

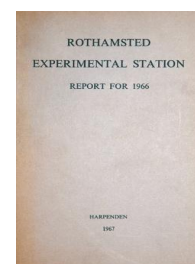
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## Rothamsted Report for 1966

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### Nematology Department

**F. G. W. Jones**

F. G. W. Jones (1967) *Nematology Department* ; Rothamsted Report For 1966, pp 137 - 160 - **DOI:**  
<https://doi.org/10.23637/ERADOC-1-14>

## NEMATODOLOGY DEPARTMENT

F. G. W. JONES

J. M. Webster left and D. L. Trudgill was appointed. Visiting workers included M. L. Mojica and C. J. Nusbaum.

Unsegmented round worms or nematodes occur in almost all natural environments. Some are parasites of animals, including man; others, commonly known as eelworms, are plant parasites or are free-living in the soil. Most eelworms are about  $\frac{1}{2}$  in. long, only just visible to the naked eye. All plant parasitic nematodes have piercing and sucking mouthparts, smaller but similar in action to the mouthparts of sucking insects.

Some members of the department identify plant parasitic nematodes, describe new species, study their life cycles and host ranges and assess whether they harm crops. Some study specific crop problems such as Docking disorder of sugar beet now known to be caused by two genera of root ecto-parasites, or the complex of nematodes thought to be injurious to cereals grown repeatedly on the same land. Others study the way in which populations of harmful nematodes increase or decrease when different crops or eelworm-resistant varieties are grown. Other lines of work include the study of chemicals, natural and artificial, that stimulate nematode eggs to hatch, sex attractants and the sexual process in nematodes, and their feeding and behaviour. All aim to increase knowledge and to suggest new ways of controlling nematode pests.

### Systematics and bionomics

Two populations of *Aulolaimus* from sandy soils in Eastern England contained both males and females and were identified as *A. oxycephalus* de Man, 1880, the first record of its occurrence in Britain. Two more populations from India lacked males and could not be identified or described fully. Study of all four populations and of some specimens from continental Europe led us to amend the definition of the family Axonolaimidae to include nematodes with reflexed ovaries, to propose the sub-family Axonolaiminae to accommodate the genera *Aulolaimus* de Man, 1880 and *Pseudoaulolaimus* Imamura, 1931, and to synonymise *Pandurinema* Timm, 1957 with *Aulolaimus*. (Hooper with Dr. M. Shamim Jairajpuri, Aligarh, India)

*Anomyctus* Allen, 1940, also from sandy soil in Eastern England, had the anterior part of the mouth spear slightly curved and unevenly thickened, a hexaradiate oral opening and was much longer than originally described. Some syntypes also had the anterior part of the spear slightly curved and were as long as some English specimens. The characteristic ring connecting the guide rings around the spear seems to be formed by a widening of the stoma lining. (Cooke and Hooper)

A new species of *Aphelenchoides* feeding on fungi and collected in soil



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around citrus roots in South India was described and named *A. goodeyi*. (Franklin with M. Rafiq Siddiqi, Aligarh, India)

For serological tests, *Ditylenchus destructor* and *D. myceliophagus* were cultured on the fungus *Botrytis cinerea* on agar plates and also on mushroom mycelium. The tests and some morphological differences suggest that the species may be distinct. Attempts to get them to interbreed failed. (Hooper and Webster)

*Aphelenchoides composticola*, *A. blastophthorus*, *Aphelenchus avenae*, *Bursaphelenchus fungivorus*, *Ditylenchus destructor*, *D. myceliophagus*, *Paraphelenchus myceliophthorus* and *P. pseudoparietinus* reproduced while feeding on the hyphae of the take-all fungus *Ophiobolus graminis* cultured on plates of potato dextrose agar. (Hooper)

### Plant parasitic nematodes on classical experiments

**Broadbalk.** In 1954 Hesling sampled the fallow strip (Section IV) and found plots 2A and 2B contained most cysts of the cereal cyst-nematode, *Heterodera avenae*, 15 and 10/100 g soil respectively (*Rothamsted Report* for 1954, p. 97). In 1955 the whole field was sampled (*Rothamsted Report* for 1955, p. 111). Section IB plot 8 was the most heavily infested plot (23 cysts/100 g soil) and, of all the sections in the 5-year fallowing cycle, the dunged strips 2A and 2B contained the greatest number of cysts (10/100 g soil). Many of the cysts were empty, and even the largest population was unlikely to injure wheat on heavy land.

In 1960 Winslow studied the migratory nematodes in 4th-year wheat (IB), and in 1961 those in continuous wheat (IA), 4th-year (II) and 1st-year wheat (III). He also took samples to below 24 in. on the side lands opposite sections I, II, III and IV. In 1966 samples were taken in continuous wheat (IA), 4th-year (II) and 1st-year wheat (III) and deep samples on the headland discards of plot 7 in sections IA, II and III. Fewer samples were taken than by Winslow, but sampling was done oftener, and results agree with his. Plant parasitic species present include: root-lesion nematodes, *Pratylenchus neglectus* and *P. thornei*; stunt nematodes, *Tylenchorhynchus brevidens*, *T. macrurus* and *T. icarus*; spiral nematodes, *Helicotylenchus digonicus* and *H. vulgaris*; and the pin nematode, *Paratylenchus microdorus*. There are also many that feed on fungi and bacteria, some minor plant parasites that feed on root hairs and some predaceous nematodes. The first three species named are associated with damage to cereals. *Tylenchorhynchus brevidens* is associated with *Olpidium brassicae* in the stunting of small grains in the United States of America. Stunting of wheat in a light soil is alleged at about 7,600/litre soil. We found up to 3,600 mixed *Tylenchorhynchus* spp./litre soil on Broadbalk. *Pratylenchus neglectus* and *P. thornei*, present in the approximate ratio of 5 : 4, can both injure wheat. *P. neglectus* is associated with *Rhizoctonia solani* in a root rot of winter wheat in Canada, and attacks wheat in Europe. *Pratylenchus thornei* injures wheat and oats in the United States of America and barley in Holland and is consistently associated with a serious root rot of winter wheat in Australia. Both nematodes thrive in heavy soils. The numbers in Broadbalk support this: together the two species constitute over 80% of the

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total of plant parasitic nematodes in the top 25 in. of soil in July, ranging from over 60% at the surface to over 90% between 20 and 25 in. deep. Numbers reached 16,700/litre and 183/g root, and exceeded those at which yield loss may be expected in Europe (*P. neglectus*—4,200–6,300/litre of soil; *P. thornei*—7,000–8,000/litre soil), but were fewer than observed in Canada in the *P. neglectus*/*R. solani* root-rot complex (430/g root in June).

Broadbalk has tile drains 2 ft deep running the length of the plots. *Pratylenchus* spp., with many other nematodes and some other soil animals, are flushed from the soil by heavy rain and can be collected in drainage water from the drain outfalls.

On Broadbalk *Pratylenchus* spp. and other nematodes increase in successive wheat crops after fallow. May is the only month for which populations were assessed in both 1961 and 1966. In both years wheat yields were inversely correlated with the numbers of *Pratylenchus*, which may be affecting the crop adversely, either alone or by aggravating damage done by some other organism.

**Hoosfield.** Hesling surveyed Hoos Permanent Barley in 1955 for *Heterodera avenae* (Rothamsted Report for 1955, p. 111). Most cysts were on Series C (24 cysts/100 g of soil) and strip 4 (21 cysts/100 g). The largest individual count was on 4C (50 cysts/100 g). Many cysts were empty, and the infestation was not thought injurious to barley. Cysts were more numerous than on Broadbalk, possibly because barley is a better host than wheat.

Migratory nematodes were estimated by Winslow in 1960 and 1961, and another survey was started in May 1966. The same plant parasitic nematodes occur as on Broadbalk, but populations were smaller. As on Broadbalk, the main plant parasitic nematodes are *Pratylenchus neglectus* and *P. thornei*, which together constitute 90% of all plant parasitic species present. The maximum number in the 1966 survey was 5,450/litre of soil in plot 4A, maximum in roots 48/g root; the maximum in Winslow's survey was 11,930/litre soil in 2A, which was not sampled in 1966. There was little evidence that *Pratylenchus* spp. were seriously injuring the barley. However, although soil around stunted plants from 4A in June contained only half as many as in the plot as a whole (1,200 compared with 2,800/litre), the roots of stunted plants contained more than four times as many as did roots of healthy plants (45 compared with 10/g root).

