plant runners in fields which have been freshly broken from old pasture.

Soil sterilisation, where not prohibitive on account of expense, would reduce the presence of mycorrhiza to a minimum.

The effect of varietal resistance remains yet to be determined, but there is preliminary evidence to show that some strawberry varieties are more resistant than others. It is possible that resistance to invasion would depend upon the ability of the exodermis of the rootlets to prevent penetration by the mycorrhizal mycelium. The possession of a thinner cortex would perhaps be an asset to the plant roots, since there would be less scope for the development of the endophyte in the tissues. Varieties or strains again, could be found which are naturally of a vigorously rooting type.

For more detailed information on the general subject of mycorrhizas, we would refer the reader to the excellent series of papers by M. C. Rayner which appeared in the New Phytologist, vols. 25 and 26. These have been collected and reprinted in book form under the title "Mycorrhiza" in the present year. This work (39), in addition to embodying her own valuable researches on the mycorrhiza of *Calluna vulgaris*, gives an account of the situation up to date. A bibliography is appended.

In conclusion, we wish to express our special indebtedness to Mr. D. V. Howells, and to his assistant, Miss Copeland, who kept us constantly supplied with freshly-collected plants from the Clyde Valley throughout the entire period of our investigation. Our thanks are also due to those strawberry growers in Scotland and in England who so kindly sent us typical diseased plants from their respective areas.

We are specially indebted to Mr. J. Ramsbottom, Deputy Curator, Botanical Section of the British Museum for valuable suggestions and the revision of proofs.

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EXPLANATION OF PLATES.

PLATE I.

Figs. 1 and 2.—Transverse sections of fine absorbing rootlets of the strawberry. The sections have been cut through an infected area and show the entering hyphae. (x200.)

ex. m., extraradical mycelium; int. m., intracellular mycelium; int. sp., intercellular space; a., arbuscules; end., endodermis.

PLATE II.

Fig. 1.—Longitudinal section of infected root, showing great development of mycelium, both intra- and intercellular, and anastomosis of hyphae. (x250.)

Fig. 2.—Longitudinal section of infected root, showing intracellular habit of mycelium in outer cortex, and intercellular habit in inner cortex. (x250.)

int. m., intracellular mycelium; int. sp., intercellular space; m., mycelium; a., arbuscules.

PLATE III.

Plate III.—Arbuscules in various stages of digestion.

Fig. 1.—Fully-developed arbuscules before the onset of digestion. (x400.)

Fig. 2.—Partially-digested arbuscules. (x400.)

Fig. 3.—The sporangiole stage. (x400.)

int. sp., intercellular space; int. m., intercellular mycelium; h., hypha-bearing arbuscules; a., arbuscules; a′., partially-digested arbuscules; s., septum; sp., sporangiole.

PLATE IV.

Fig. 1.—Vésicules of varying shape and size. (x250.)

Fig. 2.—Vésicule in intercellular space. (x250.)

Fig. 3 (after Gallaud).—Young arbuscules, diagrammatic representation.

Fig. 4.—Vésicules, showing contents. (x250.)

int. sp., intercellular space; h., hypha-bearing vésicule; n., nucleus; w′., outer wall of vésicule; w″., inner wall of vésicule.

PLATE V.

Plate V.—Longitudinal sections of infected rootlets with vésicules.

Fig. 1.—Numerous vésicules in various stages of development are seen in the tissues. (x100.)

Fig. 2.—Chain of vésicules. (x250.)

