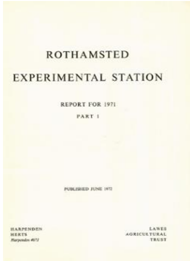
ROTHAMSTED RESEARCH

Report for 1971

Full Table of Content


ROTHAMSTED

EXPERIMENTAL STATION

REPORT FOR 1971

PART 1

Nematology Department

F. G. W. Jones

F. G. W. Jones (1972) *Nematology Department ;* Report For 1971, pp 163 - 178 **- DOI: https://doi.org/10.23637/ERADOC-1-126**

NEMATOLOGY DEPARTMENT

F. G. W. JONES

The work of the department includes the identification and description of nematodes (eelworms) that harm crops, studies of their host ranges, behaviour, structure and function, population changes when host and other crops are grown, and their control with nematicides or by other means. The potato cyst-nematode is a main study, partly because of its importance as a pest, but also because much of the information garnered applies to other species of cyst-nematodes, several of which are harmful in Great Britain and abroad.

Form and function

The activities of small nematodes depend on their internal pressure so attempts were made to measure it directly with fine glass microcannulae linked to a pen recorder by a sensitive electronic pressure transducer. With nematodes held by suction in micropipettes microcannulae could be placed in the stoma, intestinal lumen and the pseudocoelom of the larger (1–2 mm) soil-dwelling mononchs during locomotory and swallowing movements; these recorded body pressures of some tens of cm of water, fluctuating at about 0·06 Hz. (Seymour)

Observation cells were made thin enough to be used with correct Nomarski interference illumination and with all magnifications of the light microscope. *Botrytis cinerea* was cultured adjacent to an air space in the cells and provided food for *Aphelenchoides blastophthorus*, which bred successfully in the cells for more than a week. The Nomarski system uses polarised light, so much light is lost in the analyser above the specimen, and to take ciné films required extremely bright light. To prevent overheating the specimens, a filter was used incorporating two heat-filter glasses and a bath of acidified 3% ferric alum solution.

In contrast to several Tylenchoidea observed in agar cultures, *A. blastophthorus* took in water from medium free from a host fungus. R. F. Myers (*Nematologica* (1967) **13**, 323) reared *A. ritzemabosi* and *A. sacchari* (= *A. rutgersi*) on nutrient agar media alone, but *Ditylenchus* and *Tylenchorhynchus* spp. died within two weeks, presumably because the first two ingested the medium and the last two did not.

From estimated volumes of hyphae and from measured flow rates of suspended particles, *A. blastophthorus* individuals were estimated to ingest up to about 10 μ^3 of hyphal contents in one pumping, much less than the volume of their pumps at full dilation, calculated to be about 28 μ^3. The average amount of hyphal contents ingested was about 6 μ^3, but amplitudes of pump dilation, and so amounts of food ingested, differed much from one cycle to the next. Hyphal contents not only stopped flowing towards the nematode's stylet when the pump closed but sometimes flowed slightly backwards. This is to be expected because the pump has an outlet valve but no inlet valve (see also *Rothamsted Report for 1969*, 176). Instead of an inlet valve the pharyngeal lumen is much narrower in front of the pump than behind it, so it is noteworthy that, in the median bulb, the muscles took about twice as long to fill the pump as the turgor pressure did to expel its contents into the intestine (about 0·05 seconds).

During feeding and moving, the rectal lumen of *A. blastophthorus* is usually collapsed. Because the tail alternately lengthens and shortens the hind end of the intestine expands and constricts and the contents of the intestine surge backward and forward. Two or

https://doi.org/10.23637/ERADOC-1-126

three times an hour the backward flow was strong enough to force open the rectum and fill it with fluid. Then, after 1·5 seconds the *depressor-ani* muscle contracted and the contents of the rectum and of the hind end of the intestine were forcibly ejected in less than 0·05 seconds. What remained in the intestine quickly surged back to fill its hind end after the rectum closed.

It has been reported by P.-H. Yuen (*Nematologica* (1971) **17**, 1) that *A. blastophthorus* has no functional pseudocoelom or muscles in the walls of oviduct or uterus, but only vaginal muscles, and she speculated on the nature of the forces that move eggs along the reproductive tract. Our film records showed that eggs in the uterus moved backward as intestinal contents surged backward and that, in gravid females, backward surges were more powerful and rapid than forward ones. In the early stages of egg laying only a trickle of intestinal contents passed the egg in the direction of the egg's movement, but as the egg was laid a sudden surge filled the intestine in this region of the body and was associated with immediate collapse of the uterus. To all appearances, the intestine behaved as a hydrostatic organ, because it constantly applied different pressures to one side of the reproductive tract. These pressures were initiated by contraction of the body-wall muscles.

Observations on mating showed that the ventral surface of the male's tail has tactile sense organs whereby he can identify the female's vulva. Mating usually began with a male sliding along a female, parallel with her until his cloaca just overshot the vulva, when he stopped and made hooking actions with his tail until he coiled around the body of the female. The female then seemed to position the vulva opposite the spicules of the male. Individual males often attempted to mate with apparently unreceptive females. However, when one mated with a female containing two fertilised eggs, four males entwined around her, but the successful one enclosed her in the characteristic helical position. (Doncaster)

Cyst-nematodes

Potato cyst-nematode

Cement formed near the heads of* Heterodera *females. When *Heterodera* females are removed from roots and the cement that seems to hold them in position in the roots is dissected from around their heads, globules of new cement soon appear that harden and darken. Electron microscopy of sections cut through the anterior end of females of *H. rostochiensis* shows that the cement emerges through small breaks in the thin outermost layer of the cuticle. Globules seem to arise at random and not to be associated with ducts through the cuticle. The cement may be a component of the cuticle that flows through the deeper layers and is exuded through the breaks in the surface. Electron-dense material accumulates below the globules as though exposure to air induces a chemical change. What causes the outer layer of the cuticle to rupture is uncertain, but it may be friction against plant cells when the female swells or moves while its head is held fast in the root. Females of other species have globules of similar material on other parts of their bodies. Possibly these occur where contact with soil particles causes ruptures but the globules then serve only to plug the breaks and not to hold the female in position. (Shepherd, Green and Sørensen)

Sex attractants and hatching factors. Attempts to concentrate the sex attractant of the potato cyst-nematode have yielded preparations that produce detectable bands on paper chromatograms. These are impurities that have been separated from the attractant which travels with the solvent front. The impurities have Rf values corresponding to various amino acids some of which are known to be excreted by nematodes. The active fraction has a strong smell and gives a faintly alkaline reaction with bromocresol green, confirming previous evidence that it has basic properties. (Greet)

https://doi.org/10.23637/ERADOC-1-126

Purification of fractions from potato roots obtained by counter-current distribution has separated a series of hatching factors for potato cyst-nematode eggs. More than 20 have been detected, each with hatch ratings exceeding 100. The study of hatching factors has engaged many workers in several countries and proved exceedingly difficult. Many chemicals induce hatching and we do not know whether some of the factors detected in extracts of potato roots are artefacts arising from purification procedures or to what extent they are related compounds. Potato-root extracts also contain hatch-inhibiting and nematicidal materials; these accumulate in some fractions together with hatching factors, which become detectable by hatching tests only after further purification has resolved the mixtures.