*Pratylenchus neglectus* and *P. thornei* were studied further on Broadbalk Section IA (continuous wheat) and Hoosfield. In both, populations

TABLE 1  
Populations of *Pratylenchus* spp. in continuous wheat (Broadbalk Sect. IA) and barley (Hoos Permanent Barley)

	Numbers/litre of soil		Numbers/g root	
	Broadbalk	Hoosfield	Broadbalk	Hoosfield
May	1,620	920	4	5
June	2,970	1,280	14	24
July	9,380	2,530	26	5
August	4,200	1,920	89	4
September	3,270	1,250	30	9
October	3,930	2,000	No roots available	
November	7,120	3,930	No roots available	



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in the soil increased to a peak at the beginning of July, then diminished to increase again in September and October. The pattern of invasion and development of the nematodes in the roots of the two hosts differed considerably, with barley containing most in June, and wheat most in August (Table 1).

**Barnfield.** Beet cyst-nematode *Heterodera schachtii* was first reported on Barnfield when soil from plots 2AC and 8-O were examined by Mr. J. H. Stapley, School of Agriculture, Cambridge in 1935-36. The field was also surveyed in 1944, 1946 and 1954. Only in the survey by Jones and Mr. B. D. W. Morley in 1944, who found the nematode patchily distributed in all plots, were roots examined in addition to soil. This and the decline in mangold yields during the previous 8 years of the experiment's life suggested a relatively recent infestation. Poor growth was associated with a large nematode infestation in some plots. All subsequent surveys were by soil sampling, which indicates the general level of infestation in plots but takes no account of patches. In 1954 Fenwick examined areas of poor growth in plots 8A and 8AC, where parts of the mangold crop failed. The cysts were fewer in the areas where mangolds grew poorly than in the plot as a whole, and he decided that the nematode was not responsible for the poor growth. Hesling and Peters summed up the last three surveys in 1954 (*Rothamsted Report* for 1954, p. 97) by demonstrating a slight, but statistically insignificant, increase in the *H. schachtii* population between 1946 and 1954 and a correlation between plot yields and nematode numbers.

Winslow studied migratory nematodes when the field was fallowed for the first time in 1960 and sampled plots 1-O, 4-O, 4A, 5A, 6A, 8-O and 8A in February, May, August and November. The only numerous plant parasites, except for *Heterodera* larvae, were a few *Paratylenchus* (probably *microdorus*) and *Tylenchorhynchus* spp. Present, but few, were *Pratylenchus* (probably *thornei*) and *Helicotylenchus vulgaris*; most were in plot 1-O, which had 1,040 *Paratylenchus* and 760 *Tylenchorhynchus* spp./litre of soil during spring and 240 and 320/litre respectively in November. In the 6 years since, Barnfield has been fallow for 4, and should now be almost free from plant-parasitic nematodes, except the beet cyst-nematode.

### Nematodes in barley

Ten samples were sent by the National Agricultural Advisory Service from barley fields in the East Midlands, where barley was unthrifty in patches and there was no evidence of damage by cereal cyst-nematode. In all samples the commonest nematodes were *Pratylenchus* spp. With five samples there was no evidence of damage by the nematodes, and poor growth was probably from unfavourable soil conditions. Table 2 shows the numbers of *Pratylenchus* spp. in the other five. (Corbett and Webb)

### British species of root-knot nematode

**Cereal root-knot nematode, *Meloidogyne naasi*.** The biology of this newly found cereal pest was studied in pots of infested soil and in a small plot



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**TABLE 2**  
*Counts of Pratylenchus spp. in soil and in barley roots*

	Root samples		Soil samples		<i>Pratylenchus</i> spp.
	Numbers/g root	Unthrifty	Healthy	Unthrifty	
Park, Farm, Loughborough	102	155	2,350	3,400	<i>P. n.sp.</i> and <i>P. thornei</i>
Forest Farm, Papplewick	78	248	3,750	6,450	<i>P. n.sp.</i> and <i>P. crenatus</i>
Nickolls Bros. Matlock	185	229	5,000	6,000	<i>P. crenatus</i> and <i>P. n.sp.</i>
Thorlby, Ruskington	311	236	—	3,400	<i>P. neglectus</i>
Poplars Farm, Tansor	No roots		3,340	5,100	<i>P. thornei</i> , <i>P. crenatus</i> and <i>P. n.sp.</i>
MEANS	169	217	3,610	5,238	

outdoors infested with the same soil. On the plot, barley and ryegrass were sown monthly from the end of April and plants lifted every 2 weeks. In the April sowing females with egg masses were first found in ryegrass roots after 8 weeks and eggs with embryos 2 weeks later; in barley the same stages were found at 10 and 12 weeks. Development was faster in the June-, July- and August-sown plants; a few embryonated eggs appeared first in barley after 6 weeks and in ryegrass after 8 weeks, but in the September sowings adults were not found by the beginning of November (8 weeks). It seems that at least two generations can be completed during the growing season.

To compare wheat, barley, ryegrass, cocksfoot and sugar beet as hosts of *M. naasi*, seeds were sown in pots of soil with 3,000, 1,500 or 1,000 larvae/g and the roots examined after 6, 8, 10 and 13 weeks. Galling was slight and there were no differences between different infestation rates. Mature females occurred in all hosts at 6 weeks, egg masses first in cocksfoot at 6 weeks and in the other hosts at 8 weeks: numbers of nematodes were similar in the different plant species. Barley, wheat, ryegrass and sugar beet were also sown in soil uniformly infested with 25 larvae per g and the roots examined weekly. Second-stage larvae were found in all plants except sugar beet from the first to the 10th week, showing that invasion was continuous over this period. Young adult females were found in barley in the 4th week, in wheat in the 5th, in ryegrass in the 6th and in sugar beet in the 7th. Egg masses appeared in wheat, barley and ryegrass in the 7th, 8th and 9th weeks respectively. Roots of barley contained three times as many nematodes as roots of wheat or ryegrass, probably because they were larger; many sugar-beet plants were uninfested. Root galls, which began to develop soon after the larvae entered, contained from one to several nematodes. This experiment confirms that *M. naasi* develops well on barley, wheat and ryegrass and also on sugar beet. In one year numbers may increase most on barley, but ryegrass with its longer period of growth may allow more generations to be produced.

**Northern root-knot nematode, *Meloidogyne hapla*.** Soil from a field in Norfolk where carrots were damaged in 1965 by *Meloidogyne hapla* contained more than 177 larvae/100 g soil. To follow the development of *M.*



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*hapla* and its effect on the plants, carrots were sown in infested soil and the roots examined every 2 weeks. The tap root was invaded first and contained adults before they appeared in the laterals. The first adult female was found 4 weeks after sowing. Males appeared later than females; only pre-adult males were found at 6 weeks and mature males at 8 weeks, by when half the adult females had produced egg masses. Eggs with embryos were not found until 12 weeks after sowing. If eggs hatch soon after embryos are formed two generations of *M. hapla* a year would be produced in the field.

Infestation greatly affected the carrots, and at 4 weeks the tap roots of uninfested were more than five times as long as those of infested seedlings; at 6 weeks the average lengths were 396 and 87 mm respectively. All infested tap roots ended in galls, and the laterals from these became invaded and stopped growing. Even slight infestations seriously damaged seedling roots and deformed the storage root.

To assess the effect of larval invasion at different times, carrot seedlings raised in sterilised soil were transplanted into uniformly infested soil at weekly intervals after they first appeared above ground. The first seedlings were transplanted when the cotyledons had expanded and the radicle was 20–30 mm long, the second had tap roots 50–75 mm long with five or six small laterals, the third tap roots 75–150 mm long and the fourth roots longer than 100 mm. The plants grew in the infested soil for 31, 30, 29 and 28 weeks respectively. The roots of the first lot of transplants were more deformed than those transplanted later and had either three or four fangs or were abnormally short and blunt. These observations suggest that anything that delayed infestation for 6–8 weeks would benefit the crop. (Clark and Franklin)

### Cereal cyst-nematode and spring wheat

To study the adverse effect of the cereal cyst-nematode, *Heterodera avenae*, on spring wheat, the formalin experiment begun in 1964 at Woburn was continued (*Rothamsted Report* for 1965, p. 149) by superimposing a third formalin treatment (266 gal/acre) to give eight treatment sequences (Table 3). Twenty plant samples of the spring wheat variety Klocka were taken from each plot in June 1966, the tops and roots were weighed and the

TABLE 3

*Effect of formalin on yield of spring wheat, incidence of "take-all" and cyst eelworm*

Formalin treatments			<i>H. avenae</i> (larvae/g root)	"Take-all" (% plants infested)	Grain (cwt/acre)	Post cropping numbers of <i>H. avenae</i> (eggs/g soil)
1964	1965	1966				
F	F	F	187	5	25.5	37
F	F	—	163	25	21.2	42
—	F	F	133	6	25.5	24
—	F	—	173	22	24.0	23
F	—	F	119	5	25.2	23
F	—	—	140	28	20.7	14
—	—	F	82	48	22.2	7
—	—	—	88	24	20.5	5

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*Heterodera avenae* in the roots counted. "Take-all" infection was also assessed, grain yields (15% moisture content) were measured and the final egg populations of *H. avenae* estimated from soil samples taken after harvest.

