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Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

F. G. W. JONES

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Introduction

The department identifies and describes nematodes (eelworms) harmful to crops (p. 172 and publications list), and studies their structure and the way they function. The way they feed and injure crops is particularly important (p. 172). Apart from some of the cyst-nematodes (pp. 174, 182), most plant feeding nematodes attack a range of crops and weeds. This is especially true of the stem nematodes (pp. 179, 186), which rank next to cyst-nematodes in the crop losses they cause, and of virus vector and spiral nematodes which we have recently begun to study (p. 187). Although it would be possible to centre research projects on individual crops or groups of crops, individual crop-nematode problems are too many and nematologists too few to operate effectively in this manner. Therefore, we have concentrated most on potato cyst-nematodes as a model system: field infestations are widespread, the encysted nematodes readily withstand drying and are easily stored for experimental work. These nematodes are therefore convenient for many kinds of work and concentration on them enables us to enquire more deeply than if effort were spread thinly over many species and crops. Information gained is readily applied to other cyst-nematodes and to most other nematode groups.

The department does many field trials on our own and on other farms (p. 180). These are concerned with population dynamics, nematode-resistant varieties and above all with control by nematicides. Recently we have paid more attention to fungi and other organisms parasitising cyst-nematodes (pp. 178 and 180) and have studied a species of nematode found parasitising slugs (p. 188).

Identifications and descriptions

The Nematology Department is the main British centre concerned with the identification, description and taxonomy of plant nematodes. Complementary work is done at the Commonwealth Institute of Helminthology, St. Albans, which concentrates on samples received from developing countries.

Our work is concerned principally with economically important genera and groups of genera viz: cyst-nematodes (Heteroderidae), virus vector nematodes (*Trichodorus*, *Paratrichodorus*, *Xiphinema*, *Longidorus*), bud and leaf nematodes (*Aphelenchoides*), and spiral nematodes (*Helicotylenchus*, *Rotylenchus*). (Hooper and Stone)

During the year Heterodera hordecalis was identified from rough grassland in Lincolnshire and is believed to be the first record in Great Britain. It was found in association with another graminaceous cyst-nematode which resembles H. mani except in stylet knob shape (Rothamsted Report for 1976, Part 1, 202). This latter species is now known to develop on cereals, reported as non-hosts for H. mani. Because we rarely sample nonagricultural land both species may be more common than casual findings suggest. (Stone and Rowe)

H. humuli, the hop cyst-nematode, was first described taxonomically by Filipjev from accounts published by Vorgt in 1894 and Triffit in 1929. Both were based on specimens from Kentish hop gardens. Apparently Filipjev saw no specimens and no types were designated, so the species has been redescribed on specimens from Kent and a neotype designated. (Stone and Rowe)

Pratylenchoides ritteri was found associated with sugar beet in East Anglia and Paratylenchus bukowinensis with stunted celery in East Yorkshire. These are new records for Britain but both are known in Continental Europe. Longidorus laevicapitatus, occasionally reported from some tropical regions, was associated with potatoes growing in the Highlands of Kenya. (Hooper)

Whereas *Hemicycliophora* spp. have large amphidial openings flanking a small oral disc, those in an allied species collected by Mr. K. Orton-Williams, Commonwealth Institute of Helminthology are small, pore-like and completely obscured by large arc-shaped extensions of the oral disc. In other respects the allied species is typical of *Hemicycliophora*. (Stone)

Nematode feeding

Quantity of cell sap withdrawn by Ditylenchus dipsaci. The recording system described last year (*Rothamsted Report for 1976*, Part 1, 206–207) has been improved by adding an audio monitor and a locating system, a ring of light which marks the centre of the microscope field on the TV monitor screen.

The apparatus was used to study feeding of the stem nematode *Ditylenchus dipsaci*. Thrusting of the mouth stylet to penetrate a food cell and pulsation of the pump chamber in the muscular oesophageal bulb, causing ingestion of food, were two activities recorded. As pumping began, several low-amplitude pulsations occurred, a few seconds apart, when isolated muscle fibres contracted. Co-ordination later improved and pulsations became more regular and of greater amplitude. 'Missed beats' were detected, especially towards the end of pumping; these may be a regulatory response to diminishing food 172

supply or the result of fatigue. Mean pumping frequency was $6\cdot 2 \pm 0.33$ (SE) pulsation sec⁻¹, and the calculated volume of the dilated pump chamber was $5\cdot 5 \mu m^3$. A rough estimate of the volume of plant cell contents actively ingested by one *D. dipsaci* in a day is $1-2 \times 10^6 \mu m^3$. A heavily-infested bean plant may carry 100 000 nematodes, and so, assuming that passive ingestion removes the same volume as active pumping (Doncaster, *Journal of Zoology, London* (1976) **180**, 139–153), only 0.2–0.4 ml per plant day⁻¹ will be withdrawn which suggests that feeding *per se* contributes little to plant damage caused. Further work on feeding, defaecation and volume change in food cells, should lead to better estimates of amounts of cell contents lost from plants through nematode attack. (Seymour and Doncaster, with Minter, Instrument Workshops)

Stylet of *D. dipsaci.* In photographs taken with the Scanning Electron Microscope at 11 500 \times magnification, the stylet of *D. dipsaci* had a rather blunt, bullet-shaped tip. The stylet aperture was not well shown but appears subterminal. An X-ray microanalyser coupled to the microscope showed that Si, P and K were present in the cuticle and Na in the stylet. Both stylet and cuticle contained significant amounts of S. (Seymour, with Turner, Plant Pathology Department)

Structure of the anterior alimentary tract of Ditylenchus dipsaci. The oesophageal pump of D. dipsaci is smaller and weaker than that of Aphelenchoides blastophthorus described last year (Rothamsted Report for 1976, Part 1, 205). The pump chamber is triradiate with a thickened cuticular lining to which the muscles attach directly and not as described by Yuen (Nematologica (1968) 14, 385). The lumen of the procorpus and anterior metacorpus is round, but there is an additional set of radial muscles at the junction of these two regions. Just behind the pump chamber the subventral gland ducts enter the oesophageal lumen. The end-apparatus of the cuticularised ducts within the subventral gland duct ampullae is complex and incompletely cuticularised. The oesophageal lumen continues triradiate, but with a thin cuticular lining, along the whole length of the isthmus. Just behind the nerve ring, which encircles the isthmus, the oesophageal glands, incorporated with the oesophageal tissue, form the posterior bulb. The tip of the gland lobe slightly overlaps the oesophago-intestinal junction. The membranes separating the three oesophageal glands are difficult to preserve well, as are most of the membranes in D. dipsaci, but we think that the glands are separate entities and not fused as Yuen concluded. Apart from the single large nucleus in each gland, there are also groups of smaller cells with nuclei, associated with the gland tissues, which are probably supporting cells or nerve cell bodies. The oesophago-intestinal junction is of the type described in Hexatylus viviparus and A. blastophthorus. The intestinal lumen is lined with microvilli which, near the oesophago-intestinal junction, are fairly smooth-coated. From about 3 to 4 µm behind the junction the microvilli have a sculptured outer coat in the form of shallow circular flanges. (Shepherd and Clark)

Feeding of Longidorus caespiticola. Little is known about the way Longidorus spp. feed because specimens behave abnormally in observation chambers. We filmed L. caespiticola feeding on ryegrass roots in chambers which allowed the use of highest magnifications of the light microscope and differential interference contrast illumination without disturbing them. L. caespiticola feeds gregariously on cereal roots. Exploring nematodes often dislodge those already feeding but gregariousness probably favours mating, despite disturbance to feeding.

