Rothamsted Experimental Station Report for 1965

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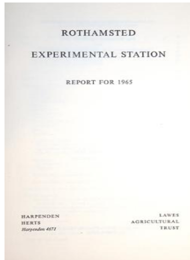
ROTHAMSTED
EXPERIMENTAL STATION
REPORT FOR 1965

Nematology

F. G. W. Jones

F. G. W. Jones (1966) *Nematology ;* Rothamsted Experimental Station Report For 1965, pp 137 - 152
- DOI: https://doi.org/10.23637/ERADOC-1-60

NEMATOLOGY DEPARTMENT

F. G. W. JONES

The death of J. B. Goodey on 30 October at the age of 51 years while on his way to New Zealand was a grievous loss to the department.

Patricia M. Cox, Pick H. Yuen and D. W. Larbey left, M. B. Lima returned to Portugal, D. C. M. Corbett and R. M. Webb were appointed, and K. Evans, D. Patel, S. K. Prasad and K. G. H. Setty joined the department as temporary workers. In September several members attended the Society of European Nematologists' Congress at Antibes, France. Mary T. Franklin became an editor of *Nematologica*.

As in previous years, help was given with the nematology course at Imperial College Field Station, and several visitors were taught to identify nematodes.

A. G. Whitehead, M. B. Lima and Pick Hoong Yuen were awarded the Ph.D. degree of London University.

The department studies nematodes or eelworms that harm crops. Broadly, these are of two kinds, those attacking stems and leaves and those attacking roots. The root parasites may roam freely in the soil and feed externally, inserting their stylets into the outer layers of root cells or sometimes more deeply, or they may enter roots, causing lesions and cavities. The females of some advanced root parasitic nematodes become immobile and enlarge greatly, whereas the males remain slender and mobile. This group contains the cyst-nematodes (*Heterodera* spp.), important crop pests, especially in Europe, North America and other temperate areas, and the root-knot nematodes (*Meloidogyne* spp.), a scourge of many crops in warmer countries or of glasshouse crops in colder ones. The aim of our work is to identify nematodes and accumulate knowledge that will lead to a better understanding of the diseases they cause and how to avoid them.

Systematics and Bionomics

The false root-knot nematode. The British false root-knot nematode *Nacobbus serendipiticus*, so far found once only in a glasshouse on tomatoes, is being studied because it is a potential pest of glasshouse crops and possibly of field crops. It resembles closely *N. batatiformis*, which is a serious pest of sugar beet in some areas in the United States of America. Both species attack a wide range of plants, including crops as varied as sugar beet, mangold, lettuce, tomato and egg plant, and common weeds. Larvae of *N. serendipiticus* also invade barley, French bean, potato and cucumber grown in pots.

Eggs are laid in gelatinous egg sacs extruded from the galls induced by the females on roots. Eggs are usually one- or two-celled when laid, but soon divide further, so that a mature egg sac contains eggs with larvae in

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all stages of development. The pattern of development resembles that of other nematodes, except that the initial cleavages are in a slightly different order and more time is required (2 weeks in water at room temperature) than previously recorded. The first larval moult occurs in the egg and the second stage larva hatches without requiring a stimulus. The third-stage larva resembles the second, except that it is larger and tends to coil within the roots. Some third-stage larvae are short with long gonads and some long with short gonads. This may be a sexual difference, but the sexes cannot be separated with certainty until the fourth stage, when males are considerably longer than females. Like third-stage larvae, the fourth also tend to coil. After the fourth and final moult the young female is long and thin, but soon swells and fills with developing eggs. Its final shape varies greatly to fit between the cells of the gall tissue it induces in the roots. The male remains worm-like and escapes into the soil.

Each of the four moults takes 3–4 days and is preceded by a period when the larva is quiescent and looks dead. In water drops the second and third moults occur in succession without the old cuticle being shed, as in the burrowing nematode, *Radopholus similis*. The final moult takes place only in plant tissue, but the second and third may occur in the soil.

Only second-stage larvae seem to feed externally on roots. Second, third and fourth stages use their mouth spears to enter roots and pass from cell to cell. The lips are pressed against a cell wall, the spear thrusts rhythmically at a selected spot until a hole is made which is enlarged until the larva passes through. The whole process resembles that by which the second-stage larvae escapes from the egg. Inside roots larvae feed and move until they enter a piliferous cell, when they leave the root, re-entering later. When conditions are unfavourable larvae coil, and do not move: they seem able to withstand desiccation. (Clark)

Stem eelworm. At Rothamsted unusually severe infestations of the stem eelworm, *Ditylenchus dipsaci*, occurred on some experimental plots of oats, causing considerable crop loss. Field beans were also attacked, especially where they followed oats in the preceding year, but also after three years of crops considered not to be hosts of *D. dipsaci*. Although many *D. dipsaci* were recovered from bean stems, the vigour of the plants was not obviously decreased. The crop histories of some sites suggest that field beans maintain and possibly increase infestations of *D. dipsaci*. The "oat race" seemed to be the one infesting the oats and beans, but the "giant race" was also found on beans from two sites. (Hooper)

An area of ground heavily infested with *D. dipsaci* (63 nematodes/100 g soil) was fumigated with methyl bromide and sampled over a period of 12 months, to observe the rate *D. dipsaci* reinvaded the fumigated area. Within 3 months a few nematodes were present in samples taken 12 in. inside the area, and after 12 months *D. dipsaci* were 6 ft from the original invasion front. The area was invaded faster when a host crop (e.g., oats) was grown than when not. (Webster and Greet)

Because the various races of stem eelworm have many minor hosts among crop plants and weeds, opportunities for hybridisation between races must be many. To study hybrids several races, namely, oat (OR),

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lucerne (LR), red clover (RCR), white clover (WCR) and narcissus (NR), were hybridised in pots, using onion seedlings as the common host.