Plots treated with formalin in 1966 yielded 24.6 cwt grain/acre, those not treated yielded 21.6 cwt/acre (significant at 5%) but formalin applied in 1964 or 1965 had no effect on yield in 1966. Yields at 0.6, 1.2 and 1.8 cwt/acre nitrogen were respectively 17.6, 23.8 and 28.1 cwt/acre (all differing significantly at the 1% level), with no interactions between amounts of N and any other treatment. The formalin applied in 1966 did not affect the numbers of nematodes invading the plant or the final populations, whereas the formalin applied in 1964 and 1965 did. Heavy rain fell during the 3 days after formalin was applied in 1966, and this may have so diluted the formalin that its nematicidal effect was lost. However, the prevalence of "take-all" was affected. Sample weights were much more strongly affected by 1966 formalin (significant at 0.1%) than were the final grain yields. The residual effects of the 1964 application, which greatly increased *H. avenae* populations at the end of that year, were reflected in the weights of plant tops in June 1966. The mean top weights from plots treated with formalin in 1964 was 35.6 g against 40.4 g from untreated plots (significant at 1%). Formalin applied in 1965 significantly increased (0.1%) the number of *H. avenae*/g root in 1966, whereas applied in 1964 it had no effect. One of the most striking features in the 3 years of this experiment has been the effect on the egg numbers of *H. avenae* after the 1966 crop (see Table 3). The most extreme differences were between the plots given formalin in both 1964 and 1965 and those not. Formalin applied in these 2 years increased the nematode populations from 6.4 eggs/g soil to 39.5/g (significant at 0.1%). Apparently by decreasing the initial infestation by nematodes and the incidence of "take-all", and by generally promoting good plant growth, formalin produced conditions favourable for *H. avenae*, an effect not produced by other soil sterilants applied at Rothamsted and Woburn. Perhaps the nematodes were too few in the other experiments for soil-population increases after the treatments, or formalin may have some unknown effect on enemies or competitors of *H. avenae*. (Williams with Slope, Plant Pathology Department)

The experiment on the effect of a range of soil sterilants (see *Rothamsted Report* for 1965, p. 150) was continued by splitting each of the plots treated in 1965 so that the residual and cumulative effects of the sterilants could be studied (Table 4). The crop was severely attacked by birds, and only straw yields are given.

Applying a sterilant in 1966 and 1965 increased straw yields and decreased nematode invasion of roots more than applying it in 1965 only. Table 4 also shows that the differences in numbers of nematodes that invade the roots are not always reflected in population differences in the soil at the end of the season. The anomalous effects of formalin also showed in this experiment. Formalin in 1965 only increased the numbers of *H. avenae* in the soil after harvest, whereas the numbers invading roots were decreased by the residual effects of the other sterilants and more so by their cumulative action. Straw yield was not significantly increased by formalin applied



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TABLE 4

Effect of fumigation on yields of spring wheat, incidence of "take-all" and cyst nematode

Treatment	Precropping <i>H. avenae</i> , 1966 (eggs/g soil)	<i>H. avenae</i> /g root	Take-all (%)	Straw (cwt/acre)	Post- cropping (eggs/g soil)
0	9	187	6	36.5	9
Formalin:					
Residual	7	180	20	37.9	16
Cumulative		60***	1	58.6***	11*
MeBr:					
Residual	3	98	5	59.7	2
Cumulative		11***	3	63.6*	2NS
Chloropicrin:					
Residual	4	54	11	45.2	5
Cumulative		14***	7	55.8***	2**
Dazomet:					
Residual	5	67	11	47.6	4
Cumulative		23***	1	72.8***	1*

in 1965, but was by the residual effects of the other sterilants. (Williams with Salt, Plant Pathology Department)

Three different rates of dazomet (dimethyltetrahydro-thiodiazine-thione) (100 lb/acre; 200 lb/acre; 400 lb/acre) were compared at Rothamsted and at Woburn (Table 5). Dazomet was applied as an 85% dust and

TABLE 5

Effect of dazomet on spring wheat (Kloka)

	0	Rotavated only	Dazomet 1 (100 lb/acre)	Dazomet 2 (200 lb/acre)	Dazomet 4 (400 lb/acre)
<i>Hoosfield, Rothamsted</i>					
<i>H. avenae</i> /g root	6.4	9.2	0.0	0.0	0.0
Take-all, %	41.9	47.8	22.6	18.2	16.0
Grain, cwt/acre	29.0	31.7**	31.1NS	32.7NS	33.6*
<i>Lansome, Woburn</i>					
<i>H. avenae</i> /g root	41.9	39.1NS	9.9NS	3.5**	0.0***
Take-all, %	19.4	17.3	9.0	2.2	0.5
Grain, cwt/acre	36.6	36.9NS	39.7*	44.1***	44.3***
<i>H. avenae</i> /g soil after 1966 crop	1.9	1.8	0.7	0.6	0.4

rotavated into the soil. On the heavy soil at Rothamsted rotavation alone increased yield 2.7 cwt grain/acre, and only 400 lb/acre dazomet further increased yield. *H. avenae* were not found in the roots from plots treated with dazomet and only few in the control plots. On the lighter soil at Woburn rotavation did not affect yield, but all amounts of dazomet significantly increased yields. More *H. avenae* larvae invaded the roots than at Rothamsted, though not enough to constitute a severe attack. "Take-all" was less than at Rothamsted. At neither place did numbers of *H. avenae* in the soil increase at the end of the year.

Dazomet at Woburn affected the response to nitrogen (see Table 6), and at 200 or 400 lb/acre eliminated the large response to 1 cwt N/acre on the other plots. The cause may be the decrease in *H. avenae* and "take-all"



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TABLE 6

*Dazomet and response of spring wheat to nitrogen (Woburn)*

	Rotavated only	Dazomet 1 (100 lb/acre)	Dazomet 2 (200 lb/acre)	Dazomet 4 (400 lb/acre)
N1 0.5 cwt/acre	31.5	32.0	39.7	41.9
N2 1.0 cwt/acre	40.8**	43.4**	46.2NS	45.3NS
N3 1.5 cwt/acre	38.5NS	43.7NS	46.4NS	45.8NS

\* and NS indicate significance of differences between adjacent means.

attack and the nitrogen released from soil by the greater amounts of dazomet.

The maximum effects of the sterilant may not have been attained in 1966, because weather was adverse when it was applied and sowing was also delayed. (Williams with Salt and Ebbels, Plant Pathology Department)

Tests with nine populations of *H. avenae* from different localities in England and Wales, including Butt Close at Woburn, suggest that the pathotype there (Andersen's race 2 in Denmark or the Netherlands' race C) is the one most common in England and Wales. (Williams)

### Docking disorder of sugar beet

Stunting of sugar beet in neutral or alkaline, sandy soils in England is called Docking disorder, after a village in Norfolk where it was first reported in 1948. Two groups of nematodes seem to be the main causal agents—stubby root nematodes (*Trichodorus* spp.) and needle nematodes (*Longidorus* spp., particularly *L. attenuatus*), but other nematodes, e.g. *Hemicycliophora* (sheath nematodes) and *Pratylenchus* spp. (root-lesion nematodes), may add to the damage in some fields, and fungi, e.g. *Rhizoctonia*, *Fusarium* and *Pythium*, may hasten the decay of injured roots. Attacked seedlings remain small because their damaged roots acquire little nitrogen, magnesium and other nutrients from the soil, and affected plants often show signs of mineral deficiencies.

The bionomics and control of *Trichodorus* spp. and *L. attenuatus* were studied at Docking, Gayton, Gayton Thorpe and Middleton (West Norfolk), Herringswell and Santon Downham (West Suffolk) and at Thornton and Wilberfoss (East Yorkshire). (See also pages 282–286.)