Nematodes fed close to root-tip meristems and their explorations were briefer when roots had been fed on before. Unlike some Tylenchida *L. caespiticola* often pulsated the oesophageal bulb as it approached roots as though 'tasting' root exudates and explored

the root epidermis less thoroughly with its lips. Also it did not use its stylet as a sensory probe, but only for penetration. Stylet thrusts quickened and shortened as the epidermis was penetrated and thrusting did not stop until the stylet was fully extended.

Although saliva was not seen flowing within nematodes or into food cells, darkening of food cells within 30–40 min of being penetrated, swelling of roots, curling and cessation of growth were probably caused by injected saliva.

Usually nematodes did not reject a feeding site until a few minutes after they had protracted the stylet fully and pulsated the oesophageal bulb a few times, possibly to sample the contents of the food cell. Accepted food cells supplied the nematode with food for up to about 6 h. Nematodes ingested by pulsating the oesophageal bulb which elongated equally and simultaneously at both ends. The tri-radiate bulb lumen always dilated and collapsed sequentially from the front backwards but dilation was only possible when liquid was available to fill it.

As in *D. dipsaci*, the lumen of the oesophago-intestinal valve was not visible until it was distended by inflowing liquid and it is probably of the same 'sticky' type as valves in other species (Seymour & Shepherd, *Journal of Zoology, London* (1974) **173**, 517–523; Shepherd & Clark, *Nematologica* (1976) **22**, 332–342). (Doncaster, with Miss Angela Towle, Reading University)

Simulating mechanical injury by long-stylet nematodes. To simulate the mechanical injury caused by the penetration of long stylets such as those of *Longidorus* spp. into root tissue, fine borosilicate glass needles were made with tip diameter of 1 μ m or less. When these needles were thrust against the root in the manner of a nematode they could not be made to penetrate; they merely bent against the root cortex. This was probably because, unlike real stylets, the needles were unsupported and because the amplitude of individual thrusts was far greater than those made by nematodes. Needles of about 3 μ m tip diameter could, however, be thrust into the root, but despite their greater thickness, they produced less drastic symptoms than nematodes do. After the needle was withdrawn, cells in its path darkened, and the root bent slightly towards the punctured side. (Seymour and Doncaster)

Cyst-nematodes (Heterodera and Globodera spp.)

Pathotypes. Pathotypes are races or sub-species distinguished by their ability or inability to multiply on cultivars or wild host plants possessing resistance genes. The distribution of these within the U.K. and of others abroad that may gain entry is important in the breeding of resistant cultivars and in their use after release. Work on these, at present mainly in connection with potato cyst-nematodes, looks ahead to possible changes in the nature of British populations as more resistant cultivars become available and begin to be planted. The population model (*Rothamsted Report for 1976*, Part 1, 207), further developed since last year, indicates that genetic changes in cyst-nematode populations (i.e. change of pathotype) following selection by cultivars with genes for resistance, are likely to take many years; a sharp contrast with the rapid changes in fungal pathogens of cereal leaves. However, because gene flow is not involved, the selection of species (e.g. *G. pallida* by potatoes with resistance gene H_1) is more rapid, although still far slower than for cereal leaf pathogens. To further the above work, we are concerned with the identification of pathotypes, whether by using test plants or morphometric methods. Whether they interbreed is also important.

New pathotypes in the U.K. The suspected occurrence of a new pathotype of *G. pallida* in Britain (*Rothamsted Report for 1976*, Part 1, 202) was confirmed at Rothamsted and the 174

Plant Breeding Institute. The population concerned, from New Leake, near Boston Docks, Lincs., was previously classified as Pa2 after testing on the differential host plants in the European Scheme. Whereas the standard Dutch population of Pa2 scarcely multiplies on clones with gene H₃ derived from *S. tuberosum* ssp. *andigena* CPC2802 × Maris Piper, the New Leake population differentiates between clones, multiplying on D47/11 and D49/1 and failing to do so on D40/8 and D42/9. Evidently progeny of CPC 2802 are heterogeneous for resistance to populations of this type. As the New Leake population can now be differentiated from the standard Pa2 population, it must be considered a new pathotype. (Stone, Rowe and Farr with Dr. H. W. Howard and J. M. Fuller, Plant Breeding Institute)

Two further populations supplied by Dr. Howard from Bickerstaffe, near Ormskirk, Lancs. (code DF in Plant Breeding Institute publications) and from Pittingdon, Durham (code CC) were also tested on European differentials and classified as Pa2. The Bickerstaffe population fails to reproduce on D 47/11 and D 49/1 and evidently is Pa2, the first confirmed occurrence of this pathotype in the U.K. The Pittingdon population has still to be tested on D 47/11 and D 49/1. Thus, all three recognised pathotypes of *G. pallida* (Pa1, Pa2 and Pa3) are present in Britain and so is a fourth represented by the New Leake population. (Stone and Valenzuela)

Behaviour of populations on potatoes with resistance genes H_1 , H_2 and H_3 . Development of new cysts of 44 South American and nine European populations of potato cystnematodes on rooted stem cuttings of potato plants with genes H_1 , H_2 or H_3 was compared with their development on stem cuttings of the susceptible variety Arran Banner. All of the European populations were unable to overcome the resistance of at least one of the genes but a population of *G. rostochiensis* from Mariglianello in Italy overcame gene H_1 (pathotype Ro2 or Ro3). On the other hand most of the South American populations were able to multiply on all the plants containing resistance genes: only one failed to overcome gene H_1 , two to overcome gene H_2 and three to overcome gene H_3 . However, gene H_3 also gave partial resistance to a further 26 populations from South America and this resistance varied in degree, perhaps suggesting that gene H_3 is not a single entity or that the South American populations are heterozygous for ability to multiply on monogenic H_3 . (Evans and Franco)

Populations from India. Collections of potato cyst-nematodes from ten sites in the Nilgiri Hills, India, included one population of *G. rostochiensis*, five of *G. pallida* and four mixtures, two predominantly *G. rostochiensis* and two predominantly *G. pallida*, suggesting that *G. pallida* is the more common species in the region. It is now known that the first finding at Ootacamund, in India (Jones, *Current Science* (1961), **30**, 187) was *G. pallida* and not *G. rostochiensis* as the females were white. (Stone and Rowe)

Nineteen European populations of potato cyst-nematode were tested on the ex Andigena CPC 2802 clone, D40/6, known to contain gene H₃ and not gene H₁. Gene H₃ does not confer resistance to Ro1 and in this test two populations of Ro2 and one each of Ro3, Ro4 and Ro5 multiplied well on D 40/6, indicating that gene H₃ is ineffective against all known pathotypes of *G. rostochiensis*. Nine populations of *G. pallida* Pa3 and one of Pa2 failed to multiply significantly on the clone; three Pa1 populations behaved similarly but that could be because D 40/6 may contain gene H₂ which confers resistance to Pa1. These results and those last year (*Rothamsted Report for 1976*, Part 1, 203) indicate that European populations of *G. pallida* respond relatively uniformly to clones with gene H₃. However, populations from South American do not. (Stone, Turner, Rowe and Farr)

Morphology of populations of potato cyst-nematodes. Morphological variations in 131 populations of potato cyst-nematodes collected from South America were compared with those in populations from Europe. The minimum numbers of observations on juveniles and cysts needed to distinguish between a *G. rostochiensis* and a *G. pallida* population were computed. Only two measurements of juvenile stylet length and four measurements of head tip to excretory pore or of tail length were required to distinguish them with 95% confidence but 40 measurements of body length were necessary. Three measurements of the length of the cyst fenestra and 12 of the distance between the fenestra and anus were required for distinguishing the two test populations with 95% confidence. It must be stressed these criteria refer only to the populations tested.