LR ♀ × NR ♂	LR ♀ × RCR ♂	RCR ♀ × NR ♂
WCR ♀ × LR ♂	OR ♀ × LR ♂	OR ♀ × RCR ♂
NR ♀ × LR ♂	NR ♀ × RCR ♂	

The ability of these hybrids to multiply on lucerne and clovers was then tested with the results shown in Table 1:

TABLE 1

Reproduction of race hybrids on lucerne, red and white clover

Race or hybrid race	Lucerne	Red Clover	White Clover
LR	+	+	−
RCR	−	+	−
WCR	−	−	+
OR	−	−	+
NR	−	−	−
WCR♀ × LR♂	−	+	−
LR♀ × RCR♂	−	+	+
LR♀ × NR♂	−	−	−
OR♀ × LR♂	−	+	+
OR♀ × RCR♂	+	−	−

+ = reproduction
− = no reproduction

Those crops and weeds in which races of *D. dipsaci* induce cells to separate and become round, so providing an environment in which reproduction is excessive, are, in a sense, "poor" hosts, for the cell changes can injure the host so severely that it dies. This is the kind of injury done by the various races to the plants from which they derive their names. These races feed on many other plants, and those on which they reproduce slowly are "better" hosts, because they are not severely injured and maintain more stable populations. Whether race hybrids will revert to the old races again, or produce new ones with a different spectrum of hosts, has still to be determined, but hybrid races made experimentally reproduced less on the "poor" host plants associated with their parents' names. (Webster)

Rice and sugarcane nematodes. *Tylenchorhynchus martini* and *Aphelenchoides besseyi* were recorded for the first time in Africa on rice plants sent from the West African Rice Research Station, Rokupr, Sierra Leone. *A. besseyi* was also obtained from several varieties of rice seed. Many *T. martini* and *Longidorus laevicapitatus* and some *Trichodorus minor* were identified in extracts from around the roots of Trinidadian sugarcane sent by Dr. D. W. Fenwick. *L. laevicapitatus* was first described from sugarcane soil in Mauritius, and this is the first record of its occurrence elsewhere. (Hooper)

Feeding and Penetration

The penetration of Tylenchid nematodes into roots, and their feeding on roots, leaves and fungi was studied photographically. Hosts are attractive at least over short distances, and feeding and penetration sites seem

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to be selected by lip contact and stylet probing. In several species one active individual often seemed to stimulate others, and the larvae of endoparasites sometimes entered roots simultaneously and close together or used the same penetration hole. In feeding, the longer stylet of *Aphelenchoides blastophthorus* extended into the endoplasm or the central vacuole of food cells, whereas the shorter stylet of *Ditylenchus* spp. penetrated only to the viscous peripheral protoplasm.

In maturing *Heterodera cruciferae*, and in the fungus-feeding nematodes *D. destructor* and *D. myceliophagus*, the subventral salivary glands were less active than the dorsal gland. In young *H. cruciferae*, and in *D. dipsaci* and *Nacobbus serendipiticus*, all glands seemed equally active, but saliva flowed fastest in *D. dipsaci*. In *Heterodera* and *Nacobbus* secretions may flow no quicker than produced by their glands, but, when *D. myceliophagus* feeds, saliva flows quickly forward as the stylet enters the food cell. At the same time the body shortens in the region of the glands, which may apply a pressure that forces saliva out through the stylet. Secretions tend to collect in well-defined reservoirs (*Heterodera*, *Ditylenchus*, *Nacobbus* but not *Aphelenchoides*) and, when feeding starts, the sporadic contractions of a few muscle fibres in the median bulb eject the contents of the reservoirs. In *D. dipsaci* the bulbar reservoir of the dorsal gland is constricted first and those of the subventral glands later.

Because *A. blastophthorus* and *A. ritzemabosi* ingest food immediately after their stylets enter a cell, saliva seems not to be injected, but ingestion by *Ditylenchus*, *Heterodera* and *Nacobbus* is delayed. Feeding by *D. destructor* and *D. myceliophagus* stops protoplasmic streaming in fungal cells and causes changes that kill the cells fed on and those around them. These changes, the formation of giant cells and the production of galls, are all evidence that nematodes inject saliva into cells they feed on. Ingestion is usually by pulsating the bulbar pump, but *D. destructor* and *D. myceliophagus* evidently need to ingest fungal contents slowly, and for this they use the posterior of the oesophagus either as a pump or a control valve. (Doncaster)

The Effects of Ultra-violet Radiation on Plant Nematodes

To improve their appearance and quality, bulbs are increasingly being washed before packing and marketing. Mains water cannot always be used, and wash-water may have to be recirculated and used over and over again, which introduces the chance of spreading stem eelworms (*Ditylenchus dipsaci*) from infested to uninfested bulbs and of spreading them throughout a grower's stocks. Therefore, experiments were done to see whether wash-water could be disinfected by ultra-violet light.