Todd *et al.* (*Biochemical Journal* (1949) **45**, 520) used the name of eclepic acid for what was considered to be a single hatching factor from potato roots, but as there are several and they include acids and neutral or weakly basic materials, we propose the name 'eclepins' instead. Highly purified preparations of two of these, eclepins 1 and 2 have been obtained in microgram amounts.

The insect moulting hormones α and β-ecdysones were tested. The α form stimulated hatching of the potato cyst-nematode but the β form was inactive (hatch ratings of 39 and 8, respectively at 0·1 mg/ml). Crude root extracts from *Nicandra physaloides* (Solanaceae) were also inactive but after hatching inhibitors were removed, they were active. (Clarke and Hennessy)

Host range. In Great Britain, potatoes and tomatoes are the only crop plants that are hosts of the potato cyst-nematode and weed hosts belonging to the same family (Solanaceae) are few. Deadly nightshade (*Atropa belladonna*) and black henbane (*Hyoscyamus niger*) are rare and seem not to be hosts for any of the known pathotypes. Earlier reports of females developing on deadly nightshade were not confirmed by our recent tests. Woody nightshade (*Solanum dulcamara*), a common hedgerow plant, was host of all populations tested and is also susceptible to other species of round-cyst nematodes.

TABLE 1

Differential hosts for pathotypes of Heterodera rostochiensis

Plants tested	British pathotype A	British pathotype B	British pathotype E
S. acaule CPC 82	−	+	+
S. chacoense	−	+	+
S. sarachoides	−	+	+
S. famatinae	−	+	+
S. multidissectum CPC 2722	+	−	+
S. tuberosum × *andigena* (Maris Piper, gene H_1)	−	+	+
S. tuberosum × *multidissectum* (P55/7, gene H_2)	+	−	+

Most plants of black nightshade (*Solanum nigrum*), a common weed of arable land, resist all populations but some are susceptible. The host range of the potato cyst-nematode seems to be confined to the Solanaceae, and to find its extent more plants in that family were tested. Of more than 100 species and varieties 57 were hosts of British pathotypes A, B and E. Table 1 lists hosts that differentiate between the pathotypes; it includes *S. chacoense* but further tests are needed to be sure of its behaviour. It would be helpful to have other than tuberous species of *Solanum* to differentiate between British pathotypes B and E and between Dutch pathotypes A, B and C, because seeds can be exchanged between countries wishing to identify their pathotypes whereas exchange of tubers is forbidden or subject to severe quarantine regulations. (Stone and Course)

https://doi.org/10.23637/ERADOC-1-126

Relationship of British and foreign populations. Last year we reported results showing that British A and Dutch pathotypes A, B and C of *H. rostochiensis* form a homogeneous group, differing in colour, stylet length and protein content from British B and E and Dutch D (*Rothamsted Report for 1970*, Part 1, 152). This year we compared further populations and our pathotype test, using resistant potato varieties grown in pots, included one variety derived from *S. tuberosum* spp. *andigena* with genes H_1 and H_3. Gene H_3 confers resistance to British pathotype E populations (Howard, H. W. (1969) *Proceedings of the 5th British Insecticide and Fungicide Conference, 1969*, 159). Larval stylet lengths, female colour and electrophoretic protein patterns (Table 2) show that the four populations from Panama, Venezuela, Bolivia and Canada belong to the British pathotype A group and three from Peru to pathotype E. The Bolivian and Venezuelan populations resemble Dutch pathotype B, producing many females on the ex *andigena* hybrid (H_1), Maris Piper. A population from Greece is British pathotype A and from India pathotype E. Tests with the ex *multidissectum* hybrids P55/7 (H_2) and K5/5 (H_1H_2) showed that none of the populations were British pathotype B. The only populations to produce many females on the H_1 and H_3 hybrids with resistance genes were the Dutch pathotype B, Bolivia and Peru 1. As the Venezuelan population resembles the Bolivian population in having yellow females and reproducing on Maris Piper, we expect it to behave similarly on H_1H_3 potatoes. The Peru 1 population reproduced poorly on all test varieties, so the results on plants with genes H_1 and H_3 may be biased. As populations similar to the Dutch B have not yet been found in Britain, or been selected where resistant potatoes with gene H_1 have been grown repeatedly, an H_1H_3 hybrid should provide adequate resistance to all British populations of the potato cyst-nematode. Our results suggest that the ancestral home of pathotype E is Peru. Pathotype A may have been introduced into Panama from Europe, as it probably was into Long Island, U.S.A., and Vancouver Island, Canada. Pathotype E in India probably came from Great Britain. (Trudgill, Parrott, Thompson, Berry and Matthews)

TABLE 2

Female colour, larval stylet length, electrophoretic protein pattern group and the number of cysts on resistant potato plants as a percentage of those on Arran Banner for four Dutch and nine non-European populations