The effects of environment on the morphology of second stage juveniles were studied in populations of nematodes raised at different population densities, in different daylengths and at different temperatures. Body length and stylet length of both species were affected significantly by all three factors, being shorter at high densities, in short-day lengths and at high and low temperatures, i.e. when conditions were adverse.

With the 131 populations raised in standard conditions, we obtained good separation of G. rostochiensis and G. pallida using measurements of body length, stylet length, tail length and distance from head tip to excretory pore on the first two axes of a canonical variate analysis. Stylet length was the most important measurement. Canonical variate analysis of measurements made on G. pallida populations from interconnecting valleys in central Peru which are separated by mountain ridges did not always group populations from the same valley together but the outlying populations tended to be those collected from the upper ends of valleys, perhaps suggesting that populations vary along the length of valleys. A further analysis of G. pallida populations from Ecuador, northern Peru, central Peru and southern Peru/northern Bolivia grouped most populations from each area closely together but one or two populations were widely separated from others of their group. (Evans and Franco)

Mating of British and Peruvian populations of potato cyst-nematodes. Single male-female crosses were made between populations of *G. rostochiensis* and *G. pallida* from the United Kingdom and Peru. Crosses between populations of the same species resulted in a greater percentage of successful matings (29%) and produced more eggs per cyst (180) than those between species (9%) and 110 respectively), irrespective of the country of origin of the parents. However, *G. pallida* males tended only to mate with their own females whereas *G. rostochiensis* males mated with *G. pallida* females more frequently. *G. rostochiensis* females seemed to prefer their own males and this, together with the ready fertilisation of *G. pallida* females by *G. rostochiensis* males to form sterile hybrids might operate to the disadvantage of *G. pallida* when both species occur together. (Franco and Evans)

Tolerance of potato varieties to potato cyst-nematode attack. Potato plants infested with cyst-nematodes have reduced root systems and suffer from water stress both because of the small root system and because in the field the roots are confined to the surface layers of the soil, the part which dries first. Monitoring water usage by 25 potato varieties grown in pots to which ample water was supplied showed that most varieties used more water per unit gain in either fresh or dry weight when infested by nematodes. Pentland Crown, a variety which tolerates attack, used least water per unit gain in fresh weight whether infested or not. Maris Piper used least water per unit gain in dry weight when uninfested and was one of the most efficient varieties when infested with *G. rostochiensis* which fails to reproduce upon it. Maris Peer, an intolerant variety (*Rothamsted Report for 1975*, 176

Part 1, 199), was one of the least efficient users of water when infested. (Evans, Grant and Ross)

Transfer cells and sexuality. Studies of transfer cell complexes induced by the feeding of *G. rostochiensis* in tomato roots indicate that those induced by females range in volume from 0.1 to 1.3 mm³. Fourteen days after inoculation they were, on average, more than six times the volume of those induced by males but 28 days after inoculation only two or three times the volume. Some complexes supported more than one nematode, e.g. two males, two females, one of each sex and adults of either sex accompanied by third stage juvenile. These findings cast doubt on the view that sex is determined environmentally. (Greet and Firth)

Effect of temperature on development. The temperature development rates of G. rostochiensis and G. pallida were studied in the hope that they would shed some light on their distribution and competitiveness. Previous work suggested that the optimum temperature for the development of G. rostochiensis was 22°C and that for G. pallida was 19°C (Rothamsted Report for 1976, Part 1, 203). This year the range of temperatures was greater: 7.5°, 10°, 22°, 27.5° and 30°C. Using the same populations and the same cv., Arran Banner, numbers of cysts produced at 22°C were similar to those found in the previous work, 824 compared with 758 G. rostochiensis and 434 compared with 418 G. pallida. Both species produced second generation juveniles free in the soil after 6 weeks at 22°C but not at any of the other temperatures. Females of G. rostochiensis appeared after 2 weeks at 22° and 27.5°C, 6 weeks at 10° and 11 weeks at 7.5°C; none developed at 30°C. Females of G. pallida appeared after 2 weeks at 22°C, 5 weeks at 10° and 7 weeks at 7.5°C; none developed at 27.5° and 30°C. At 7.5° and 10°C G. pallida produced adults earlier, and in greater numbers than G. rostochiensis, confirming that the G. pallida population is the better adapted to relatively low soil temperatures. At 27.5°C very few G. rostochiensis adults were produced and although development was rapid, second generation juveniles were not found, indicating that G. rostochiensis has little competitive advantage over G. pallida at this temperature. (Berry, Stone, Parrott and Al-Sakaff)

Hatching, osmotic stress and ion transport. When juveniles of *G. rostochiensis* were transferred from distilled water to 0.4 M-sucrose or trehalose, their water content fell from 72 to 67%, the value observed for unhatched juveniles within eggs soaked in water for 7 days. The juveniles also lost mobility. The tolerance of juveniles to osmotic stress was shown by prolonged storage (e.g. 30 days) in 0.4 M-sugar solutions followed by dilution with water to a sugar concentration <0.1 M, when many juveniles began to move actively. In hatching tests few juveniles emerged from cysts in solutions of potato-root diffusate which contained sugars at a concentration of 0.4 M but many emerged in more dilute sugar solutions. The behaviour of the unhatched juveniles within the egg suggests they are immersed in a fluid with an osmotic pressure equivalent to 0.4 M-sucrose.

Egg fluid of *G. rostochiensis* contains significant amounts of trehalose (Clarke & Hennessy *Nematologica* (1976), **22**, 190–195) and the water content of juveniles increases immediately before hatching even though the egg shell is freely permeable to water when wet (Ellenby & Perry *Journal of Experimental Biology* (1976), **64**, 141–147). This suggests that hatching is initiated when egg fluid solutes pass through the shell to the exterior. This would enable juveniles to take up water and intensify their metabolism. Such an hypothesis assumes a change of permeability in the egg shell which has yet to be established. (Clarke, Perry and Hennessy)

Calcium ions are thought to be involved in the hatching of G. rostochiensis. We ex-

amined the possibility that the hatching factor in potato-root diffusate acts as an ionophore. The transport of Na⁺, K⁺, Mg²⁺, and Ca²⁺ was determined in the presence and absence of the hatching factor in experiments similar to those with crown compounds (*Rothamsted Report for 1975*, Part 1, 185–186). We found no significant transport of any of the above cations by the hatching factor. However, three crown ethers (benzo-18crown-6, carboxymethoxy-dibenzo-16-crown-5, carboxymethoxy-dibenzo-19-crown-6) and one related compound (1(0-carboxy-methoxyphenoxy)-2-(0-hydroxyphenoxy-ethane) all caused some hatching, with ratings of 9, 3, 9 and 11 respectively, potato-root diffusate = 100. Clarke and Shepherd (*Annals of Applied Biology* (1968), **61**, 131–149) noted a pair of polar groups about 7 Å apart in some artificial hatching agents (e.g. in picrolonic acid), but the nature of the substance to which they bind has yet to be identified. (Clarke and Hennessy)

Fungi and rickettsial parasites. The Entomophthora-like fungus and Verticillium chlamydosporium usually prevent H. avenae from increasing in cereals in plots at Woburn. Although these fungi attack some other cyst-nematodes (Rothamsted Report for 1975, Part 1, 202), the Entomophthora-like fungus was found only in soil containing H. avenae but V. chlamydosporium is less specific as it occurred in Woburn soils whether cystnematodes were present or not. When the latter attacks females of H. avenae and eggs in cysts (dead females), the cuticle is not disrupted and the females do not disappear from the roots (Rothamsted Report for 1976, Part 1, 209). However, when V. chlamydosporium spores were applied to cysts the openings of which (vulva, fenestrae, anus and mouth) were artificially blocked, hyphae penetrated the cyst wall and attacked the eggs within. Penetration of the cyst wall was confirmed under the transmission electron microscope. (Kerry, Crump, Mullen and Clark)