**Stubby root nematodes.** *Trichodorus* spp. are widespread in sandy soils in England and *T. anemones*, *T. cylindricus*, *T. pachydermus*, *T. primitivus* and *T. teres* all fed on the roots of sugar beet seedlings grown in root observation boxes. *Trichodorus* spp. also fed on barley (*T. anemones*, *T. pachydermus*, *T. similis* and *T. teres*), wheat (*T. anemones*), red clover (*T. anemones*, *T. cylindricus*, *T. pachydermus* and *T. primitivus*), carrots (*T. anemones*, *T. cylindricus*), potato (*T. cylindricus*), ryegrass (*T. anemones*) and kale (*T. pachydermus*). They seem not to feed on unhealthy roots, but congregate just behind the root-tips and along the outsides of healthy roots. Affected root-tips are stubby, slightly thickened and may turn brown and grow alternately left and right to give a “zig-zag” root. Many affected root-tips grow again when seedlings are washed free from soil and nematodes and placed in a moist-chamber or in clean soil. When rootlets are killed or the



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young tap root is injured lateral roots proliferate and some of them thicken to give a shallow root system. A thousand hand-picked, washed *T. anemones* added to pots containing steamed sandy soil from Thornton caused stubby root of sugar-beet seedlings grown in the pots, and these seedlings were smaller than those in pots without nematodes or with nematode washings.

In fields where *Trichodorus* was abundant in spring there were significantly more *T. teres*, *T. anemones*, *T. cylindricus* or *T. primitivus* around tap roots of stunted seedlings during June than around vigorous seedlings. However, where *Trichodorus* were few these differences were not found. Not all stunting therefore is from *Trichodorus*, but *Trichodorus*-induced stunting of sugar beet was widespread in East Yorkshire and also occurred in other eastern counties.

Fluctuations in numbers of *T. anemones* at Thornton and a mixture of *T. cylindricus* and *T. teres* at Docking were studied in plots sown with sugar beet or barley, some unfumigated and some fumigated with "D-D" at 400 lb/acre (3.5 ml/sq. ft). Both experiments were on land where sugar beet yielded poorly in 1965. At Thornton "D-D" injected 6 in. deep into waterlogged land in December killed 93% of *T. anemones* before sowing (late March), and in these plots nematodes multiplied little in beet or barley. Before sowing there were 500–600 *T. anemones*/litre in the top 8 in. of unfumigated plots; numbers in these plots had not increased in the rows of barley by the end of May, but the rows of sugar beet then contained 1,200/litre. At the end of July there were 900/litre in the rows of barley and 1,400/litre in the rows of beet, and, in early October, 2,300/litre in the rows of barley stubble and 6,200/litre in the rows of beet. Under sugar beet two generations were produced between April and October, but under barley only one. In early October one plot had 6,200/litre close to stunted beet and 10,300/litre close to larger beet, when random sampling in the rows gave an average of 7,800/litre. At Docking "D-D" injected into moist sandy soil in December killed 99–100% *Trichodorus* in the top 8 in. At sowing time the unfumigated soil contained 625 *Trichodorus*/litre. In the unfumigated plots *Trichodorus* decreased by late July to about 10/litre along the rows of beet and barley, but then increased, and by late September there were 300 in the rows of barley and 500 in the beet rows, which had nearly twice as many females as in the barley rows. At harvest, stunted beet plants associated with *Trichodorus* showed stubby root injury, and some were damaged by *Hemicycliophora* (? *similis* Thorne, 1955), larvae of which were found attached by their stylets to swollen root tips. As at Thornton, *Trichodorus* scarcely multiplied in fumigated soil.

At Wilberfoss "D-D" at 1, 2 and 3 ml/ft was applied during ploughing in December 1964 to the bottom of the furrow in rows 10 in. apart at right angles to the direction in which the field was to be drilled. The amounts killed 76, 96 and 98% of *T. anemones* respectively before the plots were sown with sugar beet. The top 8 in. of unfumigated soil contained 700 *T. anemones*/litre at sowing, 1,000/litre at the end of May and July and 3,600/litre in early October. As at Thornton, there were two generations of nematodes during the growing season. Nematodes did not increase in the fumigated plots until the end of July, but early in October the plots given



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“D-D” at 1, 2 and 3 ml/ft row contained respectively 50, 10 and 6% of the number of *T. anemones* in the unfumigated plots. In one unfumigated plot in October there were 6,400/litre close to stunted beet and 5,000/litre close to larger beet, when random sampling in the beet rows gave an average of 4,300/litre. (Whitehead)

During the above work, stubby root nematodes, *Trichodorus* spp., were identified from 156 sites in 86 beet fields in Eastern England (Table 7);

TABLE 7

*Number of sites containing Trichodorus spp.*

Sugar-beet factory area	<i>T. anemones</i>	<i>T. cylindricus</i>	<i>T. pachydermus</i>	<i>T. primitivus</i>	<i>T. similis</i>	<i>T. teres</i>	<i>T. viruliferus</i>	No. of sites	No. of fields examined
York, Yorks	6	3	10	8	11	6		22	10
Cantley, Norfolk		5	2	13	1	1	6	27	14
Wissington, Norfolk			6	4				12	7
Kings Lynn, Norfolk		14	11	16	1	3	9	31	21
Ipswich, Suffolk			18	9	2			29	15
Bury St. Edmunds, Suffolk		1	26	4	1		6	34	18
Brigg, Lincs			1			1		1	1
TOTALS	6	23	74	54	16	11	21	156	86

of the seven species found, *T. pachydermus* Seinhorst and *T. primitivus* de Man were the most frequent. *T. anemones* Loof, 1965, recently described in Holland, numbered up to 8,000/litre of soil in fields containing diseased sugar beet in Eastern Yorkshire. (Hooper and Whitehead)

**Needle nematode.** The needle nematode *Longidorus attenuatus*, is also widespread in alkaline, sandy soils in England and associated with diffuse patches of stunted sugar beet. In observation boxes it fed on sugar beet by inserting its stylet into the tips of the lateral roots where it caused small galls. Similar galls occur on plants in fields containing *L. attenuatus*, often together with a necrotic spot behind the root-tip. The tap roots of attacked plants are not obviously injured, but although normally shaped are small.

In fields, infested sugar-beet seedlings showed typical root galling in June, and *L. attenuatus* was often found entangled in the lateral roots of gently washed seedlings. In a field near Woodbridge, East Suffolk, there were 300/litre in the soil close to much-stunted seedlings and 115/litre close to less-stunted ones. Stunting associated with *L. attenuatus* was also observed at two sites in West Norfolk.

Field experiments begun in 1965 at Santon Downham and Gayton to study the bionomics of *L. attenuatus* continued. Plots of unfumigated soil were resown with sugar beet, barley, red clover, ryegrass, kept bare or left to grow weeds. Plots fumigated with 400 lb/acre “D-D” or chloropicrin in February 1965 were resown with sugar beet. The numbers of *L. attenuatus* in the top 8 in. of soil were determined just before sowing and at 2-monthly intervals until early October. In contrast to 1965, *L. attenuatus* did not increase in the weeds, beet, barley and red clover plots, but the red clover plots contained more nematodes at sowing than during the previous autumn, probably because ploughing brought up a more heavily infested layer of soil from below 8 in. The nematode was still rare on sugar-beet plots fumigated in 1965. (Whitehead)



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**Depth distribution of the needle nematode in soil.** The distribution of *L. attenuatus* in depth was studied in 10 plots at Gayton, five sown with barley and five with beet. Three-inch-diameter cores 16 in. deep were taken in the rows and divided into four 4-in. sections. Sampling, at 6-weekly intervals, started before sowing in March. There was no evidence that migration up or down the soil profile during the growing season redistributed the nematodes. Only on one plot of barley for one period, October, was downward migration a probable explanation for larvae increasing in the 4–8-in. layer. All other changes tended to occur together throughout the profile, and so probably reflected deaths or births.

Numbers increased more with depth in additional plots growing clover or ryegrass than in those with beet or barley. Through the whole season nematodes in the 0–8-in. layer were most abundant in the clover plots followed by barley, beet and ryegrass; in the 8–16-in. layer the order was clover, ryegrass, barley and beet. During dry spells in June numbers in all plots decreased in the 0–4-in. layer. The decrease extended into the 4–8-in. layer in July under the ryegrass. In barley and clover, numbers decreased greatly until June, and in ryegrass they decreased in August and September. Then they increased, and by November the numbers in all plots resembled those in May.