The fourth-stage larvae of the bulb race of stem eelworm was used because it is the most persistent stage. At doses of radiation up to 16 μW-min/cm^2, ultra-violet light had little effect on the nematodes, and irradiated larvae inoculated into onions reproduced normally. Above 16 μW-min/cm^2, multiplication in onions declined, and at about 50 μW-min/cm^2 it stopped. Larvae irradiated with 250 μW-min/cm^2 were still able to move in water drops, but the proportion able to move decreased as the

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dose of ultra-violet radiation increased, and none moved after 15,000 μW-min/cm². Probably because of pressure from the heating effect of intense irradiation or from induced chemical changes, nematodes began to burst at 30,000 μW-min/cm², and nearly all burst after exposure to 80,000 μW-min/cm².

For comparison, larvae of the beet and potato cyst-nematodes were also irradiated. They behaved like stem eelworms, and none survived to adulthood after a dose of 250 μW-min/cm², movement was affected by 300–500 μW-min/cm² and all were still after 8,000–10,000 μW-min/cm². Possibly because the cuticles of cyst-nematodes are tougher, larvae rarely burst when exposed to very large doses, but some developed blisters.

Because stem eelworm larvae ceased to reproduce and began to die in the range 50–250 μW-min/cm², wash-water can be decontaminated by irradiation at 500 μW-min/cm². No difficulty arises in applying this radiation to clear water, but turbid wash-water contains soil particles, pieces of bulb scale and other debris, which would need to be removed. Very powerful sources of ultra-violet light now exist, around which "cleaned" water could be passed in a narrow annular space, so decontamination is a feasible proposition.

The ease with which ultra-violet light prevents nematodes from reproducing makes population control by liberation of sterile larvae a possibility. (Green and Webster)

Effect of 2,4-D on Host Resistance

Further experiments with 2,4-dichlorophenoxyacetic acid (2,4-D) showed that this plant-growth substance decreases the resistance of plant tissue to nematodes. Thus, although *Aphelenchoides ritzemabosi* normally reproduces only in the leaf and stem tissue of lucerne seedlings, it reproduces equally well on 2,4-D-induced callus derived from root, stem or foliage tissue. The 2,4-D influenced nematode reproduction indirectly by producing callus tissue, which provides a better environment than normal tissue for nematode reproduction and feeding. Witholding 2,4-D restored the original tissue specificity. When *A. ritzemabosi*, which had passed through many generations in callus culture for 2 years, was inoculated into red clover, white clover, lucerne and onion seedlings in pots they multiplied in these plants at the same rate as *A. ritzemabosi* extracted freshly from plant material.

Following from this, 2,4-D solution was sprayed (1·4 g/l/10 sq. yd) on to nematode-susceptible oats (variety Sun II) in pots previously infested with *D. dipsaci*. Eight weeks later about five times as many nematodes were recovered from the sprayed as from the unsprayed plants. Nematode-resistant oats (variety Manod) sprayed with 2,4-D contained four times as many nematodes as unsprayed oats. (Webster)

Nematodes Associated with "Docking disorders" of Sugar Beet

Docking disorder is the name given to stunted sugar beet that often occurs in patches in fields on alkaline sandy soils. It has gradually become clear that there are several disorders, of which nematodes probably cause

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two. Seedling plants seem to be attacked by nematodes produced in the preceding crop, usually barley. Overwintering nematodes begin to feed on the roots of young seedlings and prevent rootlets from developing properly, so the plant fails to obtain nitrogen and other nutrients, and remains small. Two types of stunting can be distinguished by the appearance of the roots. One is characterised by the lack of a tap root and the fangy roots tend to run horizontally; this is associated with stubby root nematodes, mostly *Trichodorus* spp., which were abundant near the roots of severely stunted plants, not only in two fields near Docking (N.W. Norfolk) but also in two in East Yorkshire. Mr. W. I. St. G. Light of the National Agricultural Advisory Service also informed us that he found more *Trichodorus* around roots of stunted than around less-stunted beet in Surrey in 1964. Within a few days of sowing beet in an observation box containing soil from a field where beet were affected, stubby root nematodes collected near the root tips, and the areas they fed on turned brown. The tips of some seedling tap roots collapsed and died and laterals began to proliferate above the lesion: the lateral roots in turn were also attacked. (Whitehead and Hooper)

Plants with the other type of stunting, which is associated with *Longidorus attenuatus* and *L. elongatus*, have small tap roots of normal shape but few lateral roots, most of which are dead or dying back from swollen tips.

TABLE 2

Numbers of needle nematodes, Longidorus *spp./litre of soil near beet tap roots in July*

	Bad areas		Good areas
	Stunted plants	Less stunted plants	Healthy plants
1964	93 (12)	30 (12)	—
1965	155 (15)	75 (12)	30 (10)

Table 2 shows numbers of *Longidorus* spp. recovered from around stunted, less stunted and healthy plants. The numbers of fields sampled are shown in brackets, and more nematodes were recovered in 1965 than 1964 because the method of extraction was better.

