Report for 1970 - Part1

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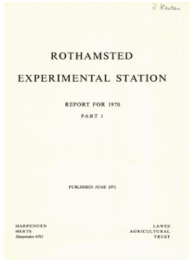
ROTHAMSTED

EXPERIMENTAL STATION

REPORT FOR 1970

PART 1

Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

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Of the many kinds of nematodes, those that harm crops, eelworms, spend some part of their lives in the soil. They are minute, translucent, thread-like creatures, with needle-like mouth parts that pierce plant cells and extract sap. Because they are so small, large populations are usually needed to damage crops. The department studies a wide range of problems but deals mainly with cyst-nematodes, root-lesion nematodes and root ecto-parasites. Most cyst-nematodes have only a narrow host range, so a suitable rotation of crops is a useful control measure, but the interval needed between crops attacked is often longer than the farmer desires, so other methods such as the use of resistant varieties and of nematicides are studied. Root lesion nematodes and root ectoparasites have wide host ranges, so crop rotation is of little value: as a control measure economic ways of using effective nematicides are being sought. In the laboratory, the life-history and biology of harmful species are being studied.

Cuticular structure of cyst-nematodes

The cuticle of second-stage larvae and males of cyst-nematodes is two-layered, whereas the cuticle of females has more to allow for the stretching and strengthening necessary when they swell to more than 100 times the volume of the larva or male. Our ideas given last year about the homologies of the cuticular layer in larvae, males and females have had to be changed after more detailed electron microscopy. Species with lemon-shaped cysts add a third layer (C) to the two (A, B) that larvae and males have; species with round-cysts add a fourth (D). Layer A has an outer electron-dense zone (A_1), which is probably lipid. Zone A_2 consists of fine fibrils, as does Zone A_3, but this also contains scattered electron-dense material. Layer B consists of characteristic symmetrical radial striations. In second-stage larvae, males and newly moulted adult females, this layer is continuous but in older, much swollen females, it is broken into small isolated blocks. The rupture of this layer may make the cuticle more permeable than it is in larvae, possibly allowing unwanted material from cell sap to be excreted. Layer C is fibrous, with zones of electron-dense deposits. Layer D occurs only in round-cyst nematodes and has distinct fibres which appear to be orientated parabolically, as in the chitin of insect cuticle and in the collagen of the eyeballs of some vertebrates. The parabolic appearance probably results from successive layers of fibres laid down at continuously changing but more or less equally spaced angles. In addition to being strong, such a structure produces a spheroid when pressure is raised inside it, and may account for the shape of the round-cyst species. Very young adult females, while still oval, have the C layer already complete, but only the beginning of the D layer. The cuticle of *H. punctata* females, which are oval, has a very thin D layer or none. *H. tabacum*, which we previously thought intermediate between lemon-shaped and round species, conforms entirely to the round. There is no obvious difference between the cuticles of round-cyst species or between the pathotypes of *H. rostochiensis*. Differences between adult females at different stages of development are greater than between species. Among lemon-shaped species, however, there are characteristic differences in the appearance of the A layer. (Shepherd and Clark with Dart, Soil Microbiology Department)

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Rickettsia-like organisms in cyst-nematodes

Thin sections of several females of the pea cyst-nematode, *H. goettingiana*, from micro-plots at Rothamsted, showed organisms resembling small bacteria of the rickettsia type. They occurred in almost all tissues, including the hypodermis, but were especially abundant in ova and cells of the reproductive tract. Sperms in the oviduct of a female of potato-cyst nematode, *H. rostochiensis*, from a Bolivian population, also contained similar organisms. Rickettsias occur in insects, mites and ticks, where they seem harmless, but they sometimes cause disease when transmitted by their carriers to vertebrate hosts. The tissues where the rickettsia occurred in the nematodes seemed undamaged, even though the rickettsias were lying free in the cytoplasm and not bounded by a membrane as they often are in arthropod hosts. In section, rickettsias ranged from rod-shaped to spherical, and the largest was 1·5–2 μm long and 0·3–0·5 μm across. (Shepherd and Clark)

The sub-crystalline layer of cyst-nematodes

The females of some species of cyst-nematodes attached to plant roots in soil are covered with waxy material called the sub-crystalline layer. The Stereoscan microscope shows this to be two layers, a thin dense basal layer overlying the nematode cuticle, and an outer layer several times thicker with radial striations and a tendency to break into polygonal plates. The inner layer also tends to fracture into patterns conforming with the fractures in the outer layer. Sometimes the outer layer consists of angular flakes but more often it is compact with radial striations. Electron microscopy of thin sections through the cuticle and the sub-crystalline layer shows that these two are separate and that a fungus, usually yeast-like but able to produce mycelia, is associated with the sub-crystalline layer and not living on the adult female cuticle. Although it may attack the cast cuticles of young females, most of the energy required to produce the wax, which is sometimes copious, must come from another source. Cyst-nematodes feed similarly to scale insects and aphids, that is they are sedentary and use their mouth stylets to obtain sap from plants. Probably some components of the sap are not used for nutrition and excess sugars or other unwanted compounds are excreted. Scale insects and some aphids produce wax but most aphids void unwanted material through the anus as honey dew. Female cyst-nematodes seem to exude unwanted substances through the cuticle over most of the body: they do not produce the wax directly.

A sample of wax from the sub-crystalline layer of a cyst-nematode of the *H. avenae* group growing on rye-grass gave an X-ray diffraction pattern suggesting that it was calcium n-tetracosanoate or perhaps a mixture of the calcium salt and free acid (see page 76). In the mass spectrometer, the volatile fraction of the wax was n-tetracosanoic acid, confirmed by comparing its mass spectrum with that of a pure sample of the acid from an outside source. A pure sample of the calcium salt was prepared and its X-ray diffraction pattern was similar to that of the wax. When a piece of the sub-crystalline layer was heated under a polarising microscope, the inner layer melted at the temperature expected for the free acid whereas the outer layer melted at a higher temperature corresponding to the calcium salt. Attempts are being made to obtain the same wax from a culture of the fungus. (Williams, Green, Shepherd, with Callow, Insecticides and Salt and Lacey, Plant Pathology)