The proportion of larvae in the population averaged 66% and changed little during the year or at different depths, except that the proportion was smaller in the topsoil of the beet plots and in soil 8–16 in. deep in the barley plots. The percentage of larvae in beet and barley plots was below average at the start, and remained so in beet but increased in barley. In ryegrass the proportion was above average in May and November. In clover the proportion dropped to below average in June, then increased steadily to 90% larvae in November. There was a general decrease in the proportion of larvae during dry spells in June and September, followed by an increase. The small changes in production of larvae suggest that under beet the nematode maintained numbers but reproduced little; under barley it reproduced a little throughout the profile; under clover it reproduced readily below 4 in. and under ryegrass below 8 in. (Green)

**Other plant-parasitic nematodes in sandy soils.** The experiment at Gayton was sampled on seven occasions and populations of several genera of small plant-parasitic nematodes estimated. In January effects from the 1965 cropping and fumigation were large. All genera increased slowly through the year in the sugar-beet plots fumigated in February 1965; *Pratylenchus* from 20% of the number in the unfumigated plots in January to 29% in November, *Paratylenchus* from 28 to 71%, *Tylenchorhynchus* from 6 to 11% and *Trichodorus* from 1 to 10%. The fallow plots maintained a small population of all common plant-parasitic genera, which presumably fed on germinating weed seeds in the soil. Numbers of *Pratylenchus* in the soil fluctuated little under most crops except for a peak of 5,100/litre in May under ryegrass. Under all crops populations were greatest in November: ryegrass 6,100/litre, barley 5,100/litre, sugar beet 3,400/litre and red clover 1,500/litre. *Paratylenchus* was the most abundant plant-parasitic nematode, and population differences under different crops



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established in 1965 were maintained through 1966. As in 1965, ryegrass plots contained most, and reached 36,800/litre by November, when barley and red clover had 3,700/litre and sugar beet 1,900/litre. Many *Heterodera* eggs hatched during spring and many larvae were released into the soil. *H. avenae* was three times as numerous in barley plots in March as in any other plots because of the extra cysts produced on this host in 1965. Many *H. trifolii* eggs hatched in the red clover plots during autumn. *Tylenchorhynchus* multiplied rapidly through the year under ryegrass and reached a maximum of 11,300/litre in November, and barley had 3,500/litre, red clover 1,000/litre and sugar beet 150/litre. (D. A. Cooke, Broom's Barn)

**Bionomics and control of stubby root and needle nematodes.** It may be possible to predict fields or parts of fields likely to suffer Docking disorder from the numbers of *Trichodorus* and *Longidorus* in the soil in mid-winter or in early spring. The soil in 133 sample sites, each 15 × 15 ft, in 67 fields of light sandy soil in East England was sampled by fieldmen of the British Sugar Corporation in mid-winter, and some sites were resampled at sowing. In mid-winter there were 500–1,000/litre *Trichodorus* at eighteen sites, 1,000–2,000/litre at eight and more than 2,000/litre at two; nine had 50–100/litre *L. attenuatus* and three 100–200/litre. At sowing there were only about half as many *L. attenuatus* as during mid-winter, and beet was less injured by *Longidorus* in 1966 than in 1965 and 1964, but the populations of *Trichodorus* remained unchanged. Stunting of beet seedlings in June by *Trichodorus* occurred where populations at sowing exceeded 500/litre and by *Longidorus* where they exceeded 50/litre. Only one site was found with damaging numbers of both *Trichodorus* and *Longidorus*.

Because *Trichodorus* and *Longidorus* can feed on a wide range of crops and weeds, they cannot easily be controlled by crop rotation, but they are killed by soil fumigation. At Gayton and Santon Downham in 1965 treatment with "D-D" or chloropicrin significantly increased yields of tops, roots and sugar (Table 8). At harvest all tops and roots were removed

**TABLE 8**  
*Effect of soil fumigants applied the previous year on yields of sugar (cwt/acre)*

Treatment	Gayton		Santon Downham	
	1965	1966	1965	1966
"D-D"	65.7***	60.9	56.7*	74.3***
Chloropicrin	67.8***	57.2	64.0**	62.3
Control	47.9	52.3	39.3	56.6
L.S.D. (5%)	5.0	12.2	12.9	8.0

from the experiment and the plots sown with beet once more in 1966. The crop was again better on the plots fumigated in 1965, particularly those treated with "D-D", and at Santon Downham the yields from these plots were significantly greater than from unfumigated plots. At Gayton, despite nettings, pheasants severely damaged the plants on the "D-D" and chloropicrin plots, and yield differences were insignificant. At Santon Downham and Gayton control of *L. attenuatus* in 1966 by the 1965 fumigation was still good in the top 8 in. of soil.



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At Docking 400 lb/acre "D-D" injected into the soil in December 1965 greatly increased yield of beet sugar but not barley (Table 9). The beet

**TABLE 9**  
*Effect of "D-D" on yields of sugar and barley grain (cwt/acre)*

Treatment	Sugar		Barley grain (15% moisture)	
	Docking	Thornton	Docking	Thornton
"D-D"	43.8***	48.5	28.3	18.1
Untreated	24.8	55.8	34.1	20.5
L.S.D. (5%)	5.3	18.6	9.6	3.9

crop was uniform on fumigated plots but uneven on unfumigated plots, where most plants were much stunted at harvest. At Thornton in a similar experiment "D-D" applied in December damaged beet sown in late March because the soil was too wet before sowing to get adequate ventilation. Beet grew more evenly on the fumigated plots, but yields were no more than on the unfumigated plots, even though yields in these seemed negatively correlated with numbers of *T. anemones* in the beet rows at harvest. "D-D" treatment did not increase yield of barley grain either (Table 9).

Although *Longidorus* and *Trichodorus* can be controlled by 400 lb/acre or either "D-D" or chloropicrin, the cost of the chemicals alone is £50 for "D-D" and £200 for chloropicrin. To see whether control could be cheapened by using smaller amounts of the chemical, in an experiment at Gayton in 1965 various amounts of "D-D" were applied in predetermined sugar-beet rows 21 in. apart. Sugar yield was increased by 2.5 and 3.5 ml "D-D"/ft of row but not by 7 ml or by 2.5 or 3.5 ml/sq ft of plot. In 1966 the plots were drilled with spring barley, which grew and yielded significantly better in treated than in the untreated plots. The loss of sugar yield in 1965 from the largest doses of "D-D" is therefore thought to have been because they damaged the beet. (Table 10.)

**TABLE 10**  
*Residual effect of "D-D" on yields of sugar and barley (cwt/acre) at Gayton*

Treatment	Sugar (1965)	Barley grain (15% moisture) (1966)
"D-D" 2.5 ml/ft row	64.2**	31.1**
3.5	61.5**	30.3**
7.0	56.6	30.5**
2.5 ml/sq ft	56.2	30.0*
3.5	57.0	31.0**
Untreated	52.3	26.9
L.S.D. (5%)	6.0	2.5

During mid-winter small doses of "D-D" were dribbled in lines about 6 in. deep and 10, 20 or 21 in. apart at right angles to the direction sugar beet was to be drilled in spring. By sowing time at Wilberfoss 1, 2 or 3 ml "D-D"/ft in rows 10 in. apart had killed 76, 96 or 98% of *T. anemones*; seedling emergence was improved and seedlings weighed 83, 56 or 61% more than those from untreated plots. However, yields at harvest were not significantly increased (Table 11). As 76% of *T. anemones* were killed when 1 ml/ft was used in rows 10 in. apart, the fumigated band seemed to



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be about 7½ in. wide. At Herringswell, with a coarser sand, 1 and 2 ml/ft in rows 21 in. apart killed nematodes well throughout the topsoil. In another field at Herringswell, where there were about 35 *L. attenuatus*/litre of soil mid-winter, beet redrilled after wind erosion in May yielded better on plots from 1 or 2 ml "D-D"/ft in rows 10 in. apart than on untreated plots (Table 11). At harvest plots given 0.5, 1.0 and 2.0 ml/ft contained

**TABLE 11**  
*Yields of sugar (cwt/acre)*

Treatment	Wilberfoss	Herringswell†
"D-D" 0.5 ml/ft, rows 10 in. apart		21.8
1.0	58.3	23.4*
2.0	59.7	26.5**
3.0	58.0	
Untreated	58.5	17.1
L.S.D. (5%)	6.5	5.8

† Yields after resowing.

only 44, 13 and 13% of the numbers of plant-parasitic nematodes in the untreated plots.