To study distribution of *L. attenuatus* and the relationships between nematode numbers, numbers of root-tip galls and yield, two fields carrying many stunted plants at the end of May were sampled during June and July. In both fields there were more *L. attenuatus* near the roots of small than large plants, and more swollen root tips on the small than on the medium-size plants and fewest on the roots of larger plants. Beet seedlings grown in pots of steam-sterilised soil inoculated with *L. attenuatus* washed in sterile, distilled water, produced swollen root tips, whereas seedlings in pots inoculated with nematode washings only did not. On both fields in June, soil from 4 to 8 in. deep contained more *L. attenuatus* 1 in. from the tap roots of the smaller plants than 2½ and 4 in. away. *L. attenuatus* became fewer in July, but again there were more near the roots of small than of large plants. (Whitehead)

The movement and reproduction of *L. attenuatus* in two infested beet

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fields were studied intensively from March to October, i.e., from before sowing beet to after harvest. Soil cores, taken against plants and at different distances from them across the rows, gave no evidence that nematodes moved from the inter-row spaces to the plants. Seedlings were probably attacked only by those nematodes already in the soil occupied by their roots, and movement towards the roots, if any, was only local, perhaps reflecting random wanderings over an inch or so. In both fields adults became fewer during April, May and June, but the number of larvae near the plants increased greatly during May and June, suggesting that only adults able to feed (i.e., near roots) laid eggs. In July soil conditions seemed unfavourable for reproduction even for nematodes near roots. (Green)

To study effects on *L. attenuatus* and other plant-parasitic nematodes of growing different crops and of soil fumigation on the growth of beet, microplot experiments were made in 1965 on two fields where sugar beet was stunted in July 1964 and stunting was correlated with the numbers of *L. attenuatus*. One experiment at each site was of five randomised blocks of eight treatments—(1) barley, (2) sugar beet, (3) ryegrass, (4) red clover (5) weeds and (6) bare fallow, all on unfumigated soil, and sugar beet after fumigating the soil during winter with (7) "D-D" (dichloropropane-dichloropropene) and (8) chloropicrin, both at 400 lb/acre. Plots were sampled for *L. attenuatus* at monthly intervals from pre-sowing to post-harvest. In the rows of sugar beet, red clover and ryegrass and under bare fallow nematodes in soil 0–8 in. deep were most abundant in June, whereas with barley numbers declined steadily to July and then increased greatly in the stubbles to attain maxima in September. Thereafter numbers declined and, in November, there were fewer than at sowing in one field and three and a half times as many in the other. In plots of sugar beet, red clover, ryegrass, weeds and bare fallow there were fewer or only as many *L. attenuatus* as at sowing. "D-D" and chloropicrin killed nearly all nematodes: no *L. attenuatus* were recovered from 8 litres of treated soil at sowing and post-harvest. Plots were harvested at the end of September, when yields of tops, roots and sugar from the fumigated plots all exceeded those from the unfumigated plots, although these yielded well (Table 3).

TABLE 3

Yield of sugar beet in two microplot trials

Treatment	Tops (tons/acre)	Roots (washed) (tons/acre)	Sugar (cwt/acre)
		Gayton	
"D-D"	22·0	21·1	65·7
Chloropicrin	27·7	22·7	67·8
Control	18·5	15·8	47·9
L.S.D. (5%)	1·9	1·5	5·0
L.S.D. (1%)	2·8	2·1	7·2
		Santon Downham	
"D-D"	23·7	18·8	56·7
Chloropicrin	26·3	21·8	64·0
Control	20·9	13·3	39·3
L.S.D. (5%)	4·3	3·3	12·9
L.S.D. (1%)	6·2	4·7	18·7

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Beet grew better and was more even on the fumigated than on the unfumigated plots, but the considerable differences evident in June diminished later.

Sodium, potassium and amino-nitrogen contents of beet from all plots were small enough not to interfere with sugar extraction, but potassium was increased by "D-D" and chloropicrin and, on one field, sodium and amino-nitrogen also by chloropicrin. Fumigation did not influence the sugar content of the roots. On the other field yields of roots and sugar (Table 4) were significantly increased by 2·5 ml and 3·5 ml "D-D" injected at 1-ft intervals in the predetermined sugar-beet rows, but not by either 7·0 ml in the rows or whole-plot treatments. (Whitehead and Greet)

TABLE 4

Yield of sugar beet after "D-D" treatments

Treatments	Roots (washed) (tons/acre)	Sugar (cwt/acre)
"D-D" Treatments		
2·5 ml/ft row	20·9	64·2
3·5 ml/ft row	19·8	61·5
7·0 ml/ft row	18·4	56·6
2·5 ml/sq ft of plot	18·5	56·2
3·5 ml/sq ft of plot	19·0	57·0
Control	17·5	52·3
L.S.D. (5%)	1·57	6·02
L.S.D. (1%)	2·14	8·21

Concurrently with the work on *Longidorus attentuatus*, other nematodes were studied. The only nematodes found 6 weeks after fumigation were lesion nematodes *Pratylenchus* (170/litre, 12% of unfumigated) and Rhabditids (800/litre, 70% of unfumigated). In fumigated soil under sugar beet *Pratylenchus* increased to 500/litre and pin nematodes, *Paratylenchus*, to 260/litre (25% and 30% of unfumigated respectively). These were the only nematodes parasitic on higher plants present in appreciable numbers, but fumigation greatly increased *Aphelenchus avenae* (to 1,500/litre) and Rhabditids (to 134,000/litre maximum, more than ten times as many as in the unfumigated).