Hatching factors and the sex attractant of potato cyst-nematode

Although attempts elsewhere (e.g. Carroll, Heyes, Johnson & Todd, *Nematologica* (1958), **3**, 154–167), to purify the hatching factors of *H. rostochiensis* in crude extracts of

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root diffusates by counter-current distribution (CCD) failed, tests with different solvents led to a system more effective than any yet devised. Analysis of root extracts by a combination of CCD with column and thin layer chromatography, indicates six components able to initiate hatching of eggs of *H. rostochiensis*. The properties of two of these are now sufficiently well defined to isolate them without recourse to bioassay. Attempts are being made to accumulate amounts needed to determine their mass spectra but as they are not volatile, they must first be modified to volatile derivatives. (Clarke)

A material produced by females of *H. rostochiensis* that attracts males ran on cellulose paper impregnated with a cation exchange resin of a weak acid type and water as the solvent at an Rf of 0·5–0·6. This suggests that it is a weak base, but attempts to separate it by electrophoresis failed and there was only a faint suggestion of movement towards the negative pole. (Greet)

Surface morphology of the vulval region of cyst-nematodes and of the lateral line of sheath nematodes

The Stereoscan microscope, which clearly displays surface structures was used to study the vulval region of cyst-nematodes. The vulva itself is a small shallow, almost circular, pit at the bottom of which lies a short transverse vaginal opening surrounded by smooth cuticle. The walls of the pit have one or two lateral ridges, which expand in front and behind into crescentic papillated areas. The size of the ridges and the form of the papillae differ in different species, as also does the pattern of ridges and folds in the region embracing the anus and the vulva. All these characters are helpful adjuncts in identifying populations of round-cyst nematodes and in separating the pathotypes of *H. rostochiensis* (see page 149).

The vulvae of lemon-shaped cyst-nematodes differ much more from those of the round-cyst nematodes than different round ones differ from one another, but have not yet been described in detail. Whether the grooves and other structures seen play a part in mating is unknown. (Green)

The lateral incisures and annular pattern of the cuticle surface of sheath nematodes are characters often used to distinguish species. Specimens from Woburn Farm were compared with specimens from Glamorgan. Both populations resemble *Hemicycliophora conida* but the annules change direction or stop where they meet the incisures and are displaced laterally, forming a pattern characteristic of other species. Published descriptions of *H. conida* show the annules crossing the incisures without changing direction and with only a slight break at the median incisure. Perhaps the lateral incisures are less reliable characters for identification than hitherto supposed. (Stone)

The cement glands of female cyst-nematodes

The head and neck of female cyst-nematodes are borne on a conical protuberance from the swollen body, which is usually called the 'neck'. To distinguish the protuberance from the true neck region the term 'thorax' is proposed. Within the thorax lies the median bulb and where the thorax narrows abruptly to the neck is called the shoulder. When females are dissected from roots, the thorax often carries a deposit of a dark hard substance. If this deposit is removed, clear globules of a sticky fluid appear that soon harden and darken. All the species of cyst-nematode examined do this and the material seems to be a cement that anchors the thorax so that the head of the female remains near the giant cells that the nematode has induced the host root to form, while the rest of its much swollen body lies outside the root. The Stereoscan microscope was used to study the

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morphology of the thorax, shoulder, neck and head and to seek the source of the fluid. The predominantly longitudinal ridges of the cuticle on the swollen body extend part way up the thorax and sometimes turn to encircle it. The cuticle on the neck and on the thorax for a short distance below the neck, is relatively smooth but that on the head is deeply sculptured. At the base of the neck next to the shoulder, the first few annules are longitudinally incised, perhaps to allow flexibility, whereas above this towards the head, the circular annulation suggests an extensible zone. On the thorax, just below the shoulder are small pores, irregularly scattered, often in groups at the bottom of fissures. All species examined had these pores through which a component of the cement may issue.

By mounting females in media with different optical properties, most of the structures in the region of the head, neck and thorax of unstained *H. rostochiensis* females can be seen and related to cuticular structures in specimens viewed under the Stereoscan microscope. The hypodermis remains thin until halfway up the thorax and is thin again on the head, but in between, it expands to occupy most of the body cross section above the median bulb. In some media and under the interference microscope, the enlarged hypodermis can be seen to be filled with vesicles, and with tubular projections directed to the surface. In the neck the cuticle has two layers as in larvae and males. (Green)

Species and pathotypes of the potato cyst-nematode

Until recently, there was thought to be only one species of the potato cyst-nematode in the United Kingdom. That populations differed, however, became evident when some were found able to multiply in tuberous species of *Solanum* resistant to others. All populations multiply on commercial potato varieties, some on plants with gene H_2 for resistance from *S. multidissectum*, some on plants with gene H_1 from *S. tuberosum* ssp. *andigena* and others on plants with genes H_1 and H_2. Populations with these three characteristics are called pathotypes A, B and E respectively. In the Netherlands, pathotypes are also distinguished by letters of the alphabet but these have a different meaning and populations are distinguished by their ability or inability to multiply on a different range of resistant potato hybrids which include *S. kurtzianum* and *S. vernei.*

To provide more information about the host plants of the three pathotypes recognised in the U.K., more than 100 species or varieties of Solanaceae were tested, about half

TABLE 1

Production of females on 12 host plants that differentiate the pathotypes of H. rostochiensis

Host plant	Pathotype A	B	E
Datura stramonium	+	−	−
D. stramonium var. *inermis*	+	−	−
Scopolia anomala	+	−	−
Solanum vernii C.P.C. 2733	+	−	−
Salpichroa mandoniana	−	+	+
Solanum leptophyes C.P.C. 4041	−	+	+
S. oplocense C.P.C. 3777	−	+	+
S. sanctae-rosae C.P.C. 2483	−	+	+
S. sarachoides	−	+	+
S. venturii C.P.C. 3715	−	+	+
S. multidissectum C.P.C. 2722	+	−	+
Saracha umbellata	+	+	−

\+ females developed on roots, − no females on roots

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being tuberous *Solanum*. Forty-four of the test plants were susceptible to all three pathotypes and a further 12 were susceptible to one or two (Table 1). Ten distinguished pathotype A from B or E but not B from E. The other two distinguished B from E but were susceptible to A. In their reactions to these hosts, pathotype A differed from B and E much more than the two differed from each other. (Stone)