Population	Larval stylet length (µm)	Electrophoretic pattern as in British pathotype:	Number of cysts on plants with genes for resistance as a percentage of the number on Arran Banner			
			Maris Piper (H_1)	P55/7 (H_2)	K5/5 (H_1H_2)	586/1 (H_1H_3)
Females yellow						
Greece	21·4	A	2	20	1	5
Panama	20·9	A	1	32	1	4
Canada	21·5	A	—	—	—	2
Dutch A	21·0	A	1	38	2	3
Dutch B	20·3	A	94	60	49	49
Dutch C	20·7	A	21	100	18	7
Bolivia	22·0	A	65	82	15	45
Venezuela	21·6	A	94	—	—	—
Mean	21·2					
Female white						
India	22·5	E	100	27	8	1
Dutch D	23·8	E	100	8	25	—
Peru 1	23·5	E	100	58	100	40
Peru 2	23·4	E	100	100	100	8
Peru 3	23·2	E	33	19	34	6
Mean	23·3					

https://doi.org/10.23637/ERADOC-1-126

The cuticular patterns of 19 populations of potato cyst-nematode from continental Europe were compared using the scanning electron microscope. The populations had previously been tested and their pathotype determined. Populations that were British pathotype E (Dutch D) had papillate vulval crescents and the vulval lips were usually also papillate, whereas British A (Dutch A) and Dutch C had dissected vulval crescents and smooth vulval lips. Dutch pathotype B tended to have papillate vulval crescents and smooth lips. The anus and vulva of Pathotype E were closer together than in the other three pathotypes, and the ratio of the distance between anus and vulva and the width of the vulva (Granek's ratio measured more precisely than possible under the light microscope) was 2·5 and 3·5 respectively. Because the distance between anus and vulva was shorter, pathotype E had fewer ridges, forks and spurs in the cuticular pattern than the other three. Dutch pathotype B populations and some C resembled a population from Bolivia in some of the characters.

The results confirm the use of vulval characters to distinguish the round cyst species of *Heterodera* and the pathotypes of *H. rostochiensis* (Abstract of papers 8.11). (Green, Jenkinson and Edwards)

H. mexicana, *H. virginiae*, and *H. solanacearum* are round-cyst nematodes from the U.S.A. that are closely related to *H. rostochiensis*. Their protein-band patterns were almost identical and similar to that of *H. tabacum*, but differed somewhat from that of *H. rostochiensis* of British pathotype E and markedly from that of A. Morphologically pathotype E was closer to the North American species. (Greet and Edwards)

Selection by a resistant potato variety. Since potato hybrids containing gene H_1 for resistance to *H. rostochiensis* were bred, interest has centred on the few larvae of pathotype A populations that succeed in maturing as females in their roots. Those females, which remain white, we now know are pathotype E (strictly a distinct species that does not interbreed with pathotype A) but those that turn yellow remain a puzzle. Are they genetically different from most other members of the pathotype A population? Or does some combination of circumstances, including perhaps some local change in the host root system allow them to develop? The answers to these questions, and knowing how the races or pathotypes of potato cyst-nematodes are distributed, are important in deciding policies in breeding and using resistant potato varieties.

Repeated growing of plants with gene H_1 on infested land sooner or later usually selects pathotype E, which then replaces pathotype A. This has happened in Butt Close, Woburn, where the resistant variety Maris Piper was grown for six years, but not after ten years in another field there, Long Mead. How soon pathotype E replaces A probably depends on the initial number of pathotype E cysts present when the resistant variety is first grown. Probably all fields infested predominantly with pathotype A also contain some E, because it has probably been spread widely in seed tubers and in soil. How well it competes with pathotype A when only potatoes susceptible to both are grown, is a matter of conjecture, but probably enough survive to multiply when susceptible potatoes are replaced by resistant ones. Selection tests on three populations of pathotype A by growing a resistant variety in pots (Table 3), showed that E had almost completely replaced A after three years. Initially these populations produced few females on the resistant variety, Maris Piper, but after three years they produced as many as on the susceptible variety Arran Banner.

Whether pathotypes similar to Dutch B and C (which reproduce on resistant varieties with gene H_1 but are the same species as British A and interbreed freely with it) could also be selected is unknown, but it must be assumed that it is possible or these pathotypes would not exist. However, as populations of Dutch pathotype B and C seem not to occur in Great Britain, selection from existing populations may be less probable than

https://doi.org/10.23637/ERADOC-1-126

TABLE 3

Number of Heterodera rostochiensis *females produced on susceptible and ex* andigena *potato plants, expressed as a percentage of the number of larvae inoculated after selection of the populations for up to three years on ex* andigena *plants*

Number of females, as a percentage of the number of larvae inoculated

Previous host	Number of years on host	Test host	Populations: Sandy	Feltwell	Woburn
Susceptible	3	Susceptible	15	24	26
		Resistant	1	1	1
Resistant	1	Resistant	7	11	8
	2	Resistant	18	—	12
	3	Resistant	25	14	16
		Susceptible	31	17	—

their introduction from continental Europe or the Andes. British E does not interbreed with A, so it probably suffers little genetic interference from A, but should individuals of Dutch B or C be introduced these would mate with males in our existing population. However, the genes they would introduce would take many years to spread through the existing populations in infested fields. (Trudgill, Parrott, Thompson, Berry and Matthews)

Effect on growth and function of potato root systems. The experiment at Woburn testing the effects of irrigation and fumigation of soil on the yields of susceptible (Pentland Dell) and resistant (Maris Piper) varieties of potato grown alternately and continuously has produced widely different populations of potato cyst-nematode in the soil and great differences in potato yields. These differences provided an opportunity to study the ways nematodes influence the growth of Pentland Dell. Table 4 gives measurements made on plants taken 10 and 11 weeks after planting from fumigated and unfumigated plots. At 10 weeks, the fresh weight of tops, roots and new tubers, leaf area and number of stems per plant from the fumigated plot were all much greater than from the unfumigated. Leaves were also much larger but not more numerous. The total weight of new tubers was also much greater, largely because the plants in the unfumigated plots had produced fewer tubers rather than smaller ones.

At 11 weeks weights of tubers and tops had increased on both plots, but whereas mean leaf area on the fumigated plots was twice that at 10 weeks, it had not increased on the unfumigated plots. Net assimilation rates (tops and tubers) were 0·0106 g/cm^2/8 days for plants on the unfumigated plot and 0·0089 g/cm^2/8 days on the fumigated plot.