Under ryegrass, barley and red clover, *Pratylenchus* free in soil increased in April from the 1,400/litre initially present to 1,750/litre, but decreased rapidly in May and June, when roots were invaded and contained; barley 500/g, ryegrass 180/g, red clover 210/g. Numbers did not decrease in soil under sugar beet in May and June, probably because the plants had fewer roots to be invaded. In August numbers in soil had again increased; barley 3,500/litre, ryegrass 1,800/litre, sugar beet 1,600/litre and red clover 800/litre. They decreased in September and October, but increased in November. In the soil of fallow plots populations decreased steadily through the year.

Except for *Pratylenchus*, larvae of cyst nematodes (*Heterodera avenae* possibly mixed with some *H. trifolii*) were the only nematodes extracted from roots in significant numbers. The 240/litre of soil before sowing increased to 1,000/litre during April after sowing, but then decreased to 40/litre in May and still further thereafter in all except barley plots. Males were extracted from roots in June; barley 33/g, ryegrass 8/g and red clover

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3/g. Eggs were produced in barley from July onwards and new larvae, which appeared in the soil from August onwards, increased numbers to 500/litre.

Paratylenchus was unevenly distributed, and plot differences were maintained throughout the year. It was the most abundant of all the plant parasitic species, and from an average of 300/litre initially it reached the following peaks in August and November: ryegrass 7,200/litre, barley 2,200/litre, red clover 1,200/litre, sugar beet 750/litre and fallow 150/litre.

From 250/litre initially, *Tylenchorhynchus* reached 3,200/litre in August under barley and then fell to 1,250/litre. Under ryegrass there were peaks of 3,000 and 4,250/litre in August and November respectively. Red clover maintained the initial level, whereas beet and fallow failed to, and the populations fell to 150/litre, about half the initial numbers. Stubby root nematodes, *Trichodorus* spp., fluctuated less than other species, but, by November, the ryegrass and sugar-beet plots contained 820/litre, barley 500/litre, red clover 400/litre and fallow 240/litre. The results confirmed that the numbers of common plant-parasitic nematodes through the year, and those remaining until the next spring are largely determined by the crop grown during the year. (Cooke)

Other work on nematodes associated with patchiness of sugar beet and barley crops was done jointly with the Plant Pathology Department (see p. 121).

Cyst-nematodes

Sexual behaviour. The possibility of controlling obligate bisexual nematodes by releasing sterile males or females needs exploring. Little has been done on the sexual behaviour of nematodes even of the important and most studied cyst nematodes. A beginning was therefore made with the beet and potato cyst-nematodes (*Heterodera schachtii* and *H. rostochiensis*).

On agar plates about half of the males respond to an attractive female. The chemical nature of the attractant was shown by comparing the response of males to living females, dead females, glass beads and to glass beads substituted for females removed just previously. Living females attracted most males; glass beads substituted for females and some dead females were also attractive, but glass beads, egg sacs of *H. schachtii* and females washed in acetone were not. Males were not attracted to females when put on agar plates at the same time, but were to females put there earlier. The site where a female had long been but from which she was removed just before the males were added was more attractive than a site containing a recently introduced female. Evidently considerable time is needed for the attractant to accumulate and diffuse, and after fertilisation it may cease to be produced for a while, or a repellent may be produced by either the female or the fertilising males.

That the movement of the males was orientated to a stimulus was shown by following their tracks. Without females present, males moved freely at about 2 mm/min., following a loosely convoluted path with few reversals or abrupt changes of direction. With an attractive female present, the path was still convoluted at first, but then became either a spiral of decreasing

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radius around the female or a zig-zag with decreasing oscillations towards her and many abrupt changes in direction. Males of both species found females by a combination of random movements, suitable for searching a relatively large area, and then by orientation to a chemical gradient when their random movements brought them near a nubile female.

Although immature females of *H. rostochiensis* seem not to attract males, males placed on them remain there longer than when placed on mature females. The number near immature females halved in 4 days and remained at a half for 8 days, whereas near mature females only a quarter remained after 4 days and one eighth after 8 days. Immature females removed from roots remain alive for more than a month without changing to a more mature, rounded shape. They are not fertilised and produce no eggs. Mature females without males, and therefore unfertilised, remain white and contain no eggs, but a proportion of fertilised females develop eggs, turn brown and become cysts. Males could mate successfully more than once, but multiple mating of females did not increase the number of eggs they produced. (Green)

Sex determination. Experimental proof was obtained for the hypothesis, advanced by Dr. C. Ellenby (*Nature, Lond.* (1954) **174**, 1016–17), that in *H. rostochiensis* sex is determined by the conditions an invading larva meets in host-plant roots, and that an important factor is larval density. When tomato seedlings were inoculated with one larva each, 11 out of 12 became females, but when inoculuated with more than one the numbers of males and females were almost equal. Similar results were obtained with potato plants. When there were fewer than 14 larvae/cm of main potato root more larvae became females than males, but when there were more than 14/cm more larvae became males than females and the number of females remained constant at about 7/cm of root. Sex determination by larval density increases the reproductive rate above what would otherwise be possible at small initial population densities and tends to stabilise the population over a considerable range of larger population densities.