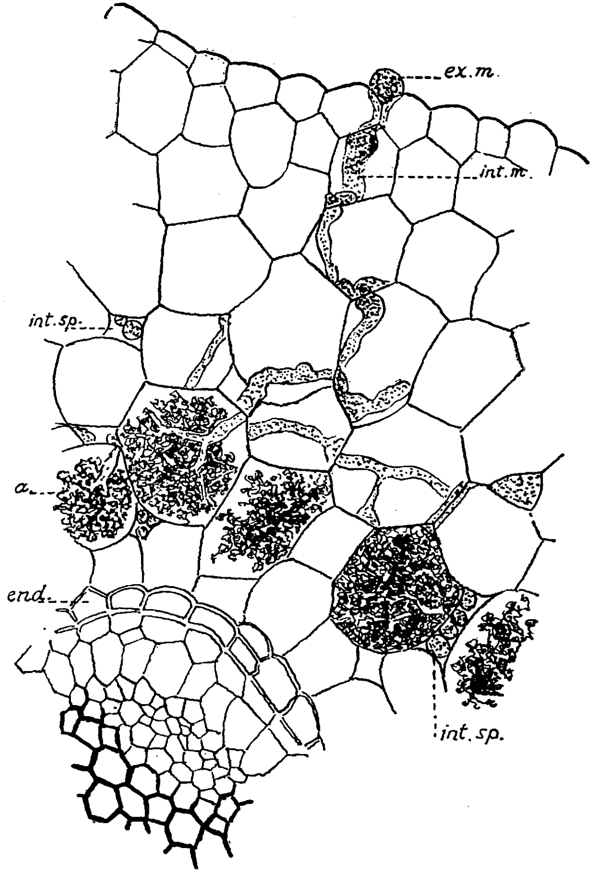
m., mycelium; a., arbuscules; v., vésicules.

PLATE VI.

Plate VI.—Longitudinal section of an infected rootlet, showing depletion of starch. Note that starch is abundant in tissues remote from the mycelium. Dissolution begins in advance of the hyphae, and cells bearing arbuscules are devoid of contents. (x165.)

int. sp., intercellular space; m., mycelium; a., arbuscules; st., starch.

PLATE I



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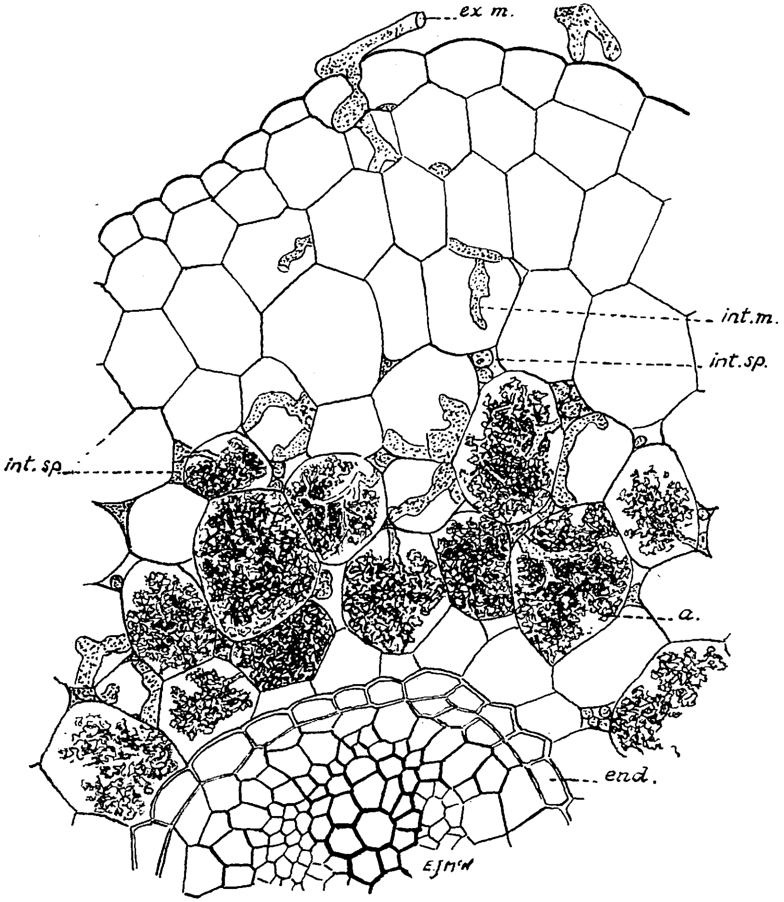

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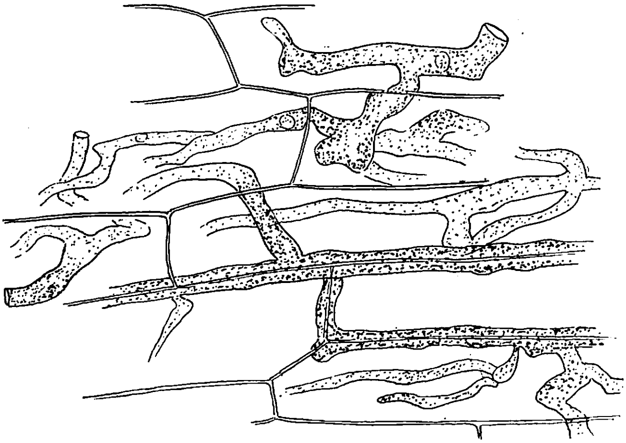