Guile (*Plant Pathology* (1966) **16**, 125–128) reported that females of different pathotypes differ in colour (A were yellow, B were cream, and E white), and Webley (*Nematologica* (1970) **16**, 107–112) that larvae of pathotype A are shorter than B and E, and have shorter stylets and the median bulb nearer the excretory pore. We measured larvae from eight British, one Bolivian and one East German population, the last from the type locality of

TABLE 2

Measurement of larvae from ten populations of H. rostochiensis *and the numbers of females formed on three resistant potato hybrids as a percentage on the susceptible potato variety Arran Banner*

Pathotype	Source of population	Percentage females on plants with gene			Larval measurements (μm)		
		H_1	H_2	H_1H_2	Body length	Stylet length	*D
Yellow females							
A	Woburn	4	100	1	463	21·2	32·2
	Feltwell	1	53	1	467	21·6	33·7
	Rostock, E. Germany	<1	87	<1	470	22·4	32·2
				Mean	466±2	21·7±0·1	32·7±0·3
A	Bolivia	65	82	15	476	22·4	32·3
Cream females							
B	Glarryford, N. Ireland	100	5	3	490	24·1	36·5
	Garvaghey, N. Ireland	81	17	4	481	23·8	35·9
				Mean	487±3	24·0±0·1	36·2±0·4
White females							
E	St. Brelades, Jersey	96	100	40	462	22·9	40·7
	Jersey	100	60	41	496	24·0	40·6
	Frampton	95	70	26	487	23·3	39·2
	Cadishead	100	84	32	492	23·8	37·9
				Mean	486±0·1	23·5±0·1	39·5±0·3

* D is the distance between the median bulb valves and the excretory pore

Heterodera rostochiensis. Table 2 shows measurements of some larvae and how the populations behaved on British differential hosts. The East German population was the same as British pathotype A. The Bolivian population differed from any so far known in Britain; its females were yellow and its measurements those of pathotype A but it multiplies freely on plants with either gene for resistance and to some extent on those with both. As reported by Webley, pathotype A larvae were shortest, had the shortest stylets and the shortest distance between median bulb and excretory pore. Pathotypes B and E had similar dimensions. (Parrott, Trudgill and Stone)

The lengths of larvae differ when they are stored or treated differently (Fenwick & Franklin, *J. Helminth.* (1942), **20**, 67), so some commonly used methods of processing them were compared. Larvae freshly hatched from a single source of eggs were killed by heat, fixed in different ways and then measured in fixative or in glycerol (Table 3).

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TABLE 3

Means of 20 measurements (µm) of heat-killed and processed Heterodera rostochiensis *larvae, pathotype E*

Coefficients of variation in parentheses

	Stylet length		Median bulb valve to excretory pore		Body length	
Heat killed in water	24·2 (4)		37·3 (8)		494 (5)	
	Fixative	Glycerol	Fixative	Glycerol	Fixative	Glycerol
Cold TAF	23·5 (4)	23·1* (4)	37·5 (10)	35·1 (7)	478 (5)	451** (6)
Hot TAF	23·9 (5)	24·1 (8)	36·1 (7)	37·9 (8)	459** (5)	469* (5)
Cold formalin	24·1 (4)	23·8 (3)	38·0 (8)	35·0 (11)	487 (4)	481 (5)
Hot formalin	23·7 (4)	23·4 (4)	36·7 (8)	36·0 (10)	464* (6)	468* (5)
Hot F.A.	23·3* (4)	22·6** (5)	36·3 (5)	36·4 (7)	466* (4)	458** (5)
Hot F.P.	23·0* (2)	23·3* (5)	35·5 (7)	35·8 (8)	465* (4)	456** (6)

*, **, significantly different from heat-killed specimens at 5% or 1% levels of probability respectively

All treatments extra to heating shortened body length, but fixing in 4% formalin shortened it less than others. Hot formal-acetic acid and hot formal-proprionic acid significantly shortened the stylet. After heat killing alone, the body length was slightly shorter than worms narcotised in 0·075%, ethylene phenoxytol. In addition to shrinking larvae the fixative TAF (formalin 7%, triethanolamine 2%, distilled water 91%) made stylet bases almost invisible after storage for several months. Ideally, larvae should be narcotised and measured immediately but heating to 65°C followed by fixing in cold formalin is the next best method. The method of preparing larvae is important because differences caused by different methods, and differences between measurements made by different observers, are of the same order as those between pathotypes. (Stone)

To see whether males of the three pathotypes could be distinguished by measuring them, samples were collected from potato plants initially infested with 4000 larvae, killed by heat, fixed in 2·5% formalin and measured. As their larvae, pathotype A males were shortest and had the shortest stylets (Table 4), but differences in stylet length were small and varied with body length. Males from resistant potato plants were shorter than those from susceptible plants and had shorter stylets. Therefore, body length/stylet length ratios were calculated and these discriminated effectively between the three pathotypes. (Stone, Trudgill and Parrott)

TABLE 4

Body and stylet lengths (µm) and body/stylet length ratios of 40 H. rostochiensis *males*

Pathotype	Population	Body length	Stylet length	Body/stylet length ratio
A	Woburn	1038	23·7	43·9
	Feltwell	1059	24·6	43·0
	Rostock	1091	26·0	41·9
	Mean	1063±7·9	24·8±0·13	42·9±0·30
E	St. Brelades	1080	25·9	41·8
	Frampton	1049	26·0	40·5
	Jersey	1071	27·3	39·2
	Cadishead	1099	26·8	41·0
	Mean	1075±6·8	26·5±0·11	40·6±0·26
B	Glarryford*	1037	26·7	38·8
	Garvaghey*	988	26·8	37·0
	Mean	1010±10·1	26·8±0·16	37·8±0·39

* Inoculum 500–1000 larvae/plant

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Last year we reported differences between the protein bands of the three pathotypes following polyacrylamide gel electrophoresis. Further tests on 11 pathotype A, four B and 14 E populations showed consistent differences in the faster moving bands. Pathotype B and E had two pairs, whereas A had only one of each of these pairs but had extra bands not found in B or E. Differences between populations of the same pathotype were small and probably caused by differences in the age and condition of females. The populations from Bolivia and from East Germany behaved as British pathotype A populations. (Trudgill)

To compare the pathotypes recognised in the Netherlands with those in the U.K., 21 Dutch populations, two from India and one from Greece were tested on the differential host plants used to recognise pathotypes in the U.K. The colour of females was recorded and stylet lengths were measured (Table 5). All populations listed in the Netherlands under the letters A, B and C resemble British pathotype A in having yellow females and short stylets. Like the Bolivian population already mentioned and unlike British pathotype A populations, some Dutch populations in this group multiplied on plants with gene H_1.