TABLE 4

Effects of H. rostochiensis *on the growth of potato, var. Pentland Dell*

	15 June 1971 (10 weeks)		23 June 1971 (11 weeks)	
	Unfumigated	Fumigated	Unfumigated	Fumigated
Fresh weight roots (g)	48·6	104·0	—	—
Fresh weight tops (g)	199	538	174	981
Leaf area (cm^2)	1598	5882	1486	11295
Plant height (cm)	34·2	43·4	43·8	64·1
No. stems/plant	4·2	8·6	2·8	9·0
Individual leaf area (cm^2)	32·8	64·5	33·0	88·0
No. leaves/stem	11·6	10·6	16·1	14·3
No. nodes/stem	13·0	11·2	17·8	15·6
Total weight of new tubers (g)	49	77	153	372
Mean tuber weight (g)	3·2	3·9	7·1	11·3

https://doi.org/10.23637/ERADOC-1-126

Plants in the fumigated plot probably had a smaller value because their leaf area index was larger, 2·18 compared with 0·59 at 10 weeks and 4·19 compared with 0·59 eight days later. Assimilation rates for new tubers only were 0·0108 g/cm^2/8 days on the unfumigated plot and 0·0043 g/cm^2/8 days on the fumigated plot, a greater difference between plots than that of tops and tubers because much of the assimilate on the fumigated plots was used for new top growth.

Final yields of tubers (mechanically harvested) were 147 g per plant on unfumigated plots and 541 g on fumigated plots. Poor growth of plants on the unfumigated plots seems to reflect lack of nutrients rather than inability to photosynthesise; for example, the potassium content of the tops was 4·0% of the dry weight in unfumigated but 6·7% in the fumigated plots. (Evans, Trudgill, Rowney, Pandé and Thompson)

Chemical control of potato cyst-nematode. Dazomet and 'Telone' (a mixture containing 1,3-dichloropropene) applied in September 1970, near Terrington St. Clement, Norfolk, to silt loam infested with potato cyst-nematode greatly increased yields of King Edward potatoes grown in 1971. The dazomet was spread evenly on the soil surface with a 'Sisag Lospred' granule distributor and mixed into the soil by a rotavator with L-shaped tines, working to a depth of 15 cm. The 'Telone' was injected 25 cm deep behind blade-type coulters 30 cm apart, and the slits were closed by harrows trailed behind the tractor-drawn toolbar. Plots were then left flat, ridged up, rolled or not rolled. In flat, rolled plots dazomet applied in two equal amounts, one before ploughing 25–30 cm deep with a digger plough to invert the furrow slices, the other after ploughing, controlled potato cyst-nematode in soil to 20 cm deep better than a double dose applied after ploughing. Two hundred and thirteen kg dazomet (98%)/ha applied after ploughing, followed by 224 kg 'Telone'/ha gave as good control as two dressings of 314 kg dazomet/ha. 'Telone' at 448 kg/ha was much less effective (Table 5). In addition to controlling potato cyst-nematodes, the larger amounts of dazomet also controlled wild oats.

TABLE 5

Dazomet and 'Telone' to control potato cyst-nematode in silt loam

Treatment	Amount (kg/ha)	King Edward tubers over 3·8 cm riddle (tonnes/ha)	Multiplication of potato cyst-nematode
Untreated	0	14·6	× 17·3
dazomet (one dose)	213	28·4***	
	314	32·6***	
	410	36·7***	× 2·4
	628	38·4***	× 1·8
dazomet (one dose) plus 'Telone' (one dose)	213 } 224 }	39·7***	× 0·7
dazomet (two doses)	175	21·6***	
	330	34·7***	
	426	37·2***	× 1·4
	628	42·9***	× 0·7
'Telone' (one dose)	448	27·4***	× 4·3

*** Significantly greater than untreated at $P = 0 \cdot 001$.

In heavily infested peaty loam at the Arthur Rickwood Experimental Husbandry Farm, Isle of Ely, applying equal doses of dazomet before and after ploughing also increased yields of King Edward potatoes more than an equivalent amount of dazomet applied after ploughing. The kill of nematodes and yield of King Edward potatoes were greater when the soil was treated during October or November than when treated during August or September, presumably because the fumigant was retained longer in the cooler, moister soil.

https://doi.org/10.23637/ERADOC-1-126

Dazomet 330 kg/ha applied after ploughing to sandy loam in Butt Close, Woburn, controlled potato cyst-nematode well to 40 cm deep and greatly increased yield of susceptible potatoes in 1970, but 168 kg/ha applied before and after ploughing these same plots failed to control potato-cyst nematode in 1971. Plots treated with two doses of 168 kg dazomet, which in autumn 1969 or spring 1970 had received 336 kg 'Telone'/ha yielded better in 1971 than those which had received 336 kg dazomet in autumn 1969 or spring 1970. Plots treated with 336 kg dazomet before ploughing and 168 kg after ploughing also yielded more potatoes in 1971. Rolling plots after applying dazomet increased neither control of potato cyst-nematode nor yields of potatoes (Table 6).

TABLE 6

Dazomet to control potato cyst-nematode in sandy loam

Treatment for 1970 crop (kg/ha)	Treatment for 1971 crop: dazomet before ploughing (kg/ha)	Treatment for 1971 crop: dazomet after ploughing (kg/ha)	1971 Majestic tubers over 3·8 cm riddle (tonnes/ha)	Eggs/g soil to 20 cm depth at harvest
Untreated	Untreated	Untreated	6·8	427
dazomet 330	330	—	37·7***	282
	330	165	48·5***	188
	165	165	35·7***	170
	165	165 (then soil ridged up)	34·7***	228
'Telone' 336	165	165	43·7***	207
	165	165 (then soil rolled flat)	44·9***	182

*** Significantly greater than untreated at $P = 0{\cdot}001$.

In pots, 5 ml of formaldehyde solution (37–40%) (the smallest dose used) well mixed in 1500 ml of infested sandy loam, prevented potato cyst-nematode from multiplying on a susceptible variety planted in the pots.