In pots infection of females by the *Entomophthora*-like fungus rarely reaches the rates observed in the field. An experiment to study the influence of pot size and watering on fungal infection revealed no significant differences in the density of females produced or their fecundity but fewer survived (p = 0.001) to form cysts in the wetter pots. This was related to an increase (p < 0.001) in the rate of parasitism by '*Entomophthora*'. (Kerry, Crump and Mullen)

Rickettsia-like intracellular micro-organisms have been found in three species of cystnematodes, *H. goettingiana*, the pea cyst-nematode; *G. rostochiensis*, the potato cystnematode (*Rothamsted Report for 1970*, Part 1, 147) and *H. glycines* (B. Endo, USDA, Beltsville, Md, USA). The micro-organisms in *G. rostochiensis* and *H. goettingiana* appear identical although the infected populations came from widely separated places; the former from Bolivia, the latter from Suffolk, England. The infected *H. glycines* population was from Tennessee, USA.

The micro-organisms are morphologically identical to rickettsiae and resemble the 'companion symbiote' of the leafhopper, *Helochara communis* Fitch (Chang & Musgrave, *Journal Cell Science* (1972) **11**, 275–293). The organism is unicellular, rod-shaped and averages $1.5 \,\mu\text{m}$ long and $0.5 \,\mu\text{m}$ diameter. Giant elongate forms measuring $6.6 \,\mu\text{m}$ by $0.8 \,\mu\text{m}$ and giant oval forms $3.2 \,\mu\text{m}$ by $2.5 \,\mu\text{m}$ have been observed in ultrathin sections of second stage juveniles. The rickettsia-like organisms contain peculiar inclusions in the form of hollow rods which appear to be attached to the cytoplasmic membrane and are often dispersed in para-crystalline fashion. These inclusions have been named 'fascicles'.

The micro-organisms have been observed in ultrathin sections of *G. rostochiensis* eggs, newly hatched second stage juveniles and adult males and females. They always occur in the cytoplasm of host cells; no extra-cellular or intra-nuclear forms have been observed. Most host cell types and tissues may be infected although the micro-organisms are most 178

abundant in muscle cells of juveniles and males and in cells of the reproductive tract of females. (Shepherd and Walsh)

Sperm cells within males and within fertilised females of the Bolivian population are infected. Reciprocal crosses between the Bolivian population and an uninfected British population of *G. rostochiensis* were made to determine the fate of micro-organisms. Infected females mated with uninfected males produced infected F_1 juveniles, whereas no micro-organisms were found in F_1 juveniles from uninfected females mated by infected males. This suggests that the infection in the Bolivian *G. rostochiensis* population is maintained by ovarial transmission. Micro-organisms in sperm cells may be lost during fertilisation or the British population of *G. rostochiensis* may be an unsuitable host. It is also possible that micro-organisms in infected sperm were too few to produce a detectable infection in the eggs they fertilise.

Attempts to see the micro-organisms under the light microscope so that populations could be screened rapidly for infections were unsuccessful. The rickettsial stains, Castaneda's, Macchiavello's and Gimenez', failed to stain the micro-organisms as did the technique for staining the endosymbionts of *Paramecium aurelia* (Beale & Jurand, *Journal of Cell Science* (1966), **1**, 3–34). The DNA-specific Hoechst 33258 stain, used for staining mycoplasma contaminants of cell cultures and chromosomes in nematodes, also failed to show up micro-organisms in infected juveniles.

Since 1970 there have been at least 20 reports of rickettsia-like organisms (RLO) causing diseases in plants, including an RLO disease of potatoes. In one a nematode is the vector. As RLOs occur in the oesophageal glands of the Bolivian potato cyst-nematode, the possibility that the nematode might transmit the organism to the potato plant was considered. No micro-organisms were observed in ultrathin sections of potato roots on which infected nematodes were feeding. Aerial parts of plants were also free of micro-organisms.

Attempts to rid second stage G. rostochiensis juveniles of their micro-organisms by soaking hatched larvae in a solution of oxytetracycline hydrochloride (0.025 mg ml⁻¹) were unsuccessful; juveniles were still infected 6 weeks later. A similar treatment of juveniles in penicillin (0.02%) for 3 weeks also failed but a few dead and dying micro-organisms were observed.

As no nematode-tissue cultures are available, the standard technique for culturing rickettsiae (the yolk sac of the embryonated hen's egg) was tried. This was unsuccessful. (Walsh)

Stem nematode (Ditylenchus dipsaci)

The cool moist spring favoured invasion of field beans by D. *dipsaci* and there were well infested bean plants on some previously infested sites of the Rothamsted and Woburn farms. However no new infested sites were found this year. More selections of V. *faba* were tested against D. *dipsaci* but none had any marked resistance.

Thiabendazole was tested on field plots for its ability to control *D. dipsaci* on beans, previous pot tests having indicated nematicidal activity (*Rothamsted Report for 1976*, Part 1, 219). It was applied as fine granules (5% a.i.) to the rows at time of sowing at 3, 6 and 12 kg a.i. ha⁻¹ or as a seed dressing using 60% a.i. wettable powder at 12 and 24 g a.i. kg⁻¹ seed.

Thiabendazole treatments had little effect on the incidence of *D. dipsaci* but there was a growth and yield response especially from treated seed; this was possibly due to control of root-rot fungi rather than *D. dipsaci*. Aldicarb granules (10% a.i.) at 5 kg a.i. ha⁻¹ were much more effective in controlling *D. dipsaci* (Table 1).

The oat race of *D. dipsaci* was tested, in pots, against some alternative leguminous

	Untreated					Ireate	a
			Т	hiabenda	zole		,
			Granules g a.i. ha-		kg a	reatment .i. kg ⁻¹ eed	Aldicarb Granules kg a.i. ha ⁻¹
		3	6	12	12	24	5
Stem height (cm) SE±	55 1·8	55 1·2	64 3·5	63 4·6	70 0·7	67 3·8	85 3·3
Infested stems (%) SE±	52 4·5	71 4·5	28 14·5	61 2·1	35 7·1	40 14·1	0 0·0
Infested seed (%) SE±	12 2·3	14 4·5	12 2·3	8 3·3	8 2·8	11 3·3	0 0·0
Seed yield (t ha ⁻¹) SED ± 0.265	0.44	0.50	0.74	0.72	1.04	0.88	1.61

 TABLE 1

 Thiabendazole and aldicarb and stem nematode on field beans

 Untreated

 Treated

crops including two cultivars of broad bean used for producing beans for canning or freezing. The cv. Beryl was very susceptible and the cv. Three Fold White moderately susceptible. Leafless peas were damaged early by a heavy invasion but plants that survived the initial attack grew away and there was little distortion of growth: the peas did not seem to be a good host. Some lupin plants (*Lipinus albus*) were severely stunted and distorted by invasion but there was virtually no reproduction in tissues. It seems unlikely that leafless pea or *L. albus* would be much damaged under field conditions. (Hooper)

Trials with nematicides

Spring oats, aldicarb and formalin. The object of this experiment at Woburn Farm, now in its second year, was to test the effects of a nematicide (aldicarb) and a fungicide (formalin) on the numbers of *H. avenae* and on the incidence of the *Entomophthora*-like fungus that destroys females in May–July. Formalin applications in March 1976 and 1977 at 3000 litres ha⁻¹ established the following sequences:

Sequence	1976	1977
1	No formalin	No formalin
2	No formalin	Formalin
3	Formalin	No formalin
4	Formalin	Formalin

Aldicarb was applied (10 kg a.i. ha^{-1}) to half the plots before sowing with oats var., Nelson (resistant, R) and Maris Tabard (susceptible, S) to *H. avenae*. Numbers of *H. avenae* juveniles were counted before planting and after harvest, as were the numbers of females in June and the incidence of parasitic fungi attacking them.