Because of its greater size and the additional energy required to produce eggs, the female obviously requires more nutrients than the male. The influence of the host's nutrient status on sex ratio was therefore examined. Decreasing the carbohydrate content of plants by shading or by removing the aerial parts increased the proportion of males, provided the plants were treated before the larvae reached the third stage, when sex is determined irreversibly. Shading and cutting off the tops of potato plants had no effect when the plants had tubers on them. Changing nutrients other than carbohydrates also affected the sex ratio. The importance of carbohydrates is not understood, but they are the most easily influenced nutrient, and their lack may limit the size of giant cells induced by larvae, and so perhaps affect the quantity or quality of the nematodes' food. (Trudgill)

https://doi.org/10.23637/ERADOC-1-60

Genetic Constitution of Females Developing on Resistant Plants

The production of *H. rostochiensis* males by hybrid potatoes containing genes for resistance derived from *Solanum tuberosum* ssp. *andigena*, *S. multidissectum* or both was examined. Some evidence was obtained that larvae from populations that produce few females on resistant plants with both genes also produce fewer males, suggesting that selection operates on both sexes.

Larvae were hatched from single cysts of the Long Mead, Woburn, population, thought to consist mainly of pathotypes 0 and 2 (*Rothamsted Report* for 1964, p. 151; *Ann. appl. Biol.* (1963) **51**, 277–294). The cysts had been raised in a pot on a resistant ex *andigena* plant, and a complication was admixture with the inoculum of old cysts raised on Arran Banner in the proportion of one old to five new, but only new-looking cysts were chosen, and from these an average of 150 larvae emerged per cyst. The larvae from 31 cysts were inoculated separately into the soil of potted ex *andigena* plants and their development followed. It seemed normal up to the third stage, but only male-determined larvae matured: with two exceptions female-determined larvae probably died and, at the end of 6 weeks, 642 males and only 2 isolated females were recovered.

This result is compatible with the hypothesis that the parent females (new cysts) were double recessives, with the genetic constitution *aa*, and the males they mated with were double dominants, with constitution *AA*. All progeny (larvae) would then be *Aa* and unable to become females. The two isolated females could have resulted from a multiple mating in which one male was *Aa* or *aa*, but several females rather than single females would have been expected. On the alternative hypothesis that females carry a dominant gene, most females would be *Aa* and most males *aa*. About half the progeny would be *Aa*, and so nearly all new cysts would contain larvae able to become females, but old cysts would not. In fact, all cysts except two behaved similarly, as to be expected if females on ex *andigena* were recessive (*aa*).

Larvae from cysts derived from a population with more than 37% of individuals able to develop into females on resistant ex *andigena* plants were also tested as described above; females developed in the progeny of nearly all cysts (11 out of 12) and there were in total 232 males to 129 females. Assuming the first hypothesis is correct, the parent females were *aa*, the males mainly *Aa* or *aa* and the progeny a mixture of *aa* and *Aa*, which on ex *andigena* would produce an excess of males. On the alternative hypothesis, the progeny would contain more individuals potentially able to become females, but the sex ratio would tend towards unity if there were competition in the root system. The second test favours the first hypothesis, but so many factors might have influenced the sex ratio that a firm conclusion cannot be drawn. The inheritance of the ability to mature on resistant plants is unlikely to be settled until controlled matings have been made between pure or nearly pure lines of known pathotypes. This is being attempted. (Trudgill and Webster)

https://doi.org/10.23637/ERADOC-1-60

Hatching of cyst-nematodes. We reported last year that sodium ethylenebisdithiocarbamate (nabam) owes its ability to hatch *H. schachtii* largely to its oxidative decomposition product ethylenethiuram monosulphide. Further work solved the question of the differences between the hatching obtained with zinc and manganese ethylenebisdithiocarbamate and with sodium ethylenebisdithiocarbamate solutions containing equivalent amounts of zinc or manganese sulphate. Zinc and manganese ethylenebisdithiocarbamates hatch more larvae than sodium ethylenebisdithiocarbamate solutions containing equivalent amounts of zinc or manganese sulphate, although neither of the inorganic salts by itself inhibits hatch. The hatch was smallest in a zinc sulphate/sodium ethylenebisdithiocarbamate solution with a mole ratio of about 1·5 : 1, in which the dithiocarbamate ion was largely removed from the solution as the sparingly soluble and more stable zinc dithiocarbamate. The increased hatch with mole ratios smaller than 1·5 : 1 is attributed to the oxidative breakdown products of the free dithiocarbamate ion. With mole ratios greater than 1·5 : 1, hatching increased with increasing zinc concentrations because of the activity of the zinc ion. The ethylenebisdithiocarbamate ion decomposed in the presence of manganese sulphate to give the usual products of oxidative decomposition, whereas sodium ethylenebisdithiocarbamate solutions containing manganese sulphate were inactive because decomposition was accelerated by the catalytic action of manganese. This produced enough dissolved carbon disulphide to inhibit hatch. Manganese sulphate/sodium ethylenebisdithiocarbamate solutions free from carbon disulphide were at least as active as solutions of sodium ethylenebisdithiocarbamate only. Solutions of manganese ethylenebisdithiocarbamate were active because carbon disulphide did not reach an inhibitory concentration.