Last year we showed that small amounts of aldicarb, 'Tirpate', 'Du Pont 1410' and 'Nemacur P' controlled potato cyst-nematodes and greatly increased yields of susceptible potatoes grown in infested soils, so further trials with different amounts of aldicarb, 'Du Pont 1410' and 'Nemacur P' were made in 1971 on infested sandy loam (Great Hill, Woburn), peaty loam (Long 24, Arthur Rickwood E.H.F.) and silt loam (Terrington

TABLE 7

Effect of aldicarb, 'Du Pont 1410' and 'Nemacur P' on yields of potatoes susceptible to potato cyst-nematode in three soils

Treatment	Amount a.i. (kg/ha)	Yields of tubers over 3·8 cm riddle (tonnes/ha): Peaty loam	Silt loam	Sandy loam
Untreated	0	32·1	17·3	12·3
aldicarb	2·8	—	20·6	36·9***
	5·6	55·0***	23·9	33·9***
	11·2	53·5***	28·9**	43·4***
	22·4	48·7***	—	—
'Du Pont 1410'	2·8	—	24·4	32·1***
	5·6	51·5***	21·8	34·9***
	11·2	47·0***	28·1**	39·4***
	22·4	51·2***	—	—
'Nemacur P'	2·8	—	24·9	24·4***
	5·6	44·9**	22·6	26·1***
	11·2	46·0**	28·1**	27·6***
	22·4	47·2***	—	—

, * Significantly greater than untreated at $P = 0{\cdot}01$, $0{\cdot}001$, respectively.

https://doi.org/10.23637/ERADOC-1-126

St. Clement, Norfolk). The crop on peaty loam soil was irrigated, which increased yields, whereas the silt loam soil was badly infested with wild oats, which probably lessened yields. Potato yields were greatly increased on all soils by all three chemicals, although 'Nemacur P' damaged the crop on sandy loam. In the silt and sandy loams, yields increased with increased dosage of nematicides but in the peaty loam, which was heavily infested, although potatoes had not been grown during the past 10 years, yields were as large following 5·6 as 11·2 or 22·4 kg a.i./ha (Table 7).

The yield of Arran Banner potatoes from plants grown for 3½ months in containers each holding 52 litres of well-mixed infested peaty loam was greatly increased by treating the soil to 12·5, 25, 37·5 and 50 cm depth with 3·5 mg a.i. aldicarb/l soil. Yields were as great when aldicarb was applied to 25 cm depth as when it was applied to 37·5 or 50 cm depth, although the nematode is controlled best when all the roots are protected from invasion. Provided aldicarb, 'Du Pont 1410' or 'Nemacur P' are not decomposed or leached too rapidly this might be achieved by mixing them into the top soil before deep ploughing during winter, followed in spring by another amount to control the nematodes in the topsoil. 'Du Pont 1410' mixed with infested sandy loam and peaty loam still controlled the nematode after 6, 12 or 18 weeks storage of the soil at 5°C and 10°C. (Whitehead, Tite, Fraser and French)

A new nematicide, used at 5 and 10 ppm in heavily infested sandy loam, allowed susceptible potato plants to grow well in pots without their roots being invaded by larvae, and so prevented the nematodes multiplying. This substance is as effective as aldicarb in controlling potato cyst-nematode but has less than a hundredth of its toxicity to mammals. (Whitehead, and Mr. S. N. Ahmed, Nottingham University)

Cereal cyst-nematode

Effect of temperature and root exudates on* H. avenae *hatch. One difficulty in working in the laboratory with *H. avenae* is to maintain supplies of larvae throughout the year, for few larvae hatch and invade roots except between February and May. To find ways of preventing hatching early in the year, to conserve larvae until needed and then induce hatching when desired, the combined effects on hatch of temperature and exudates of cereal roots were studied.

Tests over a range of constant temperatures showed that *H. avenae* hatches best from 10°C to 15°C, but hatches were larger when the eggs were exposed to diurnal fluctuations of from 10°C to 20°C. Previous workers who sought to stimulate hatching by cereal exudates did so at constant temperatures of 25°C or 20°C, and concluded that *H. avenae* did not respond to these exudates. Pooling the results of five tests at 10°C and 15°C (Table 8) showed that more larvae hatch in cereal root diffusates at 10°C than at 15°C and that hatch was stimulated more by exudates from the roots of Sun II oats than from two barley varieties tested. All cereal exudates greatly increased the hatch and the pattern of response was the same whether the cysts were stored previously at 10°C for at least six months or came from the field during spring but cysts from the field responded less

TABLE 8

The effect of temperature and exudates on the hatch of H. avenae (*per 100 cysts*)

Exudate	+10°C Larvae hatched	+10°C % cyst contents	+ 15°C Larvae hatched	+ 15°C % cyst contents
Soil water (control)	847	3·9	500	3·3
Sun II oats	6075	33·7	1895	13·7
Proctor barley	2606	13·8	1576	10·6
Var. 191 barley	3262	18·8	1696	10·0

https://doi.org/10.23637/ERADOC-1-126

to diffusate and did so most at 15°C. This suggests that eggs were affected by temperatures to which they had been exposed and that hatch in water is greater after being at winter temperatures in the field than at 1°C. In a further test, cysts from the Woburn *H. avenae* population were exposed to the action of a separately collected set of exudates (Table 9). Hatching proceeded for eight months at 10°C.

TABLE 9

Effect of exudates on H. avenae. *Means of three lots of 20 cysts*

	Larvae hatched at		Eggs remaining		% hatch	
	12 weeks	35 weeks	12 weeks	35 weeks	12 weeks	35 weeks
Soil water (control)	283	2544	3411	1150	8	69
Sun II oats	1257	2545	2264	975	36	72
Kolibri wheat	822	2412	2099	508	28	83
Proctor barley	485	2799	2672	358	15	89
Drost barley (resistant)	1789	2957	1341	174	57	95
Var. 191 barley (resistant)	1004	2794	2232	442	31	86

The differences between exudates are not easily assessed, for the vigour of the plants from which they come may influence their quality, but hatch in all exudates greatly exceeds hatch in soil water alone. This difference is very clear during the first half of the experimental period but all hatches were much the same by about 25 weeks. This suggests that, as with *H. rostochiensis*, exudates affect the rate of hatch but not total hatch. Even so, at 35 weeks when the experiment was concluded, exudates gave a greater percentage hatch. These results help to explain the previously puzzling behaviour of *H. avenae* in the laboratory and field. (Williams and Beane)

Cyst production on cereals. The production of females and cysts by six populations of *H. avenae* was studied on varieties of oats and barley, and Milford oat proved a better host than Sun II oat, i.e. more females formed on its roots. This is important because Sun II is widely used as a standard for comparing the resistance of varieties of cereals. Females on the root systems usually greatly exceeded the number of cysts extracted from soil later. Either the females were destroyed by some predator, or failed to encyst for some other reason, or many of the newly formed cysts were not extracted by the method used, which was flotation in a Fenwick can. This experiment also showed that the Rothamsted (Pennells Piece) population of *H. avenae* was nearly all race 1 (pathotype A). Almost 2000 white females developed in each Milford oat plant and none in the Drost barley. By the same standard the Woburn *H. avenae* population is estimated to contain at least 30% race 2 (pathotype C). (Williams and Beane)