All Dutch pathotype D populations had white females but multiplied to different extents on test plants with genes for resistance from *S. tuberosum* ssp. *andigena* (H_1)

TABLE 5

Female colour, larval stylet length of some foreign populations of H. rostochiensis *and the number of females on resistant potato varieties per cent of the number on Arran Banner*

	Length of larval stylet µm	No. females per cent of those on Arran Banner†		
		Maris Piper ex *andigena* Gene H_1	P55/7 ex *multi-dissectum* Gene H_2	K5/5 ex *andigena* × *multi-dissectum* Genes H_1H_2
Females Yellow				
St. Theo (Greece)	20·9	2	20	0
*C 1	—	2	162	1
C 2	—	2	37	1
A 1000	21·0	3	85	1
A 1087	20·9	1	38	2
B 1086	—	12	70	18
B 1083	20·7	7	65	11
A 1004	21·2	4	122	11
C 3	20·3	[24]	[100]	[22]
C 4	20·6	21	127	18
A 990	21·0	27	74	19
B S1	20·1	39	46	9
B S2	20·3	94	60	59
Females White				
D 1001	22·8	114	47	104
D 1068	23·3	106	43	86
D 1048	—	70	44	61
D 1057	23·8	148	8	25
D 1063	23·4	137	11	42
D 1045	23·3	55	12	32
D 1077	—	95	6	28
Kakk. (India)	22·5	124	27	8
Vijay. (India)	22·7	50	9	59
D S4	—	126	17	42
D S3	—	106	4	51

* Letters indicate the Dutch pathotype classification
† Percentages in [ ] are based on P55/6 = 100

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and *S. multidissectum* (H_2). These and the two populations from India resembled British pathotype E. Ten of the 12 foreign populations with yellow females (Table 5) were tested and found to have protein band patterns similar to those of British pathotype A (*H. rostochiensis sensu stricto*) whereas nine of the 11 populations with white females that were tested had band patterns similar to those of British pathotype E. (Trudgill and Parrott)

To clarify the relationships between British and foreign populations further, single male, single female matings were made. Three Dutch populations (A1087, B1083, C4), one from Bolivia and one from Rostock behaved as British pathotype A. That is they produced many eggs when mated among themselves or with a British A population but only a few when mated with a British pathotype E population. Similarly, two Dutch populations (D1001 and D1068) behaved as British pathotype E. Forty-three per cent of matings between populations classified as British pathotype A and 68% between those classified as British pathotype E produced eggs, whereas only 12% did when E males were crossed with A females and 34% when A males were crossed with E females. When the progeny (eggs) of similar crosses made earlier between like populations were tested, those from 127 females hatched more than 5000 larvae and produced 336 males and 1121 females on a susceptible potato variety, whereas 48 females crossed with males from unlike populations hatched fewer than 300 larvae and produced only 33 males and 10 females. Although some matings between unlike populations appear to succeed, the success rate is smaller and many fewer of their progeny can develop to adults on potato roots than from matings between like populations. (Parrott)

The differences outlined above and those in the cuticular patterns of the vulva and surrounding regions lead us to suggest that British populations of *H. rostochiensis* belong to two species. Those currently called pathotype A, with yellow females, small larvae and males, are the golden nematode, *H. rostochiensis* in the strict sense, and those known as B and E, with cream or white females and larger larvae and males, are a separate, undescribed species. Both belong to the well-defined group of round-cyst nematodes, which ought perhaps to be classified as a genus distinct from other cyst-nematodes with ovoid or lemon-shaped females, under the generic name *Globodera*. The round-cyst nematodes seem all to have originated and dispersed from high ground in Latin America where they evolved along with their solanaceous host plants isolated from one another, so leading to the speciation or partial speciation now apparent in the populations carried to Europe and elsewhere, probably after the middle of the nineteenth century. The golden nematode (pathotype A) populations from Britain so far tested behave very much alike in not multiplying much on plants with gene H_1 from *Solanum tuberosum* ssp. *andigena*. That from Bolivia and some from the Netherlands behave differently (Table 5) and are differentiated as pathotypes A, B and C there. Populations of the other species from Britain vary more than the golden nematode in their behaviour on differential hosts and in their protein band patterns: in the Netherlands these populations are usually classified as pathotype D. Although we have not been able to compare populations from other European countries with those from the U.K. and the Netherlands, we hope to do so and also to test populations from Latin America. At present it seems there are two species of the *H. rostochiensis* type in Europe and that both can be divided into races or pathotypes by their behaviour on differential hosts. (Green, Jones, Parrott, Stone and Trudgill)

Control of potato cyst-nematode and *Verticillium* wilt

In 1969 potato yields on land at Woburn infested with *H. rostochiensis* and *V. dahliae* were much increased by applying methyl bromide or aldicarb ('Temik') to the soil

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before the potatoes were planted, but not by the fungicide benomyl used as a tuber dressing. These plots were again planted with potatoes in 1970 and yields on plots treated with aldicarb or methyl bromide in 1969 were again significantly larger than yields on untreated plots. Plots treated with any of the three pesticides in 1970 all yielded significantly more than untreated plots: aldicarb, which controlled the nematodes, had more effect than the fungicide, benomyl, applied to the soil but methyl bromide, which controlled both the nematode and the fungus, increased yield by much more than the total of the increases from aldicarb and benomyl used separately, showing the damaging interaction between the two pathogens (Table 6). Plot yields in 1970 were negatively correlated with the numbers of nematode eggs in the soil before the potatoes were planted ($r = -0{\cdot}71$, $P = 0{\cdot}001$). (Corbett with Hide, Plant Pathology)

TABLE 6

Effects of nematicides and benomyl on potato yields and numbers of nematode cysts and eggs