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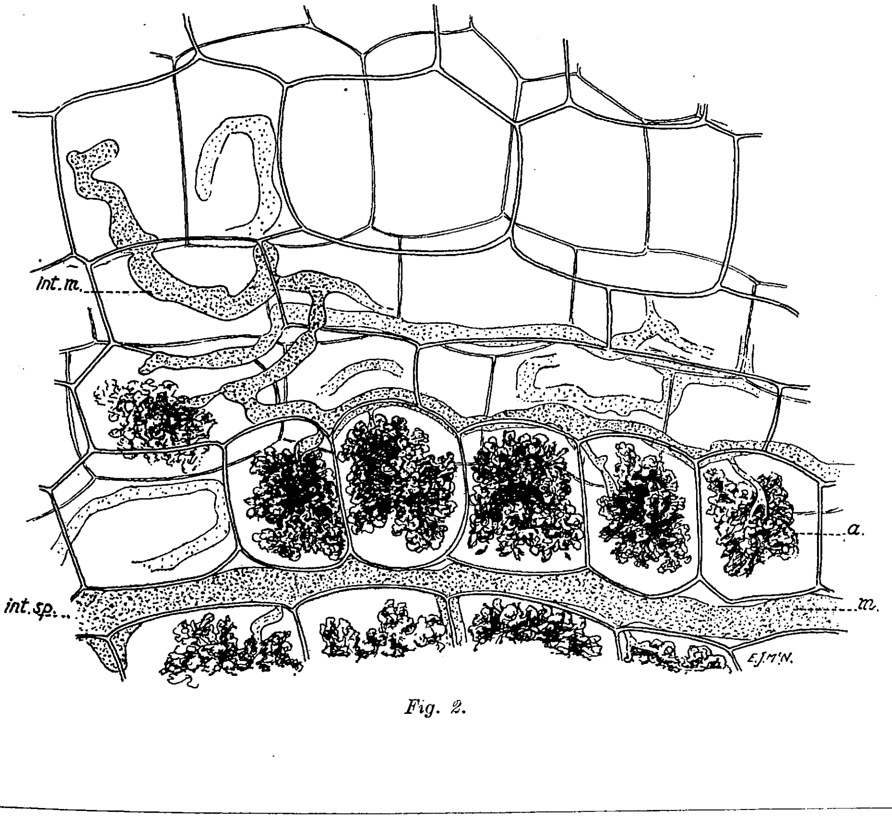

Fig. 2.

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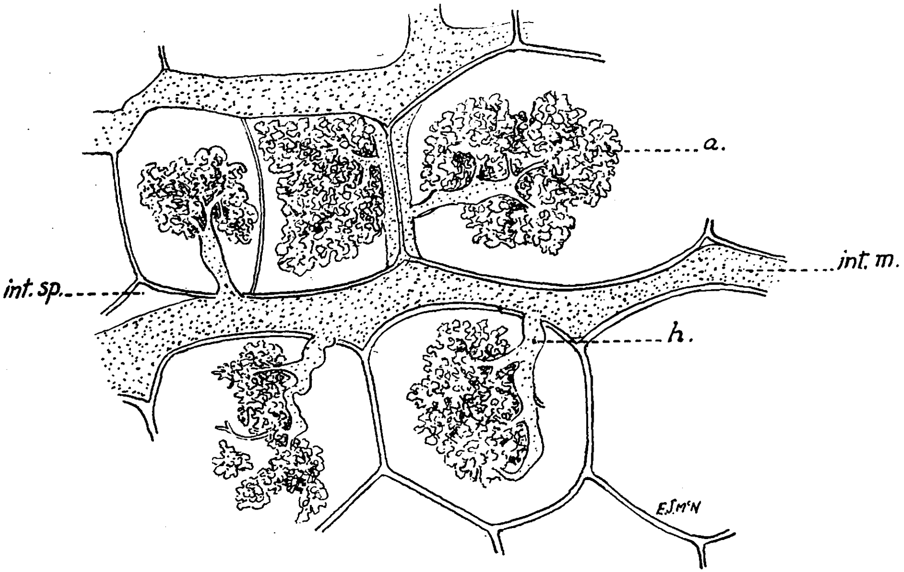