In 1976 formalin had little effect on crop growth, nematode numbers or the incidence of parasitised females (5% formalin, 6% no formalin) and no residual effect on the 1977 crop. This was attributed to the exceptional drought which presumably inhibited the fungus from attacking females.

In 1977, formalin alone slightly increased (P = 0.05) yields of resistant and susceptible oats but aldicarb almost doubled yields of both whether formalin was applied or not. That formalin alone in 1977 increased the yield of Nelson (R) more than Tabard (S) is probably because of the known sensitivity of Nelson to invasion by juvenile *H. avenae* even though few, if any, females develop. (*Rothamsted Report for 1973*, Part 1, 164)

In 1977 in the absence of formalin, 30% of females were attacked by the *Entomoph*-180

thora-like fungus and numbers of eggs after harvest were fewer by 50%. In plots receiving formalin fewer females were attacked and numbers of eggs after harvest were about the same as at planting. Evidently moister soil conditions favoured attacks by the fungus.

The effects of 1977 treatments are in Table 2. In all treatment combinations Maris Tabard outyielded Nelson, probably because the earlier maturing Nelson shed more grain. (Williams and Beane)

TABLE 2

Heterodera avenae, resistant/susceptible spring oats, formalin and aldicarb 1977

	Nelson (R)			Maris Tabard (S)				
	Formalin		No formalin		Formalin		No formalin	
	Aldicarb	No aldicarb	Aldicarb	No aldicarb	Aldicarb	No aldicarb	Aldicarb	No aldicarb
Pre-crop H. avenae eggs g ⁻¹ soil	22.5	19.0	21.6	21.4	20.9	18.6	19.5	20.5
Juveniles g ⁻¹ seminal root	5.3	23.5	7-5	66-0	10-6	175.7	21.9	271.6
White females 25 cm row ⁻¹	0	0	0	3	1	147	0	156
% Diseased		-	—			16	_	30
Carry-over popn. eggs g ⁻¹ soil	3.6	3.6	2.8	2.1	3.4	4.7	2.2	1.5
Grain 85% DM (t ha ⁻¹)	3.54	2.02	3.57	1.77	4.20	2.30	3.94	2.12
Post-crop <i>H. avenae</i> eggs g ⁻¹ soil	2.2	3.5	1.5	3.2	3.5	17.6	1.1	11.1

Rotation-fumigation experiment. This experiment, also at Woburn Farm, was intended to test the effects of fumigant nematicides applied once in the rotation potatoes, barley, sugar beet before each crop and before all crops. From the outset the site was known to be infested with *G. rostochiensis* and *H. avenae* and several species of root ectoparasitic nematodes. A number of blank plots was incorporated so that treatments with other nematicides could be introduced. Treatments with dazomet and benomyl were so inserted and in the final (9th) year, after three cycles of the rotation, aldicarb (10 kg a.i. ha⁻¹) replaced 'D-D' and dazomet, autumn and winter soil conditions having made their use impossible. Table 3 gives yields averaged over the three nitrogen rates applied (38, 75, 113 kg N ha⁻¹ to barley and 75, 150, 225 kg N ha⁻¹ to potatoes and sugar beet).

TABLE 3

Yields of barley, sugar beet and potatoes in the last year of the rotation-fumigation experiment at Woburn

Aldicarb (10 kg ha ⁻¹ a.

				.,		
Untreated	Potatoes	S. Beet	Barley	All crops (ex 'D-D')	All crops (ex dazomet)	Benomyl
Barley var. Julia t l 2.72	ha ⁻¹ 85% DM 2.96	2.99	2.68	2.63	2.82	2.66
Potatoes var. Pentl 16.8	and Crown > 3 26.4*	•8 cm diam. t 1 20•7	ha ⁻¹ 20·8	27.6**	31-2***	21.6
Sugar beet var. Kle 5.40	ein E., sugar t ha 6.08	a ⁻¹ 6·79*	6.12	6.37*	6.22	5.55

*, **, *** Significantly different from untreated at P < 0.05, 0.01, and = 0.001 respectively

Barley yields did not differ significantly, neither did the effects of the chemicals at the separate nitrogen rates. Since aldicarb does not have the phytotoxic effects noted in the 181

year when 'D-D' is applied, the beneficial effect of applying it before the previous crop observed in earlier years, was not seen in 1977.

Only aldicarb applied immediately before the current crop significantly increased yields of ware potatoes and these were greatest in plots that previously received 'D-D' or dazomet every year. Although benomyl increased yields of potatoes in the current year, the effect was insignificant: applications in previous years increased potato yields by a half. All treatments significantly increased the percentage of ware tubers.

Yields of beet sugar followed trends similar to potato yields, except that there was no significant increase after dazomet.

Whereas *H. avenae* is now so sparse as to be undetectable in most plots (max. 0.6 eggs g⁻¹ of soil) at the beginning of 1977, *G. rostochiensis* numbers have increased and this is reflected in the improved yields from plots treated with nematicide. *H. schachtii* is absent from the site. The improved sugar yields from beet plots are possibly due to the control of root ectoparasitic nematodes or of insects attacking the seedling stages. (Williams, Beane, Parrott, Berry, Webb, Tite and Finch)

Potato cyst-nematodes

Pot and field assessment of potential nematicides. Sixty-three pesticides were assessed as nematicides in pots of Kettering loam inoculated with *G. rostochiensis* Ro1. Sixteen pesticides prevented nematode increase on the roots of potato plants grown for 18 weeks. Of these compounds nine were carbamates, six were organophosphates and one was a benzimidazole. Neither acetone nor petroleum ether, used as solvents, affected nematode increase.

'AC 64475', phoxim, carbofuran, oxamyl and two formulations of aldicarb were further assessed as nematicides in trials on Great Hill, Woburn and at the Arthur Rickwood Experimental Husbandry Farm, Mepal, Isle of Ely, Cambs. At Woburn, yields were small partly as a result of drought and inadequate weed control, but 9.0 kg a.i. of 'AC 64475', 5.6 kg carbofuran and 22.4 kg phoxim greatly increased yields. At Mepal, where potatoes grew better, yields were increased most by 5.6 kg oxamyl, 3.4 kg aldicarb, carbofuran and 5 or 10 kg a.i. 'AC 64475' (Table 4).

TABLE 4	LE 4
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Effect of five pesticides on yields of Pentland Crown potatoes in soil heavily infested with potato cyst-nematode (G. rostochiensis Rol)

Treatment	kg a.i. ha ⁻¹	Tubers over 3.8 cm diam. t ha ⁻¹			
	(target)	Woburn	Mepal		
Untreated	0	3.2	29.7		
'AC 64475'	2.5	9.5*	34.9		
	5.0	10.7*	39.9**		
	10.0	14.6***	43.5***		
Carbofuran	2.5	8.4	41.0***		
	5.0	16.6***	43.8***		
	10.0	16.1***	43.9***		
Phoxim	5.0	7.9	27.9		
	10.0	9.3*	33.0		
	20.0	11.6**	38.2**		
Oxamyl	5.6		46.7***		
Aldicarb (coal granules)	3.4	-	48.1***		
(gypsum granules)	3.4	-	47.1***		
LSD (5%)		6.0	5.7		
(1%)		8.2	7.7		
(0.1%)		11.1	10.3		

*, **, *** Significantly greater than untreated at P < 0.05, 0.01, 0.001, respectively.