	Pentland Crown	Pentland Dell	Numbers after 1969 harvest	
	Tubers, tons/acre		Cysts/100 g	Eggs/g
Years of treatment	1969	1970	1969	1969
Untreated	3·49	1·96	29	60
Benomyl	3·30	4·84*	31	96
Aldicarb	7·48*	6·26*	5**	16*
Methyl bromide	8·09*	14·18*	7**	9**
Untreated, wheelmarks	3·30	—	54	98
Methyl bromide, wheelmarks	7·59*	—	8*	30

*, **, significantly different from untreated at 5% or 1% levels of probability respectively

The toxicity of two carbamoyl-oximes to potato cyst-nematode

Three amounts of two carbamoyl-oxime nematicides, aldicarb ('Temik') and Du Pont 1410, were tested for their toxicity to *H. rostochiensis* larvae; the chemicals were applied in 20 ml of water to soil in 9-cm pots containing potato plants at different intervals before and after the soil was inoculated with 3000 larvae. Batches of larvae were also exposed to solutions of the two chemicals in Petri dishes for 24 hours before they were introduced

TABLE 7

The effect of two carbamoyl oximes on the numbers of larvae of H. rostochiensis *becoming adult, expressed as a percentage of untreated*

Time of treatment, days from inoculation		Amount of chemical (mg) per 9 cm pot 0·01	0·1	1·0	Mean
In vitro	aldicarb	30	83	29	47
	1410	65	61	17	48
6 days before	aldicarb	91	7	1	33
	1410	28	21	1	17
At inoculation	aldicarb	79	37	0	39
	1410	44	56	20	40
6 days after	aldicarb	96	39	2	46
	1410	125	102	30	86
12 days after	aldicarb	105	76	73	85
	1410	66	124	68	86
18 days after	aldicarb	139	105	63	102
	1410	153	143	74	123

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to the pots. Toxicity was assessed by counting the adults produced. Both chemicals were toxic when applied directly to the nematodes *in vitro* (Table 7), but more so when applied to the soil at the time of inoculation or 6 days before (aldicarb and 1410) or after (aldicarb only). The chemicals seemingly prevent larvae from invading roots but are ineffective once the larvae have done this. Hence they would be useless as sprays applied to leaves although they are absorbed and distributed systemically in the plants (see page 158). (Trudgill)

Potatoes in the Woburn Ley–Arable experiment

Chloropicrin (400 lb/acre injected) and aldicarb (100 lb/acre of 10% granules rotavated in) were again applied before potatoes were planted in the Ley–Arable experiment, and again both increased yields (Table 8), especially chloropicrin in the ley rotation and

TABLE 8

Effect of chloropicrin and aldicarb on potato yields in the Woburn Ley–Arable experiment

Total yield (tons/acre)

Rotation	Treatments: None	Aldicarb	Chloropicrin	Aldicarb	Numbers of *H. rostochiensis* before planting (eggs/g soil)
Ley-arable[1]	14·08	15·88	20·84	19·21	2
Sainfoin-arable[1]	11·58	16·71	15·40	17·19	4
Continuous arable (H)[2]	9·67	13·49	15·88	16·86	148
Continuous arable (C)[2]	10·46	13·50	15·47	16·07	101
Mean	11·45	14·90	16·90	17·33	

[1] Potatoes every 10th year
[2] Potatoes every 5th year, and 1 year hay (H) or carrots (C) every 5th year

aldicarb in the sainfoin rotation. Populations of *H. rostochiensis* were dense in the two continuous arable rotations and control of this nematode by chloropicrin must have contributed greatly to the improved yields from these plots. However, chloropicrin also greatly increased yield of plots in the ley rotation where there were too few *H. rostochiensis* to be damaging. As migratory nematodes were few in the top 20 cm of soil, after harvest, soil was sampled down to 60 cm, in 10 cm fractions, in the ley and arable with hay sequences (i.e. those in which the response to chloropicrin was greatest). *Longidorus leptocephalus* were plentiful 30 to 60 cm deep in untreated plots but not in plots treated with chloropicrin (Table 9). As the summer was dry, *Longidorus* attack on

TABLE 9

Longidorus leptocephalus *in the potato plots of the Woburn Ley–Arable experiment*

Rotation	Treatment	Numbers 200 ml of soil, Depth in cm: 0–10	10–20	20–30	30–40	40–50	50–60
Ley–arable	Chloropicrin	0	0	0	0	0	2
	None	0	2	3	44	39	50
Continuous arable (H)	Chloropicrin	0	0	0	0	0	4
	None	0	0	1	32	35	29

deep roots in the unfumigated plots may have caused some yield loss. Responses were smaller to aldicarb than to chloropicrin (except in the sainfoin sequence), presumably because chloropicrin kills pathogens other than nematodes and releases nitrogen, and

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perhaps also because the *Longidorus* in the ley sequence were too deep to be reached by aldicarb. The large response to aldicarb in the sainfoin sequence is unexplained, but those yield differences attributable to nematodes are probably from control of both *H. rostochiensis* and *L. leptocephalus*, with chloropicrin having its greatest effect where both occur together.

All species of migratory ectoparasitic nematodes were affected by both chloropicrin and aldicarb and may have contributed marginally to yield losses in untreated plots although the two commonest genera, *Paratylenchus* and *Tylenchus*, were fewer than usually associated with damage to crops. Other factors may have contributed to yield improvements, and although neither fungi nor other micro-organisms were identified that might have decreased yields, there may have been some. The treatments may also have increased the availability of nutrients to the crops. (Evans with Salt, Plant Pathology)

Chemical control of potato cyst-nematode

In Butt Furlong, Woburn, where the sandy soil (about 10% clay) is heavily infested with potato cyst-nematode, 300 lb dazomet ('Basamid', a 98% a.i. prill formulation)/acre rotavated during autumn 1969 into the surface 15 cm of soil, which was then ridged, greatly decreased the number of larvae infesting Majestic potatoes planted in the ridges and greatly increased the yield of tubers. This was on plots treated in 1969 and 1968 with 32 gal 'D-D'/acre (Table 10). In heavily infested peaty loam (about 15% organic matter)