Experiments at Gayton Thorpe and Middleton (Table 12), on land infested with *L. attenuatus*, measured effects on sugar yields of treating land in winter with various amounts of "D-D" and their interaction with nitrogen. Either 150 (N<sub>1</sub>) or 300 (N<sub>2</sub>) units of nitrogen was applied as "Nitro-Chalk". Amounts of "D-D" less than 2.0 ml/ft could not be applied satisfactorily because the feed pipe blocked. At Gayton Thorpe "D-D" at 3 ml/ft every 10 in. significantly increased yield of sugar, as also did 2 ml/ft every 10 in. with 150 units of N but not with 300 units. On average, extra nitrogen also significantly increased yield of sugar, but the nitrogen effect was greatest on untreated plots. At Middleton 3 tons of sugar/acre was obtained from untreated soil: neither "D-D" nor extra nitrogen increased sugar yield.

**TABLE 12**  
*Effects of "D-D" applied by blade coulter (A) or plough sole applicator (B) on sugar yields (cwt/acre)*

Treatment	Gayton Thorpe (A)	Middleton (A)	Treatment	Gayton Thorpe (B)	Middleton (B)
<i>Whole Plots</i>					
Control	52.7	60.5	Control	44.4	Control 52.1
"D-D" 0.5 ml/ft every 10 in.	53.7	59.6	"D-D" 0.5 ml/ft every 10 in.	53.1**	(0.7 ml) 51.4
"D-D" 2.0 ml/ft every 10 in.	55.6	65.0	"D-D" 1.0 ml/ft every 10 in.	55.0**	(1.4 ml) 54.9
"D-D" 3.0 ml/ft every 10 in.	58.8*	60.7	"D-D" 2.0 ml/ft every 10 in.	52.3**	(2.8 ml) 55.4
"D-D" 0.5 ml/ft every 20 in.	53.9	59.8			
L.S.D. (5%)	5.4	6.2		4.9	5.5
<i>Half Plots</i>					
N <sub>1</sub>	50.8	62.6***		48.7	56.1*
N <sub>2</sub>	55.0***	59.0		53.8	50.7
L.S.D. (5%)	1.5	1.8		6.5	4.3

Small amounts of "D-D" were applied effectively in smaller experiments on the same fields with a plough-sole applicator. During reploughing, using



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constant-head gravity-flow and a controlled land speed, "D-D" was metered into the bottom of 10-in.-wide furrows at 0.5, 1.0 and 2.0 ml/ft at right angles to the intended direction of drilling. There were 70–90 *L. attenuatus*/litre soil during mid-winter on the experimental sites, but only half as many in May. At Gayton Thorpe in May there were more seedlings/yard of row in treated than in untreated plots, and although extra nitrogen greatly increased the weight of seedlings on unfumigated plots, "D-D" fumigation doubled seedling weights and significantly increased sugar yield. At Middleton, where nematodes were less well controlled, seedling weight was little affected by extra seed-bed nitrogen or "D-D". "D-D" did not increase sugar yields, and extra nitrogen decreased them. In October there were 3,000 *Trichodorus*/litre of unfumigated soil at Gayton Thorpe but only 300/litre at Middleton. (Whitehead and Greet)

In four trials on sandy soils where beet had previously grown poorly only slight improvements in growth and yield were obtained from fumigation with "D-D" or chloropicrin. Neither needle nematodes (*L. attenuatus*) nor stubby root nematodes (*Trichodorus* spp.) were abundant on these four sites. (Greet)

### Host-parasite relationships

**Plant-growth substances and reproduction.** Natural plant-growth substances affect the rate the bud and leaf nematode, *Aphelenchoides ritzemabosi*, reproduces in tissue cultures. Proteolytic enzymes secreted by the nematodes may release from plant tissue substances that stimulate cell activity and provide a nutritional environment favouring nematode reproduction, for they multiply fastest when plant growth or cell activity is greatest.

Applying plant-growth substances and their inhibitors to leaves of growing plants affected the multiplication of nematodes. Both gibberellic acid (2.0 mg/plant) and indole-3-acetic acid (IAA) (1.5 mg/plant) increased root weight and doubled the number of female *Heterodera rostochiensis* on potato (Arran Banner) roots. A gibberellin antagonist, (2-chloroethyl)-trimethylammonium chloride (CCC), applied to the soil (47 mg/plant) doubled the number of males but did not significantly alter the number of females or size of the root system. CCC greatly decreased the number of *Ditylenchus dipsaci* in oats, whereas tryptophane (an IAA precursor) increased the number. (Trudgill and Webster)

Extracts of lucerne callus infected with *Aphelenchoides ritzemabosi* contained more growth substances than extracts of the artificial tissue or of the nematodes. The active substance had an Rf value similar to that of tryptophane. (Webster with Burnett, Botany Department)

**Races and pathotypes.** Hybrids between the lucerne race (LR), red clover race (RCR), white clover race (WCR), narcissus race (NR), tulip race (TR) and oat race (OR) of *Ditylenchus dipsaci* were produced and reared on onion seedlings, and tested on three bulb hosts, narcissus, tulip and hyacinth (Table 13). On average, the hybrids multiplied less than their parent races (Table 14). RCR and TR inoculated together into tulip



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**TABLE 13**  
*Reproduction of race hybrids on narcissus, tulip and hyacinth*

	Narcissus	Tulip	Hyacinth
LR	1	3	1
RCR	2	1	1
WCR	1	2	1
NR	3	3	1
TR	3	3	2
OR	1	3	2
LR♂ × WCR♀	1	3	1
RCR♂ × LR♀	3	2	1
NR♂ × LR♀	3	3	1
LR♂ × OR♀	2	3	1
RCR♂ × OR♀	1	2	1

3 = Rapid, 2 = Slow, 1 = Entry but no multiplication

produced significantly fewer nematodes than did TR alone and more than RCR alone. Hybridisation probably occurred. As RCR males do not induce RCR females to lay eggs in tulip (Table 13), it is unlikely that TR males would do so. Competition between the RCR and TR males to fertilise TR females would result in progeny derived from TR♂ × TR♀ and the hybrid RCR♂ × TR♀. The mixed inoculum may have produced fewer offspring than TR alone because the hybrid was infertile and numbers depended on the multiplication of the TR × TR crosses.

**TABLE 14**  
*Mean number of nematodes extracted from tulip after 12 weeks*

Race	Number of nematodes inoculated per plant	Mean numbers produced per plant
RCR	273	68 ± 12
RCR	546	60 ± 30
TR	262	21,953 ± 1,057
TR	524	21,945 ± 1,136
RCR + TR	273 + 262	5,902 ± 2,399

Antisera produced from saline extracts of five *Heterodera* species, two pathotypes of *H. rostochiensis*, three *Ditylenchus* species (see p. 158) and four races of *D. dipsaci* were tested against their homologous and heterologous antigens. All *Heterodera* extracts reacted with their homologous antisera, and some reacted with antisera to other *Heterodera* spp. *H. schachtii* has common antigens with *H. trifolii* and *H. cruciferae* with *H. goettingiana*. Antisera to both *H. cruciferae* and *H. goettingiana* precipitated extracts of *H. carotae*. The populations of *H. rostochiensis* reacted similarly, although two were mainly pathotypes 0 and 2, and the other mainly pathotype 1. The cyst wall or egg shell was probably the source of the antigen in the cyst extracts, because the antisera did not react with extracts of the homologous free-living, second-stage larvae, but did react with extracts of homologous mature white females. The tests between the nematode extracts and heterologous antisera showed that the *Heterodera* species tested formed two serologically distinct groups. One contained *H. rostochiensis*, *H. schachtii* and *H. trifolii* and the second group *H. goettingiana*, *H. cruciferae* and *H. carotae*. The four races of *D. dipsaci* were serologically indistinguishable from each other, but were distinguishable



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from *D. destructor* and *D. myceliophagus*. *D. myceliophagus* had more antigens in common with some *D. dipsaci* races than with *D. destructor*. (Hooper and Webster with Gibbs, Plant Pathology Department)

**Amino-acid antimetabolites.** Eight amino-acid antimetabolites were tested for their effects on four nematode species and on the growth of their host plants in pots. DL- $\beta$ -phenylalanine and DL-valine had little effect on either plants or nematodes. DL-ethionine decreased the number of *Ditylenchus dipsaci* in onions by 98%. DL-aminobutyric acid greatly decreased the number of *Nacobbus serendipiticus* galls on tomato, DL-alanine decreased *Heterodera avenae* females on wheat by 86% and *Aphelenchoides ritzemabosi* on lucerne by 82% without harming the plants. Similar tests with *A. ritzemabosi* in lucerne callus in sterile culture showed that all antimetabolites decreased the number of nematodes and depressed plant growth more than in plants in pots of soil, probably because the antimetabolites were not broken down by microbes. (Prasad and Webster)

### Clumping of *Ditylenchus dipsaci* in water

Films made of *D. dipsaci* clumping in water suggested that prerequisites for clumping were: (a) some moving vigorously; (b) the culture must contain enough nematodes per unit volume for encounters to be frequent, because clumping is by random interlocking and seems not to depend on sensory stimulation. Individual nematodes in a sparse population did not change behaviour when touched by other nematodes. However, when nematodes entered clumps their movement was obstructed by those in the clump and was restricted to bending or hooking actions that favoured interlocking and consolidation of the clump. Clumps were essentially dynamic aggregations whose formation or dispersal could be assisted by any external factors that favoured directional movement of nematodes.