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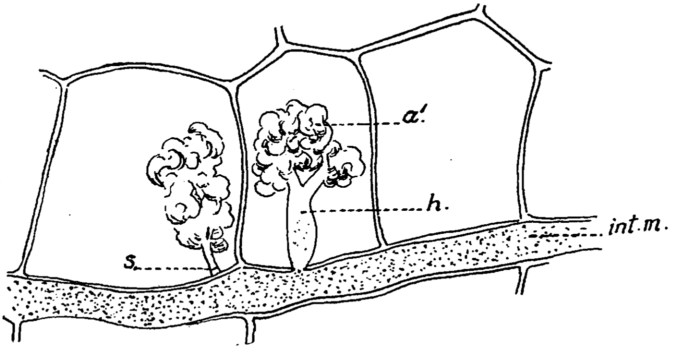

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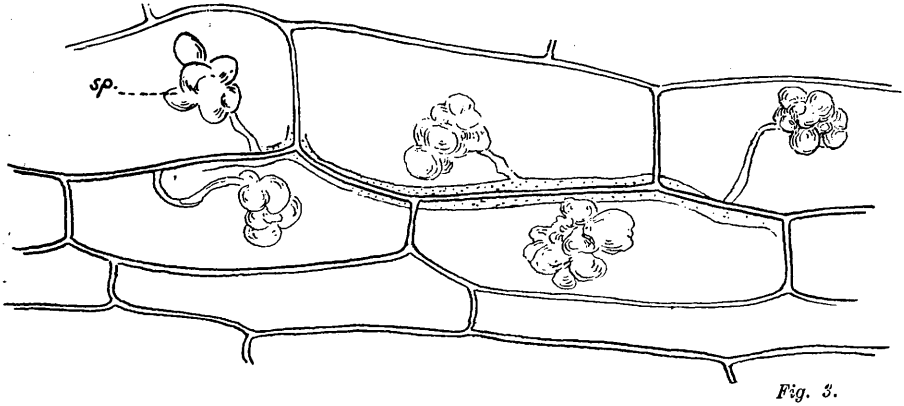
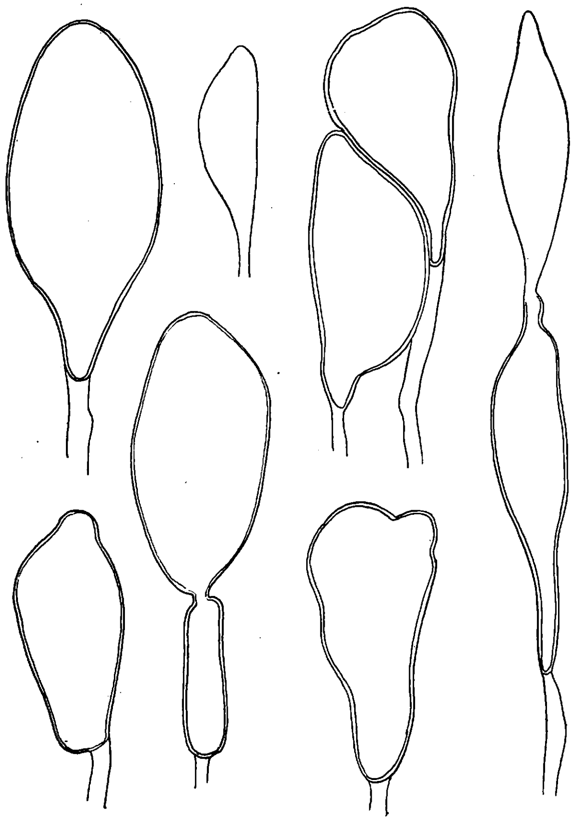


Fig. 3.