TABLE 10

Yields of spring wheat and numbers of cereal cyst-nematode after aldicarb, dazomet and formalin treatments; Woburn 1971

	Untreated	aldicarb 9 kg/ha (a.i.)	dazomet 381 kg/ha	formalin 290 litre/ha
Grain yield (85% DM, tonnes/ha)	2·20	3·21***	2·46	2·18
H. avenae (larvae/g seminal root)	97·0	14·0***	41·1*	109·3
H. avenae, (eggs/g soil, post-crop)	10·3	2·0***	8·6	9·9

*, *** Signficantly greater than untreated at $P = 0{\cdot}05$ and $0{\cdot}001$ respectively.

https://doi.org/10.23637/ERADOC-1-126

Nematicide trials. Aldicarb, dazomet and formalin were applied during winter or spring before sowing spring wheat, var. Kolibri, on light sandy loam soil in Butt Close, Woburn containing an average of 4 eggs/g soil of *H. avenae*. Table 10 shows the yields of grain harvested, numbers of nematode larvae in roots during June and eggs after harvest. Whereas in previous years dazomet gave the largest yields and the best control of the nematode, aldicarb was best in 1971. The reason is unknown but may be related to soil conditions when the nematicides were applied. (Williams and Beane)

In another experiment in the same field, formalin was applied during autumn or spring before planting spring wheat var. Kolibri and winter wheat var. Cappelle. Each variety was sown during both autumn and spring. Table 11 gives grain yields, numbers of nematodes in the roots during June (spring wheat only) and the numbers of eggs after harvest.

TABLE 11

Grain yields and H. avenae *in spring and winter wheat in plots treated with formalin; Butt Close 1971*

	Sown	Formalin	Formalin sequence				Mean
			— —	— F	f—	fF	
Winter wheat							
Grain, tonnes/ha	Autumn	Autumn	2·57	2·70	2·98	2·84	
Post-harvest eggs/g			1·3	7·3	6·1	2·5	4·3
Grain, tonnes/ha	Spring	Autumn	1·59	2·20	1·82	2·33	
Post-harvest eggs/g			3·7	10·8	6·8	14·0	8·8
Spring wheat							
Grain, tonnes/ha	Spring	Autumn	2·45	2·44	2·06	2·54	
H. avenae/g root			151·5	99·5	135·8	92·7	119·9
Post-harvest eggs/g			9·6	12·7	5·4	3·9	7·9
Grain, tonnes/ha	Spring	Spring	2·31	2·42	1·95	2·65	
H. avenae/g root			330·7	41·1	473·3	129·3	243·6
Post-harvest eggs/g			13·2	12·1	26·5	10·7	15·6

— — No formalin 1970 or 1971; — F, formalin 1971; f —, formalin 1970; fF, formalin 1970 and 1971.

Some plots that were treated with formalin in 1970, were treated again, and effects of the early treatments compared with the treatments in 1971 imposed on them. Most eggs after harvest (26·5/g soil) were in the spring-wheat plots treated with formalin in 1970 only: these plots also yielded least grain (Table 11). However, this was so only where formalin was applied during spring. Over the whole site grain yields were small and were not obviously correlated with incidence of take-all or numbers of nematodes in roots suggesting that other factors limited yields. Formalin had little effect on take-all attack in May or July but significantly increased the yield of autumn-sown winter wheat. Formalin applied during autumn had no effect on the number of nematodes in the roots of spring wheat, but applied during spring made them fewer without increasing yield significantly. (Williams and Beane with Salt, Plant Pathology Department)

Stem nematodes

Stem nematode (*Ditylenchus dipsaci*) again occurred on field beans on the Rothamsted farms, mainly where beans were infested in 1970. Presumably these attacks were initiated by soil-borne nematodes. The most damaging attack was in Great Field II, where beans were last grown in 1968 and the oat and 'giant' races occurred together: yield was lost and the seed was infested.

Broad-bean stems, received from Eastern England, were severely distorted by the 'giant' race. Some pods bore small necrotic lesions that were heavily infested and the seed from one of them was also infested. (Hooper and Pike)

https://doi.org/10.23637/ERADOC-1-126

At Woburn an attempt was made to control stem nematode in onions, a very susceptible crop, with nematicides. The site, light sandy soil, was infested by adding the chopped stem bases of field beans, and was watered whenever the weather was dry, to ensure a moist soil surface so that the nematodes could move and invade the onion seedlings. Large amounts of dazomet controlled *D. dipsaci* better, and increased yield of dry bulb onions more, than did small amounts of aldicarb (Table 12). Dazomet also suppressed weeds until the onion plants were well established. (Whitehead, Tite, Fraser and French)

TABLE 12

Control of Ditylenchus dipsaci *and yield of dry bulb onions* (*var. Robusta*) *with dazomet and aldicarb in irrigated sandy loam, Woburn*

Treatment	Amount (kg a.i./ha)	Onions over 3·8 cm riddle (tonnes/ha)	*D. dipsaci*/litre soil in rows at harvest
Untreated	0	9·3	1229
aldicarb	2·2	20·8*	100
	4·4	16·1	217
	6·6	17·3	25
	8·8	23·9**	67
dazomet	220	31·6***	100
	440	42·4***	0
	660	47·5***	8
	880	51·2***	0

*, **, *** Significantly greater than untreated at $P = 0{\cdot}05, 0{\cdot}01, 0{\cdot}001$ respectively.

Root lesion nematodes

Damage to cereals apparently associated with *Pratylenchus* spp. often manifests itself as plants that grow poorly in the same patch year after year. To study the survival of *Pratylenchus* populations within such patches, roots of commonly occurring weeds were examined from different places. The *Pratylenchus* species parasitising the cereal roots at each site were also found in roots of weeds. *Pratylenchus* has a wide host range but does much better on some than others. *Equisetum arvense*, a weed of increasing importance in cereal crops, from Broadbalk and from Butt Close (Woburn) was a poor host for the *Pratylenchus* spp. present there and the most found was 5/g of root. Most broad-leaved weeds were also poor hosts, but some grasses harboured large populations, e.g. annual meadow grass (*Poa annua*), couch grass (*Agropyron repens*) and common bent grass (*Agrostis gigantea*). On a field at Papplewick, Notts, *A. gigantea* had as many as 1475 *Pratylenchus*/g root in March; as it spreads in patches of crops that grow poorly, it may be important in maintaining the nematode populations in these patches and explain their persistence. (Webb)