Of 12 single *D. dipsaci* in micro-chambers recorded continuously for 20 hours by time-lapse cinematography, some were more active than others, but all rested from time to time. Resting was followed by a period of body bending that sometimes developed into locomotory movement. Nematodes were little more active when in pairs than alone. (Doncaster and Webster)

### Yields of potato varieties resistant to potato cyst-nematode

At Woburn, on land that had not carried potatoes for more than 10 years, the eelworm-resistant variety Maris Piper yielded 13.04 tons/acre of ware tubers and the susceptible varieties Pentland Dell, Majestic and paracrinkle-free King Edward yielded 7.72, 9.55 and 10.71 respectively. In another experiment in the same field Pentland Dell and Maris Piper were grown on land slightly and moderately infested with potato cyst-eelworm. Some plots were irrigated and some were fumigated with "D-D" at 400 lb/acre. Irrigation depressed yields by about 20%. Fumigation doubled the yield of Pentland Dell on moderately infested land and increased it by a fifth on slightly infested land; it increased yield of Maris Piper by a quarter and a fifth respectively.



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TABLE 15

The effect of crop sequences on pre-cropping nematode population and on the yield of resistant (R) and susceptible (M) potatoes

Treatment effects								
Preceding crops	M	R	R	R	R*	M	M	R
Crops to which results refer	M	M	M	R	R	R	R	R
Average precropping numbers, years 2-7, means of 24 plots, egg/g	85 ±0.7	71 ±0.6	51 ±0.6	39 ±0.7	78 ±0.7	107 ±0.6	63 ±0.6	105 ±0.6
Average yields, years 2-7, means of 24 plots, lb/plot	11.5 ±0.9	20.1 ±0.5	23.9 ±0.5	26.1 ±0.9	21.5 ±0.9	14.8 ±0.5	19.9 ±0.5	11.1 ±0.5
Seasonal effects								
Years	1 1960	2 1961	3 1962	4 1963	5 1964	6 1965	7 1966	Means
Average precropping numbers, eggs/g								
M, means of 12 plots	67	81	59	75	61	71	68	68.3 ±8.6
R, means of 20 plots	68	127	69	89	52	62	70	74.1 ±6.7
Average yields, lb/plot								
M, means of 12 plots	34.3	10.5	15.6	5.7	19.1	38.6	21.6	20.79 ±1.8
R, means of 20 plots	40.1	6.7	16.8	5.0	27.5	32.8	24.7	21.98 ±1.3
Sowing dates								
	29 May	24 March	26 April	7 May	7 May	8 April	3 May	

M = variety Majestic.

R = NY4/27, ex *andigena* hybrid.

\* = M not R grown in first year to start sequence at a higher population density than average (i.e. 216 eggs/g) compared with an average of 65 eggs for the remainder. Statistical analysis based on four-plot treatment means.

Table 15 summarises the results of a long-term trial on heavily infested land at Woburn where the experimental resistant variety NY24/7 was compared with the variety Majestic. Like Maris Piper, NY24/7 was bred by Dr. H. W. Howard, Plant Breeding Institute, Cambridge, from *Solanum tuberosum* ssp. *andigena*. Standard errors apply to means over all four replicates and the 6 years from 1961 to 1966. They are estimated from plot-to-plot variations only, and make no allowance for seasonal variations in yields of varieties or their effects on nematode numbers. Yields after the crop sequences follow expected trends, i.e. are greatest when pre-cropping egg counts are least. The varieties Majestic and NY24/7 yielded similarly over the 7 years of trial. Season affected tuber yields more than cropping sequence. Nematode numbers were less influenced by season than yields, partly because, even when potatoes are grown, a large fraction of the encysted eggs is carried over unhatched to the succeeding year. Mean seasonal yields and sowing dates were not correlated.

Table 16 summarises results of a trial in which a susceptible potato variety was compared with one with a gene (A) for resistance derived from *S. tuberosum* ssp. *andigena*, another with a gene (B) from *S. multidissectum* and a third with both (AB). Tubers were supplied by Dr. Howard. Before planting, half the plots were fumigated with methyl bromide to destroy



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TABLE 16

*Yields of resistant potato varieties on infested land before and after fumigation with methyl bromide (lb/plot)*

	<i>ab</i>	<i>Ab</i>	<i>aB</i>	<i>AB</i>
Unfumigated	15.2	20.3	21.6	27.1
Fumigated	47.2	65.5	76.3	81.1 ± 3.84***
Mean effect	31.1	42.9	48.9	54.2 ± 2.71***
O versus MeBr		<i>a</i> versus <i>A</i>		<i>b</i> versus <i>B</i>
20.9	67.5	39.9	48.5	37.0 51.6 ± 1.92

nematodes and other pathogens. Assuming the better performance of the resistant varieties reflects their possession of genes for resistance and not other genetical properties of these hybrids, gene *A* increased yield by 20%, gene *B* by nearly 40%. On the unfumigated plots the variety with genes *A* and *B* gave a yield almost equal to the sum of expected improvements, but on the fumigated plots, where pathogens were eliminated, it gave much less.

Resistant varieties are invaded by larvae and injured by them, but the greater yield on infested land of varieties with gene *A* or *B* shows that varieties with these genes suffer less than varieties without. However, at Woburn the increases in yield were less than found with gene *A* in some trials by the National Institute of Agriculture Botany, and fumigation with methyl bromide at Woburn increased yields so much that pathogens other than potato cyst-nematodes may also have been diminishing yields. (Jones and Parrott)

### Hatching of cyst-nematode eggs

Quiescent larvae of potato cyst-nematode in eggs freed from cysts were stimulated by perfusing them with 0.0025*M*-sodium metavanadate or potato-root diffusate and time-lapse films made during the next week. Even in water, some larvae alternated occasional movements with hours or days of quiescence. Sooner or later, larvae in both hatching stimulants began moving forward and backward and swung their heads. Emergence from the eggs was always preceded by some minutes or hours of stylet thrusting, during the later stages of which the head was thrust against one end of the egg or against both alternately. The stylet was never thrust at the side walls. The force with which the body thrust the head against the egg shell shortened and flattened the head but did not distort the egg shell.

By the time a larva emerged, the distensible distal ends of its pharyngeal gland ducts were swollen with globular secretions. The dorsal glands of those stimulated by root diffusate were active. Larvae stimulated by sodium metavanadate behaved differently: they had distended sub-ventral gland ducts, but the dorsal glands were less active and larvae emerged sooner. Larvae stimulated with root diffusate used their stylet tips to make a line of perforations so close together that each split through to the previous one and made a continuous cut. One larva, while probing with its stylet, found the beginning of an earlier cut suggesting the association of proprioceptors with the stylet protractor muscles. Larvae stimu-

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lated with metavanadate thrust their stylets more randomly, and only with this stimulant were the sides of the stylet used in a sawing action to cut short slits. Two larvae that perforated one end of the egg with their stylets moved to the other end; later, returning to the first, they slowly pushed their heads through the seemingly softened egg shell without further stylet thrusting. (Doncaster and Shepherd)

Various inorganic ions and organic compounds were tested for their ability to stimulate hatching of eggs of seven common British species and two foreign species of cyst-nematode. Ions of 23 elements, mostly metals, hatched some or many eggs of at least one species.  $Zn^{2+}$  hatched many eggs of six species and  $[PtCl_6]^{2-}$  four. *H. schachtii* was stimulated by more ions than any other species. No ion increased the hatch of *H. avenae* to above that in water. The response to ions did not suggest any obvious affinities between species. Each species shared at least one active ion with at least one other, but no two had exactly the same range of active ions. However, differences between the response of *H. tabacum* and *H. rostochiensis*, thought possibly to be pathotypes of the same species, suggest that they are distinct species (see, however, p. 158). This is borne out by differences in their response to some organic compounds. Hatching of *H. rostochiensis* was stimulated as much by vanadate ions as by potato-root diffusate; the only other compounds equally stimulatory are anhydrotetrionic acid and picrolonic acid (4-nitro-3-methyl-1-*p*-nitrophenylpyrazolone). These last two were among four organic compounds whose ability to hatch eggs of all the species mentioned except *H. avenae* was compared. The other two were picric acid (2,4,6-trinitrophenol) and flavianic acid (2,4-dinitro-1-naphthol-7-sulphonic acid). Each hatched many eggs of at least one species. Picric acid hatched *H. schachtii* and *H. tabacum*, picrolonic acid *H. rostochiensis*, *H. schachtii* and *H. glycines*, anhydrotetrionic acid *H. rostochiensis*, *H. schachtii* and *H. tabacum*, and flavianic acid *H. cruciferae*, *H. glycines*, *H. schachtii*, *H. tabacum* and *H. trifolii*. Thus *H. schachtii* was the only species stimulated by all four compounds. Flavianic acid also hatched a few more eggs of *H. goettingiana* than hatched in water. None hatched *H. carotae*. Flavianic acid and zinc and ferrous salts are the only compounds that hatch many eggs of *H. glycines*, for which no root diffusate is known to stimulate hatching *in vitro*.