PLATE IV



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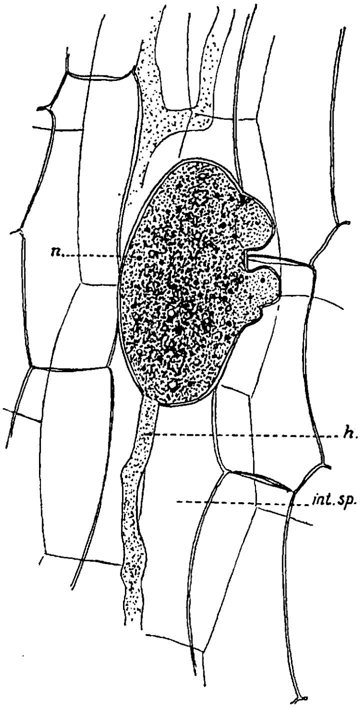

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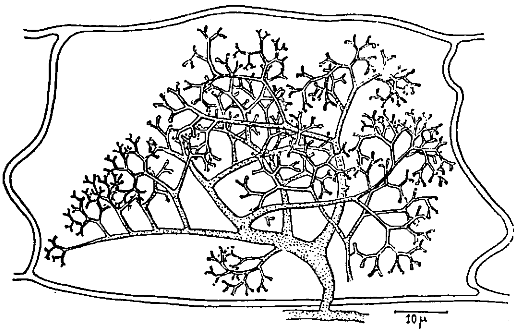

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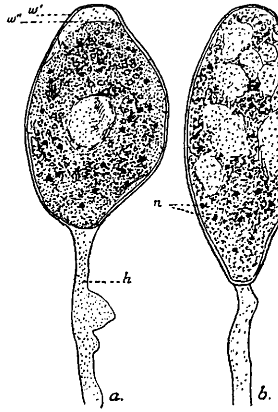
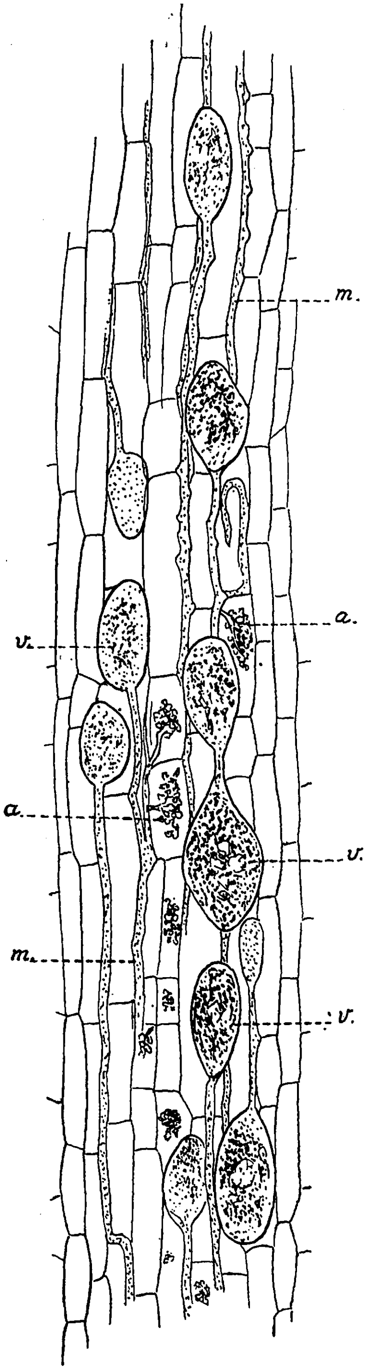


Fig. 4.

PLATE V



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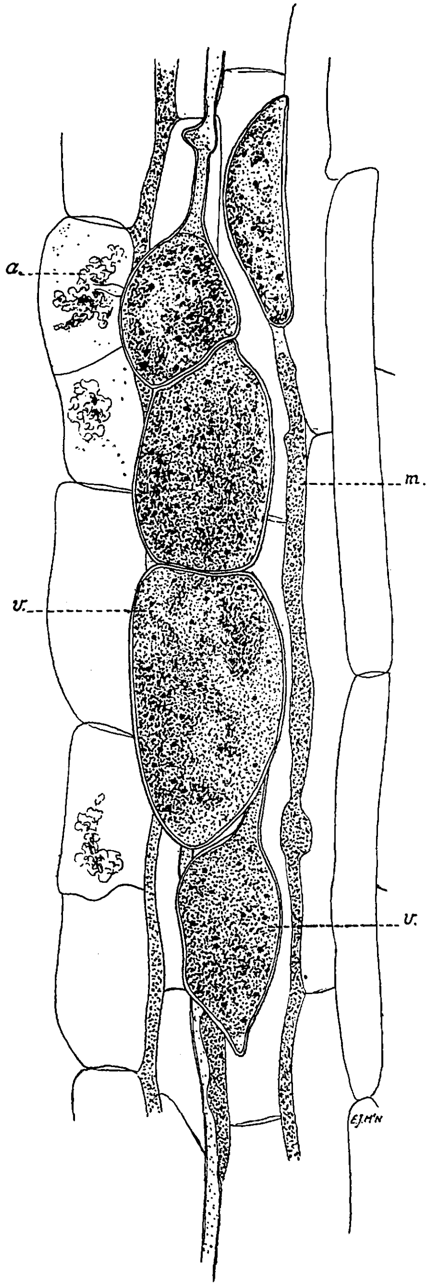