TABLE 10

Placement of soil fumigants to control potato cyst-nematode

		Ware tubers tons/acre; (larval invasion % untreated plots)		
Treatment	Amount (lb/acre)	Butt Furlong (sandy loam) Majestic	King Edward	Ancaster Farm (peaty loam) Maris Piper
Untreated	0	4·6	4·7 (100)	11·6***
Dazomet (98%)	38	5·3		
	75	6·7*		
	150	7·4*	8·4* (7)	
	300	10·3***	10·2** (1)	
	450		12·6*** (1)	
	300†		12·6*** (1)	
'Telone'	140		9·5** (27)	
	280		11·3*** (14)	
	560		13·6*** (7)	

*, **, ***, significantly greater than untreated Majestic or King Edward at 5%, 1%, or 0·1% levels of probability respectively

† Half rotavated in before ploughing, half rotavated in after ploughing

at Ancaster Farm, Isle of Ely, dazomet similarly applied to ridges controlled the nematodes better than did 'Telone' injected into the ridges during autumn. Rotavating 150 lb dazomet/acre into the surface 15 cm of soil before ploughing and then rotavating another 150 lb into the surface 15 cm after ploughing controlled the nematode well to plough depth and increased the yield of ware-sized tubers by as much as did 450 lb of dazomet/acre in the ridges. In sandy loam of Butt Close, Woburn, dazomet controlled potato cyst-nematode whether it was applied during autumn or the spring and whether the plots were ridged up immediately after treatment or not. Here also, dazomet was more effective than 'Telone' injected into preformed ridges (Table 11).

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TABLE 11

Control of potato cyst-nematode in sandy loam, Woburn

Treatment	Amount	Ware tubers var. Majestic tons/acre (larval invasion % untreated plots)
Untreated (autumn ridged)		7·5 (100)
(not ridged)		6·8 (100)
Dazomet (98%)	300 lb/acre, autumn in ridges	16·0*** (2)
	300 lb/acre, autumn not ridged	18·1*** (1)
	300 lb/acre, spring in ridges	20·1*** (4)
	300 lb/acre, spring not ridged	19·1*** (1)
'Telone'	300 lb/acre, autumn in ridges	13·5** (35)
	300 lb/acre, spring in ridges	11·6* (36)

*, **, ***, greater than untreated at 5%, 1%, or 0·1% levels of probability respectively

Up to 10 lb a.i. aldicarb/acre, rotavated into the top 15 cm of soil during spring just before planting potatoes, killed many of the potato cyst-nematodes in peaty loam at the Arthur Rickwood Experimental Husbandry Farm, Isle of Ely, gave a good crop of King Edward potatoes and also increased the yield of Maris Piper. In contrast 'D-D' killed fewer of the nematodes and dazomet, even when applied in autumn, damaged the King Edward crop, although it killed the nematodes in the top 15 cm of soil.

Ten pesticides, spread on the soil surface at 10 lb a.i./acre and rotavated in just before

TABLE 12

Assessing nematicidal activity of ten pesticides in two soils

Treatment	Amount	Ware tubers var. King Edward tons/acre; (larval invasion % untreated plots): Arthur Rickwood E.H.F. (peaty loam)	Leverton Grange (silt)
Untreated	10 lb a.i./acre	2·7 (100)	12·5 (100)
Aldicarb	10 lb a.i./acre	10·5*** (1)	18·8** (2)
'Tirpate'	10 lb a.i./acre	9·7*** (1)	19·0** (1)
Du Pont 1410	10 lb a.i./acre	8·8*** (7)	21·0*** (8)
'Nemacur P'	10 lb a.i./acre	8·5*** (2)	18·3* (1)
'Terracur P'	10 lb a.i./acre	3·3 (82)	15·0 (21)
Thionazin	10 lb a.i./acre	7·8*** (4)	14·3 (75)
Phorate	10 lb a.i./acre	4·3 (37)	17·1* (31)
Diazinon	10 lb a.i./acre	5·0 (95)	14·1 (78)
Chlorfenvinphos	10 lb a.i./acre	1·3 (38)	12·3 (50)
'Mocap'	10 lb a.i./acre	6·8** (15)	16·9 (95)

*, **, ***, greater than untreated at 5%, 1%, or 0·1% levels of probability respectively

TABLE 13

'Du Pont 1410' and potato cyst-nematode, Butt Close, Woburn

Treatment applied to: Soil	Leaves	Ware tubers var. Pentland Crown tons/acre
Untreated		8·5
4·3 lb a.i./acre		17·0***
8·6 lb a.i./acre		18·3***
17·2 lb a.i./acre		17·7***
4·3 lb a.i./acre +	2 lb a.i./acre	15·3***
4·3 lb a.i./acre +	4 lb a.i./acre	15·7***
8·6 lb a.i./acre +	2 lb a.i./acre	17·4***
8·6 lb a.i./acre +	4 lb a.i./acre	16·1***

***, significantly greater than untreated at 0·1% level of probability

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planting King Edward potato were compared on silt soil at Leverton Grange, Lincolnshire, and on a peaty loam soil at the Arthur Rickwood E.H.F., both infested with the potato cyst-nematode. Three carbamoyl-oximes (aldicarb, 'Tirpate' and Du Pont 1410) and 'Nemacur P', an organo-phosphorus pesticide, were most effective in controlling nematodes and increasing yield. Thionazin was also effective in peaty loam but not in silt; phorate was moderately effective in silt (Table 12). In sandy loam on Butt Close, Woburn, 'Du Pont 1410' rotavated into the surface 15 cm of soil controlled the nematodes and greatly increased yield of Pentland Crown (Table 13). Neither in pots nor in field plots did Du Pont 1410 sprayed on the leaves of young potato plants kill the nematodes in the roots.