Tubers over 3.8 cm diam. (t ha-1) in 1977

Integrated control. An experiment to control *G. rostochiensis* Ro1 by a combination of nematicides, crop rotation and a resistant potato variety (Maris Piper), was started in 1972 on sandy loam in Stackyard, Woburn. The rotations were (a) resistant potatoes, sugar beet, barley, susceptible potatoes, and (b) susceptible potatoes, sugar beet, barley, susceptible potatoes. The nematicides tested were the soil fumigants dazomet and 'Telone' (dichloropropene) applied in autumn or winter and the oximecarbamate oxamyl applied to the soil in spring before potatoes were planted. The rotations were completed on the last of the three series of the experiment in 1977 and the results show that nematicide treatment increased yields of tubers by 10–20 t ha⁻¹, whether resistant or susceptible potatoes had been grown in 1974. Nematode increase was controlled best by oxamyl (Table 5).

TABLE 5

Yields of Pentland Crown potatoes and increase of potato cyst-nematode in soil treated with different amounts of three nematicides, following a three-course rotation beginning with resistant or susceptible potatoes in 1974

		(nematode increase, times)			
		Potato va	riety in 1974		
Treatment	∮ kg ha−1	Maris Piper resistant	Pentland Crown susceptible		
Untreated	0	31.1 (7.3)	26.3 (2.2)		
Dazomet	224	43.4 (3.4)	43.2 (4.1)		
Dalomet	336	47.4 (1.0)	45.6 (1.7)		
	448	50.9 (0.9)	46.2 (2.5)		
	672	51.2 (0.9)†	48.2 (1.6)		
Dazomet and 'Telone'	224 224 224 224	51.2 (0.8)	48.8 (2.5)		
'Telone'	448	42.3 (1.4)	37.5 (3.3)		
Oxamyl	5.6	40.5 (0.8)	43.1 (0.9)		
Oxamyl and 'Telone'	5.6	47.0 (1.4)	46.9 (0.9)		
Terono	Mean	45.0	42.9		
$ \begin{array}{c} \text{LSD} (5\%) \\ (1\%) \\ (0.1\%) \end{array} $	vertical comparisons of	yields only	5·8 7·6 0·1		

 \dagger excludes the apparent increase in one replicate from 1 to 7 eggs g⁻¹, which is probably not significant.

Methods of incorporating granular nematicides in soil. In many earlier experiments we have shown that populations of potato cyst-nematodes can only be controlled reliably by granular nematicides, when they are thoroughly mixed in the top 10-15 cm of the soil. This can be done by spreading the granules on the soil surface and incorporating them in the soil by rotary cultivation (rotavation) or by spreading the granules on the soil surface and at 5 and 10 cm deep in the soil followed by rotary harrowing. Rotavation is slow and may harm the structure of some soils. Applying the granules in layers in the soil by blowing them out of the back of large A-blade coulters requires considerable traction and rotary harrowing does not mix granules well in the vertical plane. Harrowing soil on which granules have been spread usually leaves about 70% of the granules in the top 5 cm of the soil. A new technique of applying and mixing granules in the top 12-15 cm of the soil has been developed which overcomes all these problems. The granules are blown through vertical band distributors which are attached to the back of spring- or fixed-tine cultivators mounted 12.5 or 25 cm apart on a tractor-drawn toolbar. The soil is then worked over with a rotary harrow (Lely 'Roterra') which mixes the granules evenly in the horizontal plane. Chemical analyses of soil samples collected just after

application showed that the nematicide was incorporated to a depth of 15–20 cm with a slight preponderance (about 40%) of granules lying between 10 and 15 cm deep. Putting one-third of the granules on the soil surface and two-thirds of the granules through vertical band distributors 12.5 cm apart on the toolbar followed by rotary harrowing distributed the granules uniformly to 15–20 cm, as did the rotavator. There was little difference between the granule distributions obtained with the narrow or wide-spaced tines after the subsequent 'Roterra' cultivation. Field trials at Woburn and at the Arthur Rickwood EHF showed that *G. rostochiensis* Rol was controlled as well by applying oxamyl granules by the new technique as when they were incorporated by rotavation (Table 6). The vertical band distributor has been patented by the National Research Development Corporation.

TABLE 6

Control of potato cyst-nematode (G. rostochiensis Ro1) by different methods of applying granular nematicide to the soil

	Method of application	Method of	increase	
kg a.i. ha ^{−1}	to soil†	in soil	Woburn	Mepal
0	-	Roterra	11.7	28.2
0		Rotavator	11.5	20.2
5.4	VB 25 cm	Roterra	1.1	0.9
	VB 12.5 cm	Roterra	2.2	0.6
	SVB 12.5 cm	Roterra	0.4	0.8
10.1		Roterra	0.9	0.6
		Roterra	0.6	0.4
	SVB 12.5 cm	Roterra	0.5	0.3
	S	Rotavator	0.7	0.8
10.1	S	Rotavator	1.0	0.8
			1.8	12.6
			2.4	17.0
			3.2	22.5
	0 0	kg a.i. ha ⁻¹ application to soil† 0 — 0 — 5·4 VB 25 cm VB 12·5 cm SVB 12·5 cm 10·1 VB 25 cm VB 12·5 cm 5·4 SVB 12·5 cm 5·4 SVB 12·5 cm	kg a.i. ha^{-1}application to soil†incorporation in soil0—Roterra0—Rotavator5·4VB 25 cm VB 12·5 cm SVB 12·5 cm VB 12·5 cm VB 12·5 cm VB 12·5 cm Roterra VB 12·5 cm VB 12·5 cm Roterra VB 12·5 cm Roterra SVB 12·5 cm Roterra SVB 12·5 cm Roterra SVB 12·5 cm Roterra SVB 12·5 cm Roterra SVB 12·5 cm Roterra	Method of application to soil†Method of incorporation in soilincrease woburn0—Roterra11.70—Roterra11.70—Roterra11.55.4VB 25 cmRoterra1.1VB 12.5 cmRoterra2.2SVB 12.5 cmRoterra0.410.1VB 25 cmRoterra0.9VB 12.5 cmRoterra0.6SVB 12.5 cmRoterra0.710.1SRotavator1.01.82.41.8

 $\dagger S = to soil surface; VB 12.5 cm or 25 cm = in vertical bands 12.5 cm or 25 cm apart in top 12 cm of the soil; SVB = 1/3 to soil surface, 2/3 in vertical bands 12.5 cm apart in top 12 cm of the soil.$

Depth distribution. The effect of two susceptible varieties of potato and extra NPK fertiliser on the depth distribution of potato cyst-nematodes in lightly infested land was studied at Terrington St. Clement, Norfolk, Kenny Hill, Cambs. and at the Arthur Rickwood E.H.F., Mepal, Cambs. In silt loam at Terrington and in peaty loam at Kenny Hill little increase in numbers of potato cyst-nematodes on susceptible potatoes occurred below 30–40 cm. At all three sites Pentland Crown yielded better than King Edward and in the top 20 cm of the soil King Edward potatoes increased nematode numbers twice as much as Pentland Crown did. At Mepal extra NPK incorporated in the seedbed shallowly or deeply lessened nematode increase but irrigation had no effect.

In another experiment at Mepal, yields of Pentland Crown potatoes were not correlated with numbers of potato cyst-nematodes 12.5–25 cm deep in land treated with oxamyl to 12.5 cm deep.