The rate cyst-nematode populations decline in the field is of practical importance. Some of the decline is from spontaneous hatching not stimulated by root diffusates. Several metal ions common in soil stimulate beet cyst-nematode eggs to hatch, but their effect on the eggs of other cyst nematodes was unknown. We therefore tested the effect of Al^{3+}, Ba^{2+}, Cd^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}, K^{+}, Na^{+}, Sn^{2+} and Zn^{2+} on the hatching of the following species: *H. schachtii*, *H. rostochiensis*, *H. trifolii*, *H. cruciferae*. *H. glycines*, *H. tabacum*, *H. carotae* and *H. goettingiana*.

Ions that hatched the eggs of one species did not necessarily hatch others; *H. schachtii* eggs were hatched by many ions, but those of other species by only a few. With one exception, the ions that hatched the eggs of other species also hatched those of *H. schachtii*. The exception was Mg^{2+}, which was slightly active for *H. trifolii* (hatch rating 22) but not for *H. schachtii* (hatch rating −30). Zinc ions hatched eggs of most species of *Heterodera*, except *H. avenae*. Hatch with zinc salts was more than with water and often equalled or exceeded that in the root diffusate of their host plants. Hatch of *H. schachtii* was significantly greater than in water with 0·6 mM Zn^{2+}, and gave a maximum hatch with 3–10 mM.

The wide activity of the zinc ion suggested it might be a co-factor for the active materials in plant root-diffusates. We therefore tested the effect of mixtures of purified potato cyst-nematode hatching factor and zinc

https://doi.org/10.23637/ERADOC-1-60

chloride on the hatching of eggs of *H. rostochiensis*. For the mixtures tested, the hatch obtained was usually greater than the sum of the hatches of the individual components.

The production of potato root-diffusate and the first stages of extraction of the hatching factor from it were made automatic to avoid watering and collection by hand. The potatoes are grown in a greenhouse in pea-shingle on sloping tiers of corrugated asbestos. The shingle is supported on a plastic mesh so that water drains through along the whole length of the beds and down the corrugations into gutters. Potato eyes are sown thickly in the shingle to give dense root growth and watered by spaced mist jets. The leachate is collected in a reservoir, acidified with dilute hydrochloric acid (5% v/v) and trickled through a mixture of silver sand and charcoal (4 : 1), from which the hatching factor is eluted with acidified acetone. The equivalent of 20 tons of root diffusate, three times last year's total, was produced with less labour. (Shepherd and Clarke, Biochemistry Department)

In connection with the work on hatching, the composition of the egg-shell of *H. rostochiensis* was studied jointly with the Biochemistry Department. (See p. 113)

Cereal cyst-nematode. The formalin experiment was continued (*Rothamsted Report* for 1964, p. 150) by superimposing a second formalin treatment, again at 266 gal/acre, so that four treatment sequences were obtained. Twenty-plant samples of the spring wheat, variety Opal, were taken from each plot in May 1965, the tops and roots weighed and *Heterodera avenae* in the roots counted. Grain and straw yields (15% moisture content) were recorded at harvest. Table 5 shows yields and other results.

TABLE 5

Yields of spring wheat and nematode numbers after formalin treatments

1964	1965	Grain (cwt/acre)	Straw (cwt/acre)	Sample wt (tops g)	*H. avenae* (/g root)	Root wt (g)	"Take-all" incidence (%)	Initial *H. avenae* (popn/g soil)
—	—	10·3	17·4	18·6	264·3	6·5	67·0	12·2
F	—	14·1***	21·4*	17·0	545·1	7·2	45·3	42·2
F	F	24·0***	30·7***	30·7	298·3	5·2	0·6	25·1
—	F	32·5***	41·5***	48·5	111·7	4·4	5·0	7·2

F = formalin applied as general biocide.

Formalin applied before the 1964 crop decreased grain and straw in 1965 by 2·3 and 3·4 cwt/acre respectively (both sig. at 0·01). As the 1964 formalin controlled root disease but increased the soil population of the cereal cyst-nematode, *H. avenae*, it seems that this nematode was mainly responsible for the yield decrease in 1965. Nabam applied in 1964 had no residual effect in 1965, and irrigation in 1965 was also ineffective. Increasing nitrogen from 0·6 to 1·2 cwt/acre increased yields of grain and straw, but 1·8 cwt/acre gave no further increase. Formalin applied before the 1965 crop increased grain by 16·1 cwt/acre and straw by 16·7 cwt/acre (both sig. at 0·001).

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The incidence of "take all" where formalin was not applied in either year was 67%, but less than 1% where it was applied in both years, whereas about the same number of nematodes invaded the root systems whether or not formalin was applied. The increase from formalin in both years, 13·7 cwt/acre of grain and 13·3 cwt/acre of straw, mainly reflected the elimination of "take all". Formalin in both years and in 1965 only brought the incidence of "take all" to less than 1% and 5% respectively, and yields were inversely proportional to the number of nematodes invading the roots. Regression analysis showed that yield varied inversely with *H. avenae* numbers when "take all" affected few plants, but not when it was prevalent. (Williams, Slope, Plant Pathology Department, and Widdowson, Chemistry Department)

The relationship between *Heterodera avenae* and yield of spring wheat, variety Opal, was studied in another experiment at Woburn. Methyl bromide (436 lb/acre), "D-D" (800 lb/acre), chloropicrin (400 lb/acre), dazomet (400 lb/acre), formalin (200 gal/acre) and mercuric chloride (5 lb. Hg/acre) were applied when the initial *H. avenae* population was 9 eggs/g soil. Except mercuric chloride, all sterilants significantly increased yields. Chloropicrin gave the best grain yield and dazomet the most straw. Nitrogen at 1·8 cwt/acre had no significant effect on the grain yields combined for all treatments, but significantly increased (0·001) straw yields. The effect of N at 1·8 cwt/acre varied between sterilants, sometimes decreasing yields, sometimes increasing (Table 6). An attack of mildew, nitrification effects of the sterilants themselves and bird damage may have contributed to these results.