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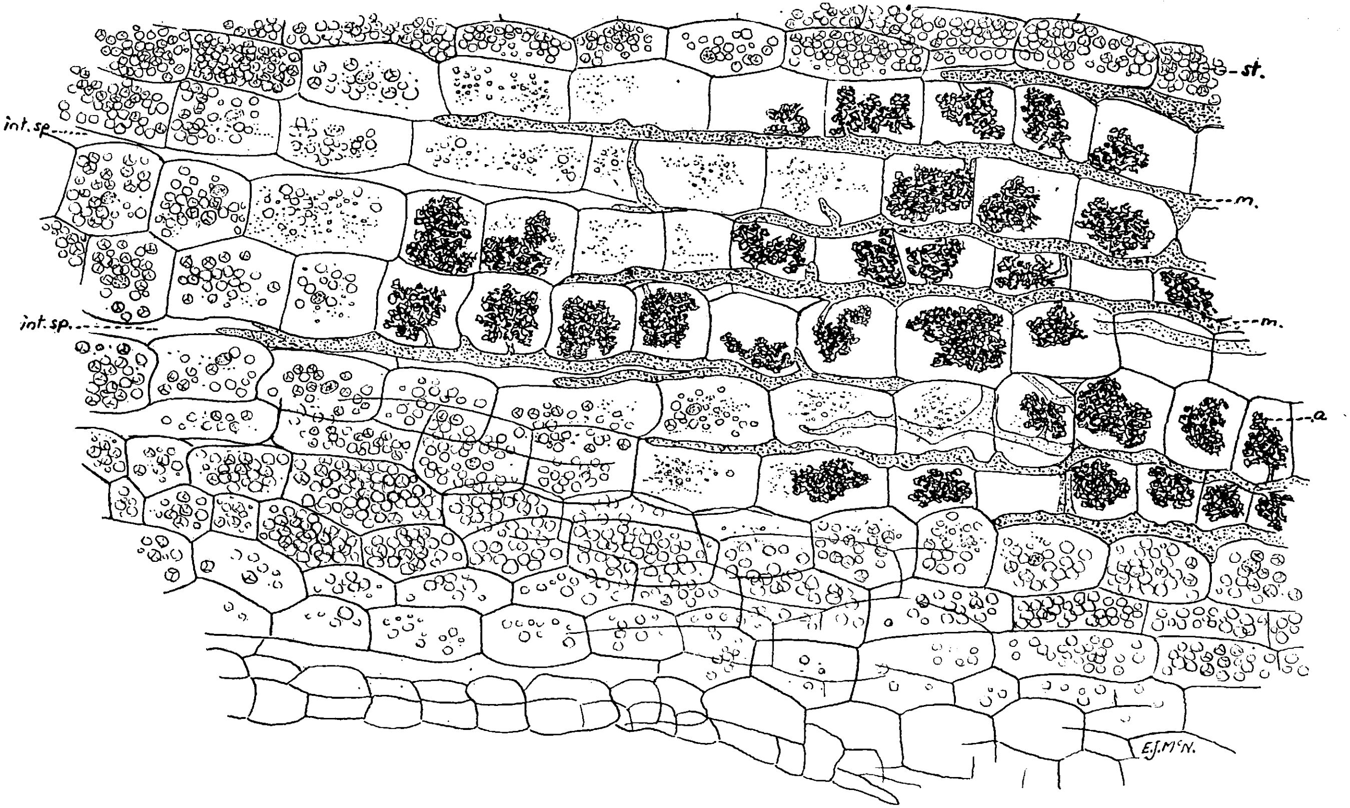
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