Many redox dyes, including indamines, indoanilines, indophenols, viologens, azines and thiazines, stimulate *H. schachtii* eggs to hatch. None except the indamine tolylene blue, the azine phenosafranine and phenol-indophenol hatched the other species. *H. cruciferae* and *H. schachtii* hatched well, and *H. glycines* and *H. rostochiensis* moderately in tolylene blue; *H. tabacum* hatched moderately in phenol-indophenol and *H. trifolii* moderately in phenosafranine.

A characteristic that recurs in organic compounds active in hatching *H. schachtii* is an extended chain in the molecule, with terminal polarisable atoms; many of the most active have a chain of five atoms. The activity of anhydrotetrionic and picrolonic acids with *H. rostochiensis* may be associated with a similar but longer chain of seven to nine atoms. Vanadate solutions may also provide a chain, with polarisable terminal atoms, of similar length to that of these two acids. Too few compounds are known



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that stimulate the other species to suggest any association between structural features and activity.

The diversity of ions that stimulate hatching makes it unlikely that ions participate directly in an enzyme system within the egg or larva. This seems even more unlikely in view of the many organic compounds that stimulate *H. schachtii*. More probably ions and other hatching agents are taken up by some constituent of the egg or larva and alter the structure and function of the binding material. There is, however, no correlation between the hatching of *H. schachtii* by ions and the known stability sequences of various biological metal-binding systems, which suggests that there may be several sites of action that differ in their response. (Shepherd with Clarke, Biochemistry Department)

### Sexual behaviour of cyst-nematodes

Females of most species of cyst-nematodes probably secrete substances that attract their males, for, in tests, females of *H. schachtii*, *H. glycines*, *H. cruciferae*, *H. tabacum* and *H. rostochiensis* all did so. The specificity of these attractants is of interest because it may indicate natural groupings of species, also because it probably influences ability of species to hybridise. The males of *H. schachtii* and *H. rostochiensis* were not attracted to the females of the other species. About a fifth of the males of *H. schachtii*, *H. glycines* and *H. cruciferae* were weakly attracted to each other's females. Some males of *H. schachtii* were also attracted to females of the parthenogenetic *H. trifolii*. Males of *H. tabacum* were attracted weakly by their own females and more strongly by females of *H. rostochiensis*, but males of *H. rostochiensis* were strongly attracted by their own and by females of *H. tabacum*, suggesting a close relationship between the species (see, however, p. 153). Males from a "Woburn population" of *H. rostochiensis* bred on Arran Banner potato (pathotypes 0 and 2) were attracted by their own females and by females from a population that had passed two generations on an experimental ex *andigena* × ex *multidissectum* potato variety (pathotype 1,2). It has been assumed that pathotypes of *H. rostochiensis* interbreed freely: these observations show that males and females of some are brought together.

As a male can successfully mate with many females, it would be biologically advantageous if its behaviour changed after mating so that it moved away and was attracted to another female. Such a mechanism exists in males of *H. schachtii*, for those that had access to females overnight, or access limited by a dialysis membrane, were less active and less responsive to attraction than virgin males. After 5 hours separation from females, however, ability to respond tended to return. Females of *H. schachtii* also attracted males less after being mated and, again, the decreased attractiveness was transitory, for after 5 hours separation the females were as attractive as the virgin females. The change in male behaviour may be explained by fatigue of the sensory organs. This behaviour pattern, which favours males mating with several females, and the fact that males mature before females, encourages outbreeding, but does not prevent interbreeding when populations are sparse.



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A start was made in studying the purification of the attractant produced by females of *H. rostochiensis* and *H. schachtii*. Neither volatilised *in vacuo* or in dry air at 40° C. Both were slowly inactivated in solution by U.V. irradiation but not by boiling for a short time nor by 90° C for 20 minutes, 80° C for 20 minutes, 35° C for 3 days or 25° C for 6 days. From dried preparations they were soluble in water but not in acetone, methanol or ether. When an aqueous solution was shaken with ether the attractants remained in the aqueous phase but passed into the ethereal layer if the solution was made acid or alkaline. The attractants appear to be polar organic compounds. (Green)

### The effects of gamma-radiation on potato cyst-nematode

Work on the sterilisation of insects with gamma-radiation showed that most are greatly harmed by 20,000 rads and are sterilised by much smaller doses. Adult males and females of *H. rostochiensis* seem to be more resistant, as their ability to mate and produce eggs was unaffected by 16,000 rads, though the viability of the eggs was not tested. Also, larvae irradiated at doses up to 16,000 rads, both before and after inoculation on to tomato seedlings, developed into adults, although the seedlings suffered badly. Irradiation of cysts had a marked effect on hatching and fecundity, but the effect was dependent on pre-irradiation treatment as well as dose. If the cysts were dry at irradiation, hatching was almost doubled and none of the eggs were killed. However, other treatments decreased hatch and, in some tests, killed eggs. The formation of new cysts (females) on potato plants corresponded approximately with the hatchability of the larvae. Even the smallest radiation doses caused some sexual sterilisation, but after 16 kilorads almost 5% of the eggs in new cysts still contained viable larvae. (Evans)

### Sex determination in the potato cyst-nematode

The sexual differentiation of the second-stage larvae is influenced by the number of larvae per unit length of host root and its nutrient supply (*Rothamsted Report* for 1965, p. 146). As the number of invading larvae increases, the percentage that become male increases from 10% to nearly 100%, which is best explained by suggesting that larvae develop into females only when they feed in one of a limited number of sites able to contain a giant cell large enough and rich enough in nutrients. These sites may be fixed points in the stele or, more likely, wherever there is room for such a giant cell. Measurements of giant cells associated with females in the primary roots of tomato seedlings show that, when distribution is not random, there is room for about 25/cm of roots. Lateral roots have fewer sites, and more larvae that enter them become male. When young potato plants growing in pots with few lateral roots were inoculated with 5,000 larvae they produced an equal number of males and females, whereas inoculating older plants with abundant lateral roots produced 40 males to every female.

Plant-growth substances also affect the sex ratio, presumably by altering



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the rate the giant cells develop. Growth stimulators, such as gibberellic acid, indole acetic acid and tryptophan, increase the proportion of females, whereas chlorocholine chloride (CCC) and *N*-dimethylamino-succinimic acid, which slowed cell expansion, increase the proportion of males or prevent larvae reaching maturity. Slowing cell division in the host plants by irradiation or treatment with maleic hydrazide did not affect the sex ratio. Nematodes feeding in plants can be killed by adding unnatural D-amino-acids or an excess of some L-amino-acids to the soil. DL-Methionine is especially effective, kills all the feeding larvae of *H. rostochiensis* and is only slightly toxic to potato plants. Plants treated with DL-Tyrosine produced more males and fewer females than untreated plants. (Trudgill)

### Effects of "Nemafos"

A test was done to find whether treating tomatoes with the organophosphorus pesticide, "Nemafos", affected attack by the root-knot nematode, *Meloidogyne incognita*. Emulsions of "Nemafos" at four concentrations of the active ingredient *O,O*-diethyl-*O*-2-pyrazinyl phosphorothioate were either sprayed on 6-week-old plants or drenched into the soil when the plants were transplanted into infested soil. Some plants were transplanted into infested soil at the same time without any treatment, and 4 weeks later they were similarly sprayed or drenched. Treatments were replicated four times. After 13 weeks differences between root weights in the differently treated pots were insignificant, but the protective drench applied at transplanting time greatly decreased the amount of root galling. Spraying the leaves of transplants affected galling much less than the drench, but decreased it significantly. (Greet)