Root ectoparasitic nematodes

Nematode populations in wheat following direct seeding and ploughing. During the fifth year of an experiment at Woburn, comparing winter wheat directly seeded in unploughed land sprayed with herbicide and sown conventionally after ploughing, pin nematodes (*Paratylenchus* spp.) were more, and stunt nematodes (*Tylenchorhynchus* spp.) and Dorylaims fewer in unploughed land, but total nematodes were similar. During the first four years of the experiment, a mixture of DDT, diazinon, chlordane and thionazin was sprayed on to some plots before planting, but this had no effect on nematodes. However, phorate granules, 10 lb a.i./acre, applied during the fifth year made all nematodes, except Dorylaims, significantly fewer. Since the experiment started in land ploughed

https://doi.org/10.23637/ERADOC-1-126

from pasture, nematodes have decreased greatly, so the steady decrease in yield has some cause other than nematode attack. (Corbett, Webb and Howe)

Nematodes in the potatoes of the Ley–Arable experiment at Woburn. Chloropicrin injected at 400 lb/acre during 1967 greatly increased yields of potatoes in the Ley–Arable rotation experiment at Woburn in 1971. Similar injection of chloropicrin during autumn 1970, into plots of potatoes treated with aldicarb (100 lb/acre 10% granules rotavated in) during the spring greatly increased yields, despite the fact that yields from the untreated plots were larger than usual. Numbers of needle nematodes (*Longidorus leptocephalus*) were estimated at 40 to 60 cm deep in soil, where they are most numerous, and those of other ectoparasitic nematodes and of potato cyst-nematode (*H. rostochiensis*) to plough depth. Cyst-nematodes were too few to have influenced yields but *L. leptocephalus* were most abundant where yields were smallest (Table 13). Populations were still small where chloropicrin was applied during 1967, probably because the needle nematodes have protracted life cycles and reproduce slowly. Populations of other root ectoparasitic nematodes had evidently recovered from any effects of chloropicrin applied during 1967, but they were only partly controlled by chloropicrin and aldicarb applied for the 1971 crop.

TABLE 13

Effects of chloropicrin and aldicarb on potato yields and numbers of nematodes

Woburn Ley-Arable Experiment 1971

	Treatments			
	None	chloropicrin residues (applied autumn 1967)	chloropicrin and aldicarb	chloropicrin and aldicarb + chloropicrin residues
Yield (tonnes/ha)	59·3	69·1	78·6	74·1
Longidorus (No./litre, at 40–60 cm deep)	180	5	55	2
Ectoparasitic nematodes (No./litre)	8410	8110	2905	3305
H. rostochiensis (eggs/g soil)	2	1	1	4

As potato cyst-nematodes were too few to be damaging and most of the other root ectoparasitic nematodes were pin nematodes (*Paratylenchus* spp.), most of the yield increases from treating soil with nematicides seems to come from controlling *Longidorus leptocephalus*. Because this nematode is most numerous deep down, the damage it causes to the root system becomes apparent after a prolonged dry spell when it shows above ground as a premature yellowing of leaves. In 1971 these symptoms became evident late in July towards the end of a dry spell of weather, when plants on the untreated plots began to yellow whereas those in treated ones remained green until autumn. (Evans and Pandé)

Stubby root nematodes. Two new species of *Trichodorus* were described. One was found in sandy soil around roots of Sitka spruce seedlings in a forestry nursery at Kennington, Oxon, and associated with arable crops and herbaceous plants in several places in East Anglia. The other was found only in the wet, sandy soil of a woodland in Essex, associated with the roots of elder (*Sambucus nigra* L.). (Hooper and Pike)

***Tylenchorhynchus dubius* and field beans.** In pots of steamed sandy loam from Butt Furlong, Woburn large numbers of *Tylenchorhynchus dubius* severely stunted field beans. (Whitehead and Fraser)

https://doi.org/10.23637/ERADOC-1-126

Docking disorder of sugar beet. In 1971, Docking disorder, the stunting of sugar-beet seedlings by root-ectoparasitic nematodes, was rare. Nevertheless, in three of four large-scale experiments in fields in West Norfolk infested with the needle nematode (*Longidorus attenuatus*) and stubby-root nematodes (*Trichodorus* spp.), fumigating the row positions with 'D-D' before sowing beet significantly improved seedling vigour (Gayton I and II, Docking I). At Gayton 'D-D' was injected into the soil half a day or a day before sowing; at Docking a week before sowing. Sugar-beet plants in untreated rows were healthy in all four fields. In neither field at Docking did 'D-D' treatments increase sugar yields. At Gayton, where the soil contained a little more clay, more nematode damage, especially to deeper roots, probably occurred and the value of the increase in sugar yield was more than twice the cost of 'D-D' treatment. Pulling the injector tines through the soil 15 or 25 cm deep in the row positions before sowing beet did not increase sugar yields significantly (Table 14). (Whitehead, Tite, Fraser and French)

TABLE 14

Effect of row fumigation with 'D-D' on sugar yield in four fields prone to Docking disorder

	Sugar (tonnes/ha)					
	Docking			Gayton		
Treatment	l/ha	I	II	l/ha	I	II
Untreated	0	—	—	0	—	7·13
Tines only (to 15 cm depth)	0	6·49	6·43	0	7·10	7·37
Tines only (to 25 cm depth)	0	—	—	0	6·89	7·33
'D–D' + tines (to 15 cm depth)	0	—	—	48	8·12**	8·21**
	72	6·77	6·69	64	8·17**	8·03**
	107	6·84	6·51	97	8·17**	8·26***
	143	6·78	6·59		—	—
Nematodes/litre soil, 1 April, 1971, in untreated plots						
Longidorus		71	21		70	134
Trichodorus		1171	830		1196	612

, * Significantly greater than 'tines only (to 15 cm depth)' or (Gayton II) 'untreated' at $P=0{\cdot}01$ and $0{\cdot}001$ respectively.