The influence of cultural technique on potato yields in infested soil. An experiment in Longmead, Woburn in 1976 and 1977 investigated the effects, on yield of Pentland Crown potatoes grown in soil heavily infested with G. rostochiensis Ro1, of oxamyl (10 kg a.i. ha⁻¹ in the seedbed), spacing of seed tubers (25 v. 50 cm apart in the ridges), fertiliser (F1 = 245 kg N: 245 kg P₂O₅: 377 kg K₂O v. F2 = 245 + 125 kg N: 364 kg P₂O₅: 754 kg K₂O ha⁻¹) and date of haulm destruction (mid-September v. October).

In both years oxamyl greatly increased yield but extra fertiliser did not. In 1976 spacing the seed tubers 25 cm apart in the ridges increased yields compared with spacing them 50 cm apart. In 1977 there was no significant difference in yields between the two spacings. Late destruction of the haulms increased yields especially in soil treated with oxamyl (Table 7).

TABLE 7

Effects of oxamyl, tuber spacing, extra fertiliser and late destruction of potato haulms on yields of Pentland Crown potatoes in 1976 and 1977

			Tu	ibers ove	er 3.8 cn	n diam.	(t ha ⁻¹)
Tuber spacing	Fartilizant	τ	Untreate Harvest			Oxamyl	(10 kg a.i. ha ⁻¹) Harvest
cm apart)	Fertilisers†	1st		2nd	V	1st	2nd
					1976		
25	∫F1	9.6		10.4		34.9	35.4
25	}F2 F1	13·9 9·1		12·7 10·9		32·4 26·8	39·6 32·0
50	F1 F2	8.6		10.1		25.6	36.7
	Mean	1	10.7				32.9***
	LSD (0	(5%) (1%)			7·2 9·6		
	(0	·1%)			12.6		
					1977		
25	{F1 F2	22·0 15·8		18·9 21·2		34·7 23·8	35·8 43·2
50	{F1 F2	18.4		11.4		34.7	36.4
50		18.9	17.2	11.7		39.8	47·4 37·0***
	Mean	(5%)	17.3		12.4		370
	1.50	(1%)			16.8		
	LSD (0	1%)			22.2		
† For amo	ounts in F1 and F2 s	ee text.					

Tubers over 3.8 cm diam. (t ha-1)

*** Significantly greater than untreated at P < 0.001.

Pea cyst-nematode (Heterodera goettingiana). In the second year of a multifactorial experiment at Witham, Essex, pea yields and pea cyst-nematode increase were greatly affected by the amount of granular oxamyl applied to the seedbed and the method by which it was incorporated in the soil. Yields were increased most when the nematicide was incorporated in the top 15 cm of the soil by rotavation and by the largest amount of nematicide (9.8 kg ha-1). Irrigation with 3.4 cm of water, applied in four equal doses by watering cans, increased yields but stimulated nematode increase in plots treated with the smallest amount of oxamyl (2.4 kg ha-1) or with none (Table 8). Chemical analysis by gas-liquid chromatography of oxamyl in soil cores showed that granules applied in the seed furrow resulted in most of the oxamyl being placed in the top 5 cm of the soil, and little chemical moved from this region in the 3 weeks after application. Substantial movement of oxamyl to 10 cm deep, with traces to 15 cm, had occurred after 6 weeks in both irrigated and unirrigated plots, but no further movement occurred. So it is not surprising that, as in 1976, rotavation, which distributes the granules throughout the top 15 cm of soil increased pea yields more and controlled pea cyst-nematode better than did either the 'Roterra' or seed-furrow application. The rate of breakdown of oxamyl increased as the soil temperature rose, 50% being lost after 4 weeks and 90% after 9 weeks.

Beet cyst-nematode (*H. schachtii*). In the second year of an experiment on the chemical control of beet cyst-nematode at Little Downham, Cambridgeshire, oxamyl or aldicarb

TABLE 8

Control of pea cyst-nematode (Heterodera goettingiana) by different amounts of granular oxamyl applied to the soil in different ways

Method of incorporating	kg oxamyl	Yields of fresh peas (nematode inc	
oxamyl in soil	ha ⁻¹	Unirrigated	Irrigated
Rotavator	0 2·4 4·9 9·8	$\begin{array}{c} 0.03 & (2.7) \\ 1.4^{**} & (3.1) \\ 3.8^{***} & (1.8) \\ 5.5^{***} & (1.4) \end{array}$	$\begin{array}{c} 0.4 & (5.4) \\ 1.4^* & (4.4) \\ 3.6^{***} & (2.1) \\ 5.5^{***} & (1.2) \end{array}$
In seed furrows	0 2·4 4·9 9·8	$\begin{array}{c} 0.1 & (2.8) \\ 1.1* & (2.5) \\ 1.7*** & (3.1) \\ 1.9*** & (2.2) \end{array}$	$\begin{array}{c} 0.1 & (3.3) \\ 1.6^{***} & (3.9) \\ 2.4^{***} & (2.2) \\ 2.9^{***} & (1.9) \end{array}$
Roterra	0 2·4 4·9 9·8	0·1 (1·7) 0·7 (1·3) 1·7*** (1·9) 2·7*** (1·8) 1·71	$\begin{array}{c} 0.1 & (2.7) \\ 0.8 & (2.5) \\ 1.9^{***} & (1.4) \\ 3.0^{***} & (1.1) \\ 1.98 \end{array}$
Yields LSD vertical comparis	sons only (5%) (1%) (0.1%)	0. 1. 1.	8
Nematode increase LSD, ver	tical compariso (5%) (1%) (0·1%)	2: 2: 3:	9

*, **, *** Significantly greater than untreated at P < 0.05, 0.01, 0.001 respectively.

incorporated in the top 15 cm of the soil greatly reduced the number of larvae invading sugar-beet seedlings in peaty loam containing on average 34 eggs g^{-1} soil. Soon after the stand of seedlings had been thinned, treated plots had bigger plants than untreated plots. When the crop was harvested in early November such differences were not apparent though sugar yields were increased by the larger amounts of nematicide. Even the largest amounts of aldicarb or oxamyl did not significantly affect the increase in nematode numbers, which was much less than had been expected in a season favourable to sugar beet. We conclude, therefore, that although these nematicides can prevent serious injury to sugar beet seedlings and so greatly increase yield in heavily infested soil they are not persistent enough, even when applied in very large amounts to the seed bed, to prevent increase of this nematode which passes two generations in one season. In view of the large numbers of females seen on the roots of sugar-beet seedlings and well grown plants in untreated plots the slow population increase recorded after harvest suggests the intervention of an enemy that destroys females (Table 9).

Stem nematode (D. dipsaci). The study of control, by granules containing 10% aldicarb, of 'bloat' disease in onions, caused by stem nematode, continued in autumn- and springsown onions on Great Field, Rothamsted. In autumn-sown onions (var. Imai Early Yellow) aldicarb applied in the seed furrows alone failed to protect onions from late attacks by stem nematode and so failed to increase yields. When the same amounts of aldicarb were applied half in the seed furrows and half as a top dressing in the onion rows in spring, yields were greatly increased and 'bloat' disease was contained. In springsown onions (var. Robusta) aldicarb applied either in the seed furrows alone or in the seed furrows and as a top dressing prevented 'bloat' disease. In untreated plots the seedlings were 'bloated' while still very young and died in early summer. The nematode 186

numbers, which were around 25 litre⁻¹ of soil before sowing failed to increase either in untreated or in treated plots (Table 10). (Whitehead, Tite, Finch, Fraser, French and Nichols, with Bromilow and Hudson, Chemical Liaison Unit and Mr. J. Smith, Arthur Rickwood EHF)

TABLE 9

Effect of rotavating granules containing 10% aldicarb or oxamyl into the top 15 cm of soil infested with beet cyst-nematode on sugar yield and on nematode increase

Treatment	kg a.i. ha ⁻¹	Sugar (t ha ⁻¹)	Increase of nematodes, times
Untreated	0	6.39	2.3
Oxamyl	2·3 4·6 9·2 18·4	6·46 7·06** 6·97* 6·95*	1.6 2.7 3.2 4.4
Aldicarb	2·4 4·8 9·6 19·2	6·70 7·11** 7·29*** 7·49***	2·9 3·1 2·3 1·5
LSD (5 %) (1 %) (0·1 %)		0·44 0·60 0·81	2·0

*, **, *** Significantly greater than untreated at P < 0.05, 0.01, 0.001, respectively.