TABLE 6

Yields of spring wheat after fumigation treatments

	0	MeBr	"D-D"	Chp.	Daz	For.	Mer.	Rotavated	Mean
				Grain (15% moisture), cwt/acre					
N_1	21·8	24·9	33·0	32·3	29·1	30·7	19·6	19·3	26·4
N_3	22·9	31·5	28·1	31·0	24·4	29·7	25·9	22·1	27·0
Mean	22·4	28·2	30·6	31·7	26·8	30·2	22·8	20·7	26·7
		**	***	***	*	***	NS	NS	
				Straw (fresh weight), cwt/acre					
N_1	55·1	63·6	66·1	70·4	89·2	64·0	31·9	36·1	59·6
N_3	49·4	82·6	85·7	89·6	79·5	71·6	53·8	48·9	70·2
	52·3	73·1	75·9	80·0	84·4	67·8	42·8	42·5	64·9
		***	***	***	***	***	**	**	

When the grain, straw and sample yields were adjusted for regression on the number of *H. avenae* in the roots they differed little from the unadjusted yields. The fact that the original differences in yield were maintained after adjustment and that the variance ratios for fumigant effects remained significant indicated that these treatments were in general having significant effects other than nematicidal ones. The same feature was observed when the yields were adjusted for "take all" disease ratings, demonstrating the difficulty of ascribing the decreases in yield to one pathogen in particular. The general similarity between the effect of the sterilants on "take all" and *H. avenae* are shown in Table 7.

https://doi.org/10.23637/ERADOC-1-60

TABLE 7

Comparisons of effects of sterilants on "take-all" and H. avenae

"Take-all" rating, %

	0	MeBr	"D-D"	Chp.	Daz	For.	Mer.	Rotavated	Mean
N_1	71·6	25·5	25·4	45·8	2·4	19·2	64·2	61·8	39·5
N_3	32·6	11·5	0·5	13·8	0·8	4·3	14·4	28·8	13·3 ***
Mean	52·1	18·5	12·9	29·8	1·6	11·8	39·3	45·3	26·4
		***	***	***	***	***	*	NS	

H. avenae/g root

	0	MeBr	"D-D"	Chp.	Daz	For.	Mer.	Rotavated	Mean
N_1	217·3	20·5	12·1	26·8	7·5	44·5	457·5	332·0	139·8
N_3	182·3	10·9	11·7	8·9	15·0	26·2	233·4	193·0	85·2 NS
Mean	199·8	15·7	11·9	17·9	11·3	35·4	345·5	262·5	112·5
		***	***	***	***	**	NS	NS	

An interesting feature of this experiment is the effect that *H. avenae* seems to have on the development of the root system within the depth sampled (approx 4 in.). The weights of crown and seminal roots of the 20-plant samples differed greatly in plants from plots given different treatments (Table 8).

TABLE 8

Effect of H. avenae *on growth of seminal and crown roots of spring wheat*

Treatment	Nematodes/g root	Fresh weight in g: Seminal roots	Crown roots	Total
None	199·8	4·8	2·8	7·6
Rotavated	262·5	5·5	2·8	8·3
Mercury	345·5	5·6	2·7	8·3
Dazomet	11·3	2·0	5·0	7·0
MeBr	15·7	2·2	4·4	6·6
Formalin	35·4	2·7	4·4	7·1
Chloropicrin	17·9	1·8	4·4	6·2
"D-D"	11·9	2·5	6·6	9·1

Where many nematodes invaded, the seminal roots proliferated extensively and weighed more than the crown roots, whose development seemed to be retarded at this stage of growth by many nematodes invading the seminal roots. Very few nematodes were found in the crown roots in these May samples. (Williams and Salt, Plant Pathology Department)

Potato cyst-nematode. An experiment at Woburn tested machinery for applying and mixing solid formulations of soil fumigants and the influence of depth and intimacy of mixing on control. Dazomet powder was mixed with a fluorescent tracer and applied evenly at 1·6 lb/100 sq ft. The plots were then rotavated and ridged in various ways. None of the cultivations mixed the powder uniformly to full depth, and from 50 to 80% remained in the top 3 in. Distribution was poorer the greater the depth. The number of *H. rostochiensis* larvae subsequently in roots and the crop yields failed to show consistent differences between treatments, but on average treated plants yielded 4·9 tons/acre more than untreated, which yielded

https://doi.org/10.23637/ERADOC-1-60

10·4 tons/acre. The nematode populations after harvest averaged only 10% of those on untreated plots, and ranged from less than 1 to 33%. Dazomet controlled the nematode satisfactorily however it was incorporated into the soil, but would probably have done better with better mixing. (Greet)

https://doi.org/10.23637/ERADOC-1-60