Thus the potato cyst-nematode can be controlled chemically by treating the soil with various pesticides, of which the most effective are dazomet, aldicarb, Du Pont 1410, 'Tirpate' and 'Nemacur P'. (Whitehead and Tite)

Docking disorder of sugar beet

In 1970, Docking disorder, the stunting of sugar-beet seedlings caused primarily by root-ectoparasitic nematodes, was rare. Nevertheless in two of three large-scale experiments in fields in West Norfolk infested with stubby-root nematodes (*Trichodorus* spp.), fumigating the row positions with 'D-D' or 'Telone' to kill the nematodes before sowing beet significantly increased sugar yield (Table 14). At East Winch pulling the injector tines

TABLE 14

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

Treatment	Gal/acre	Sugar (cwts/acre) Docking	E. Winch	Gayton
Untreated	0	—	52·6	—
Tines only	0	55·1	55·7	59·6
Tines + 'D-D', 1 week or	5	—	—	67·0***
more before sowing	6	58·1	58·5(*)	—
	7	—	—	68·9***
	8	—	58·0(*)	—
	9	—	—	65·6**
	10	52·7	58·5(*)	—
	13	53·1	—	—
Tines and 'Telone' 1 day before sowing	5	—	—	67·5***
Tines and 'D-D' 1 or 2 days	5	—	—	66·9***
before sowing	6	56·3	60·7*(**)	—
Beet harvested from each plot		6 rows × 50 yd	2 rows × 360 yd	2 rows × 480 yd

*, **, ***, significantly greater than 'Tines only' treatment at 5%, 1%, or 0·1% levels of probability respectively; (*), (**) significantly greater than untreated at 5% or 1% levels of probability respectively

alone through the soil 15 cm deep did not increase yield significantly. The light sandy soil at Docking probably dried out too quickly after sowing for these nematodes to injure sugar-beet roots much but at East Winch and especially at Gayton where the soil contained a little more clay some nematode damage, especially to deeper roots probably occurred. The soil at Gayton was heavily infested with both *Trichodorus* spp. and a needle nematode (*Longidorus attenuatus*). These results show that fumigating the rows into which the sugar-beet seeds are later sown may more than repay the cost of treatment

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in fields prone to Docking disorder, even in a year when climatic conditions are unfavourable for the activity of the nematodes responsible. (Whitehead and Tite)

Nematicides against migratory nematodes in sugar beet at Woburn, 1967–69

Poor growth of sugar beet on Butt Furlong in 1966 was thought attributable to migratory nematodes, which were studied there again in 1967 when 'D-D' was applied at 2·6, 5·8 and 9·7 ml/m to the plough sole, 12 weeks before sowing beet. In 1968 sugar beet was again sown with nematicides applied across the 1967 treatments: 'Telone' at 3·3 ml/m injected before sowing; 'Lannate', 5% granules at 22 lb/acre, and aldicarb 10% granules at 11 lb/acre, both 3 days after sowing. The three amounts of 'D-D' all significantly decreased numbers of the chief migratory root nematodes present (*Tylenchorhynchus dubius*, *Pratylenchus neglectus* and *Trichodorus primitivus*) (Table 15). In 1968, nematodes

TABLE 15

'D-D' and numbers of migratory nematodes in Butt Furlong. Means of monthly counts per litre of soil, April–October 1967

Treatment	*Tylenchorhynchus dubius*	*Pratylenchus neglectus*	*Trichodorus primitivus*
Untreated	813	214	398
2·6 ml 'D-D'/m	398	145	251
5·8 ml 'D-D'/m	389	48	182
9·7 ml 'D-D'/m	324	56	93

were again significantly fewer in plots treated with 'D-D' in 1967. Of the other nematicides 'Lannate' was least effective in controlling nematodes and damaged the beet, aldicarb was the most effective and greatly decreased the numbers of *Trichodorus*, the most abundant migratory nematode (Table 16). 'Telone', was intermediate and most

TABLE 16

Nematodes in Butt Furlong, numbers per litre of soil, 1968

	Tylenchorhynchus dubius		*Pratylenchus neglectus*		*Trichodorus primitivus*		Total nematodes	
Treatment	May	Aug.	May	Aug.	May	Aug.	May	Aug.
Untreated	302	174	98	34	288	871	5754	5248
Aldicarb	132	56	115	11	123	282	5012	2570
'Lannate'	195	36	123	11	339	437	4365	2455
'Telone'	30	17	22	26	59	479	2188	3162

effective against *Tylenchorhynchus* but stunted the beet. Nematicides were not applied in 1969, when barley was grown without responding to any treatments applied during the two previous years. Soil samples taken after harvest showed that 'Telone', 'Lannate' and aldicarb applied in 1968 in combination with the largest amount of 'D-D' applied in 1967 made nematodes significantly fewer in 1969. *Tylenchorhynchus* and *Trichodorus* were significantly fewer in plots treated with 'D-D' and aldicarb. Nematodes in all plots were few in 1969 probably because of the long dry period during late summer. (Mojica and Stone)

Rotation–fumigation experiment, Butt Close, Woburn, 2nd year

During the first year of this experiment (1969), 'D-D' had no significant effect on barley yields but it significantly increased the yield of potatoes given the least N, and there was

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also a significant increase ($P = 0{\cdot}05$) when potato yields for all three nitrogen rates were averaged. The yield of sugar beet was not significantly affected by 'D-D' treatment in 1969. Table 17 summarises the *H. avenae* egg counts (per g air-dried soil) before and after the 1969 crops.

TABLE 17

H. avenae *in rotation–fumigation experiment, eggs/g air-dried soil 1969*

	Barley		Potatoes		Sugar beet	
	Pre-	Post-crop	Pre-	Post-crop	Pre-	Post-crop
Untreated	1·3	1·3	3·9	1·5	2·5	1·8
Treated plots ('D-D')	0·7	0·4	1·4	1·4	2·4	0·6

TABLE 18

Barley grain yield from Rotation–Fumigation experiment, 1970

Yields of grain at 85% D.M.: cwt/acre

	No treatment	'D-D' before potatoes	'D-D' before sugar beet	'D-D' before barley	'D-D' before all crops	Dazomet 1970	Mean
0·3 cwt N	6·8	13·8**	6·3	9·8	13·5*	14·8**	9·9
0·6 cwt N	13·8	14·8	13·0	14·6	16·9	17·3	14·1
0·9 cwt N	12·6	18·6*	11·3	14·8	14·6	19·0*	14·7
Mean	11·1	15·7**	10·2	13·1	15·0*	17·0***	

*, **, ***, significantly different (horizontal comparisons) at 5%, 1% or 0·1% levels of probability respectively

During the second year (1970), dazomet was applied to some plots and, as Table 18 shows, increased the yield of barley. There was also a significant increase in barley yield from fumigation with 'D-D' for the previous potato crop but not with 'D-D' used before the barley crop. 'D-D' applied in both years increased yield significantly only with fertiliser at 0·3 cwt N/acre.