TABLE 10

Effect of granules containing 10% aldicarb in onion rows on yields of autumn- and spring-sown onions

Treatment	kg a.i. ha ⁻¹	Healthy onions (t ha ⁻¹)	
		Autumn-sown onions	Spring-sown onions
Untreated	0	12.8	0
Seed furrows only Mean	2·2 4·4 8·8	2·4 10·6 10·8 7·9	37·1 39·7 36·5 37·8
Seed furrows and top dressing	$1 \cdot 1 \\ 1 \cdot 1$	41.6	37.3
	2.2	40-0	44.3
	4·4 4·4	34.0	40-5
Mean		38.5	40.7
LSD (5%) Untreated v. (1%) any other		9·8 14·9	_
(0.1/0))		23.9	
LSD (5%) Treated		11.3	8.0
(0·1%) only		17·2 27·6	_

Miscellaneous studies

Spiral nematodes. The spiral nematode *Helicotylenchus vulgaris* was associated with poor growth of sugar beet seedlings at Swaffham Prior, Cambridge (*Rothamsted Report for 1976*, Part 1, 61–62). This site is on the Wantage soil series (silty clay loam) and a survey of other fields in the area showed that the nematode was present, although usually in smaller numbers (c. 500 litre⁻¹) than at the original site (c. 3500 litre⁻¹).

The *H. vulgaris* population at ten of these sites was sampled at monthly intervals until the crop (a cereal or sugar beet) was harvested. Numbers fluctuated during the period but were usually little changed at the end, although the sites had a wide range of initial numbers.

A nematicide trial at Swaffham Prior sown with sugar beet (see p. 66) was sampled for *H. vulgaris*. Granules of phorate, aldicarb and 'AC 64475' at 1 kg a.i. ha⁻¹ although significantly reducing numbers, did not give an acceptable kill. The other chemicals applied at the same rate were: bendiocarb, thiofanox, oxamyl and carbofuran. At harvest there was no significant difference in the populations from any treatment. (Spaull)

Nematodes attacking oilseed rape. The pathogenicity of four *Pratylenchus* spp. to the roots of oilseed rape varieties, Lair and Nevin, was tested in sterile culture and compared with a known good host, maize. Both rape varieties were as good hosts as maize for *P. neglectus* but only moderate hosts for *P. crenatus*, *P. fallax* and *P. pinguicaudatus*. The last two failed to maintain themselves on Nevin rape. Slight symptoms of root damage occurred on both rapes infested with *P. fallax* and *P. pinguicaudatus* which caused severe damage to maize but there was no visible reaction to *P. neglectus* or *P. crenatus*. Rape therefore seems to tolerate attack by *Pratylenchus* spp.

Both rape varieties were sown in seed boxes and inoculated with juveniles of the oat race of *Ditylenchus dipsaci*. Neither variety became infested although the stem bases of other rapes are sometimes attacked.

In pots of soil infested with *Heterodera schachtii*, cyst counts at harvest were similar to those on sugar beet indicating that both rape varieties were good hosts for this nematode. (Webb)

Nematode parasites of slugs and insects. The nematode Angiostoma limacis is an obligate gut parasite of several species of slug. The two free-living stages feed on bacteria and have a stoma typical of bacterial feeders. The parasitic juveniles develop progressively larger, stronger and more cup-shaped mouthparts until the organ of the adult is formed which is typical of vertebrate parasites which browse on gut linings. Adults of *A. limacis* have been found attached by the head to the host gut wall, probably feeding there. The distribution of the three parasitic stages in separate parts of the gut could be due to feeding site being determined by mouth size. Infestation of individuals of the slug Arion hortensis on one site increased from 55% in September 1976 to around 100% during January to June 1977, then decreased to 45% by August 1977. Whether changes in incidence are due to seasonal changes in host population density or to changes in the physical environment is unknown. The number of nematodes per host appears to be regulated by a mechanism giving a consistent monthly average of about three adults per slug. Sometimes the host envelops adult nematodes in mucus which appears to be a defence reaction.

Mermithid parasites were recovered from leatherjackets in Wales and Suffolk, 12% of individuals being infested in February 1977 on a damp Welsh site. A sheath produced by the host insect, presumably defence reaction, surrounded some of the living juvenile nematodes, others being melanised and dead.

Large numbers of *Neoaplectana carpocapsae* strain DD 136, which attacks many insects, were applied to plots of beet seedlings at Broom's Barn to observe its effect on the soil fauna. During the rearing of the 18 million nematodes required, centrifugation at 136 G to concentrate suspensions for storage had no harmful effect. Bioassay of soil after application into the seed-rows showed that the rate of infection of insects decreased exponentially with time but some nematodes persisted for at least 115 days. Soil moisture restricted the nematodes' movements and few dispersed more than 30 cm from the row in 60 days. (Oswald)

Conjoint work, visits and visitors

R. H. Bromilow studied the leaching and degradation of oxime carbamate nematicides in soil and pesticide uptake to plants (Chemical Liaison Unit, p. 155). F. G. W. Jones was Clive Behrens Lecturer in the Department of Pure and Applied Biology, Leeds, 1977–78 and was a member of the Advisory Board to the Research Council's Taxonomy Review Committee which has now reported. Members of the Department attended numerous meetings and gave many lectures and demonstrations, notably at the Royal Society, the British Museum of Natural History, the Linnean Society, Imperial College, Southampton University, Birmingham University and at meetings and workshops of the Association of Applied Biologists.

F. G. W. Jones attended the 10th Meeting of the Organisation of Tropical American Nematologists in Lima, visited the International Potato Centre and other centres in Peru and a number of agricultural establishments in Brazil in connection with the Anglo-Brazilian scheme for co-operative research. He also attended the European Plant Protection Organisation Conference on forecasting in crop protection held in Paris. A. G. Whitehead attended a cereal nematode conference in Amsterdam in December, W. J. C. Oswald the Soil Pests Working Group of the IOBC in Zurich and B. R. Kerry the USDA, Nematology Laboratory, Beltsville Agricultural Research Centre, Maryland, and the 16th Annual Meeting of the Society of American Nematologists at East Lansing, Michigan, both in the U.S.A.

Miss Adelina Valenzuela, University of Chile, Santiago, Mr. Aziz Al Sakaff, Ministry of Agriculture, Aden, Yemen, Mr. A. S. I. Ghorab, Institute of Plant Pathology, Giza, Egypt, Dr. N. C. Kyrou, Inspectorate of Agriculture, Salonica, Greece, and Miss Angela Towle, University of Reading, worked in the Department for various periods. A. N. Bateson, N. Clarke, Jacqueline M. Forrest, Antoinette S. Paul and I. C. Ross were sandwich course students and Rosemary Brind, J. L. Day and Isobel McGeachie were vacation workers.

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