Needle nematodes at Woburn. To find out whether the needle nematode *L. leptocephalus* occurred more widely at Woburn, a number of experiments on different fields were sampled 60 cm deep in 20 cm fractions. *Longidorus* spp. were abundant only on the site of the Ley–Arable experiment in Stackyard and the direct seeding experiment in White Horse Field. The concentration between 40 and 60 cm below the soil surface occurred only under potato crops. *L. leptocephalus* was by far the commonest species found, with some *L. elongatus* and *L. caespiticola* in White Horse Field and a few *L. caespiticola* in Stackyard. Although Butt Close had the same soil type as where *Longidorus* is common in Stackyard, only isolated individuals could be found in two experiments and in the hedgerow nearby. *Longidorus* was also virtually absent from one site in Road Piece, three sites in Butt Furlong and Long Mead hedgerow. Most were found in the Brown Earths on re-sorted Lower Greensand in Stackyard and White Horse Field, but almost as many occurred in the gley soils of Stackyard on Lower Greensand/Oxford Clay.

The large numbers deep under potatoes suggest that cultivations kill the nematodes nearer the surface, and this is supported by the distribution in the Direct Seeding experiment on White Horse Field, where numbers in mechanically cultivated and sod-seeded plots averaged 423 and 606/litre respectively.

As most of the experiments sampled grew the same crop for at least three years, the numbers found should indicate the ability of the crops grown to support *Longidorus* spp.

https://doi.org/10.23637/ERADOC-1-126

On this basis winter wheat was a good host in White Horse Field, yet in the Intensive Cereals experiment on Stackyard I (slightly heavier soil) there were few. Despite the many found in White Horse Field there was no correlation between numbers and yield of wheat. (Evans and Pandé)

Examination of grass fields at Woburn revealed that they contain up to 900 *Longidorus*/litre soil. In fields where grass is cut for hay or where the surface layers tended to be dry, *Longidorus* were more numerous below 15 cm. (Trudgill and Thompson)

Long-term effects of TAF fixative

To aid in identifying species, we maintain a large and unique collection of type slides. In preparing specimens for this and other purposes, TAF fixative (formalin 7%, triethanolamine 2% in water) has been used for the last 15 years. Nematodes remain lifelike after fixation. The triethanolamine both neutralises any free formic acid and, being hygroscopic, prevents the specimens from drying should the fixative evaporate. However, when nematodes kept in it for eight to ten years were rapidly processed to glycerol, their cuticles suffered degeneration or distortion. The cuticle of *Tylenchorhynchus ornatus* separated into two closely adpressed layers resembling *Hemicriconemoides* spp., and the thin outer layer of the cuticle of a *Trichodorus* sp. swelled and sometimes ruptured, giving the appearance of an incomplete moult. The outer cuticle of *Aphelenchoides sacchari* degenerated so much that the body annulation and lateral incisures could not be seen. Specimens fixed in TAF at the same time as these, but processed to glycerol within a year or so of fixation, showed no such abnormalities. The alkaline reaction of TAF (pH 8·2–8·5) may cause this slow degeneration of the cuticle. (Hooper)

Preparation of nematodes for scanning electron microscopy

To prepare nematodes for examination in the scanning electron microscope involves removing volatile materials (chiefly water) before subjecting them to a vacuum. Of several methods, impregnation with glycerine is best, but the nematodes are flaccid, difficult to handle and easily damaged by the electron beam. Other methods require elaborate preparation and give less good results.

Impregnation with acetone enables specimens fixed by the methods used for light microscopy to be used. Fixed nematodes are washed in several changes of distilled water and put into glass blocks with 3 cm diameter cavities containing some distilled water. The blocks are placed in an atmosphere of dry acetone for a day, when the water in the cavities is slowly replaced with acetone by vapour exchange. The nematodes are removed from the acetone, dried and placed on 'Stereoscan' stubs coated with an adhesive film, prepared by washing of 'Sellotape' in ether and coating the stub surface with the solution; the ether evaporates leaving a film of adhesive. The stubs are then evenly coated with a film of gold, 200 Å thick.

Specimens in glycerol were damaged when the electron beam was concentrated on a small area. 'Catalcon K5' doubled the time before damage began and coating with gold gave further protection but, once coated, specimens could not be withdrawn, reorientated, cleaned or dissected. Because they were coated with gold the electron beam rarely damaged specimens in acetone except when sometimes it penetrated their surfaces. Specimens in mixtures of acetone and glycerol were damaged.

Larvae and males of *Heterodera* spp. prepared in this way show cuticular details clearly, including the annulations, lateral field patterns and head structures. The cuticle surface is finely crinkled but otherwise undistorted and can be viewed at magnifications up to 25 000 times. (Stone, Green and Clark)

https://doi.org/10.23637/ERADOC-1-126

The heads of many species of *Heterodera* larvae have a structure undetected by light microscopy. It is an extension of the disc surrounding the mouth to the position of the dorso-lateral and ventro-lateral pairs of lips. Most species with lemon-shaped cysts have it, but not those with round cysts, in which the disc is almost a circle with the dorso-lateral and ventro-lateral pairs of lips visible above and below. *H. weissi* and *H. cacti*, which have lemon-shaped cysts, resemble the group with round cysts. Nevertheless this further difference between the round-cyst and the lemon-cyst strengthens the view that the genus *Heterodera* and its sub-groups need revising. (Stone and Course)

Conjoint and other work

Work with other departments included a study of the interaction between potato cyst-nematode and *Verticillium* wilt in field trials at Woburn (Corbett with Hide, Plant Pathology Department, see page 158), estimation of bromine residues in the same trials (Corbett with Hide, Plant Pathology Department, Lord, Insecticides Department and Brown, Pedology Department) and identification and counting of nematodes surrounding the roots of grain maize (Janet Fraser and Corbett with Barnard, Field Experiments Section). With Nutman and Bell, Soil Microbiology Department, Doncaster produced a film on 'Nitrogen fixation in lucerne' which was one of those shown at the XVIth International Film festival of Scientific and Educational Films at Padua, Italy in November. Jones gave six lectures, part of an international course on plant nematodes, in the Agricultural University, Wageningen, the Netherlands, and several members of staff gave lectures to students at Imperial College Field Station.

During April and May the work of the department was disrupted by the move from the old nematology building, now demolished, to temporary quarters while permanent laboratories are being constructed. Dr. Mary T. Franklin retired at the end of March after a decade of service in the old nematology department at Winches Farm, St. Albans, under the late Professor R. T. Leiper and the late Dr. Tom Goodey and a further 24 years service in the department at Rothamsted. Drs. M. K. Seymour and R. Bromilow joined the staff in October. The head of department was appointed deputy director of the Station in April.

https://doi.org/10.23637/ERADOC-1-126