Significant increases in potato yields in 1970 were gained after two consecutive applications of 'D-D' and one of dazomet, averaged over nitrogen dressings. Again, as with the barley, there was a significant yield increase when 'D-D' was applied in 1969 only, before the previous sugar-beet crop. These results may indicate that in the year it is applied, 'D-D' damages plants, which detracts from its beneficial effects as a fumigant. (Williams)

Cereal cyst-nematode at Rothamsted

In some of the cereal disease reference plots, spring wheat grew poorly and the roots were much knotted. After harvest the soil was sampled and the largest population of cereal cyst-nematode ever found at Rothamsted was recorded. Average populations in plots 19 and 21 (respectively, after eight and three crops of spring wheat) were 13 eggs/g of soil and in some parts of plot 19 there were local populations of as many as 130 eggs/g soil. No reason can be given for the sudden and unexpected local infestation, which is mainly concentrated in plots 15, 16, 18–21. (Williams with Slope, Plant Pathology Department)

Stem eelworms on field beans

The 'giant race' of the stem nematode, *Ditylenchus dipsaci*, apparently introduced with the seed for the 1968 crop of beans on Summerdells field, severely affected the second

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(1969) and third (1970) bean crops grown there. The 'giant race' injures beans in Mediterranean regions but has only been recorded once before on beans in Britain. This is puzzling because observations on the beans in Summerdells show that it can over-winter in our soil. Stem eelworms occurred this year on field beans on the Rothamsted farms mainly where the beans were previously infested and nematodes were presumably soil-borne. There was little evidence of seed-borne infestations in the crops.

Aldicarb granules (11 kg a.i./ha), applied before sowing beans on Barnfield, killed more than half the Tylenchid nematodes and three-quarters of the total nematode population. Only 5% of plants was infested with stem eelworm on the aldicarb plots in August, whereas 31% were in the untreated plots (see also p. 136). On Fosters Field, plants on plots with aldicarb were free from stem-eelworm, whereas the other plots had infested plants (see p. 186). (Hooper)

Nematodes associated with Sitka spruce

Populations of nematodes were studied in plots sown annually with Sitka spruce or Grand fir in a forestry nursery at Kennington, Berks. The plots are on light sandy soil and the pH is adjusted from 3·8 to 7·0 by adding finely ground limestone. Although 4·5 is the optimum soil pH for Sitka spruce growth, *Tylenchus emarginatus* and *Trichodorus teres*, that feed on Sitka roots, were most common in plots at pH 5·4–5·6. An *Aphelenchoides* sp., which has been cultured on fungi, was most common in plots at pH 3·8 and did not occur in plots of pH 5·3 or more. *A Mononchus* sp. (predatory nematode) was most common in plots at pH 5·6–6·0 but rare at pH 3·8. In a second experiment, an undescribed species of *Trichodorus* occurred in Sitka spruce beds at pH 5·2–5·5 but not in beds at pH 4·2–4·7. Some half-plots of the first experiment were treated with aldicarb granules (0·24 g a.i./sq m) during March 1969, just before sowing, and the plots sampled down to 15 cm for nematodes early in June. There were only 12% as many plant-parasitic nematodes and 33% other free-living nematodes as in untreated plots. Seedling growth by October was only slightly improved by aldicarb, even with a second dressing of 0·48 g a.i./sq m applied to the soil surface during June but growth response was very patchy possibly because of the very dry season. (Gowen and Hooper, with Salt, Plant Pathology)

Taxonomy

A species of *Aphelenchoides* much used in research at Rutgers University, U.S.A., and previously thought to be *A. sacchari*, is an undescribed species in which body length and gonad length differed greatly depending on the age of the cultures and the fungus host on which it was cultured. Body length of females ranged from 318 to 569 μm and their ovaries are 18–77% of the body length. The shape of the female tail also varied greatly. Another undescribed species of *Aphelenchoides*, originally from rice stems in Sierra Leone, reproduces fastest on *Botrytis cinerea* at 35°C, a temperature lethal to many other nematodes. Adults and larvae of this nematode survived more than 12 hours in water at 38°C but they did not reproduce. (Hooper)

Conjoint and other work

Doncaster made a colour ciné film about nitrogen fixation by lucerne, based on work by P. S. Nutman and F. Bell of the Soil Microbiology Department, in connection with the International Biological Programme. The film shows infection of root hairs by nodule bacteria that lead to the development of root nodules, and illustrates the planning,

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execution and results of a field experiment designed to identify the main factors limiting nitrogen fixation.

D. J. Hooper cooperated with Dr. C. E. Taylor, Scottish Horticultural Research Institute, in the identification of many specimens received from the N.A.T.O. sponsored survey of the occurrence of *Longidorus* spp. and *Xiphinema* spp. in the U.K. and Eire.

The long-term experiment on potato varieties resistant to *Heterodera rostochiensis* sited in Long Mead, Woburn, ended with a crop of the resistant variety Maris Piper. The yield depended on the number of nematode eggs left in the plots by previous treatments and averaged 13 tons/acre, although late sown. Tubers were healthy although potatoes were grown on almost all plots each year for the last ten. On plots that grew marginal hosts (Solanaceae) or potato hybrids resistant to the eelworm every year there was little evidence of a change of pathotype or species (see page 149) to the one able to multiply on them. The other long-term experiment, in Butt Close, Woburn, testing irrigation and fumigation, and growing a resistant and susceptible variety continuously or alternately, continued. In this land, the species with white females (pathotype E) has begun to appear in some of the plots that have grown the resistant potato varity Maris Piper each year for five years.

Staff

Five members of the department attended the Xth International Symposium of the Society of European Nematologists held at Pescara, Italy, in September.

In November, Mary Franklin gave an invited paper on 'Interrelationships of nematodes, weeds, herbicides and crops' at the Xth British Weed Control Conference, Brighton, and in January F. G. W. Jones gave the John Curtis 'Woodstock' lecture on 'The control of the potato cyst-nematode' at the Royal Society of Arts.

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