Report for 1960

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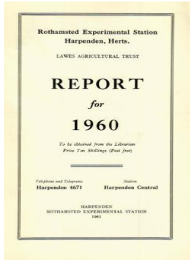

Rothamsted Experimental Station
Harpenden, Herts.

REPORT
for
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Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

F. G. W. JONES

F. G. W. Jones gave a paper at the Twelfth International Symposium on Plant Protection in Ghent; he also spent ten weeks in India, where he gave a course of lectures and practical work at the Muslim University, Aligarh, and visited other centres to advise on plant nematology. Miss K. Pawelska (Poland) joined the Department in March to work on problems associated with *Heterodera* spp. for one year. Mr. C. D. Blake (Australia) arrived in August and will spend two years working on host–parasite relationships in *Ditylenchus dipsaci*. Miss P. M. Nelson left in July and was replaced by Miss M. C. Gander. J. J. Hesling left in November to join the staff of the Glasshouse Crops Research Institute.

SYSTEMATICS AND BIONOMICS

The revised text of the late Tom Goodey's book *Soil and Freshwater Nematodes* is almost complete, but many drawings still have to be done. *Aphelenchoides cocophilus*, the nematode associated with "red-ring" disease of coconut, has been newly described from fresh material sent from Trinidad by Dr. D. W. Fenwick. Because of a combination of morphological characters, this nematode has been transferred to a new genus, *Radinaphelenchus*. While examining many genera and species in the Dorylaimoidea, new ideas were formed on the nature of the so-called spear guiding apparatus in this group. The old concept of the guiding ring as a single or double structure has often appeared rather arbitrary. The guiding apparatus is essentially a sheath fused outwardly as a fixed ring to the wall of the stoma in front and inwardly round the spear base near its junction with the spear extension. This sheath is also fused outwardly to the stoma wall about half-way down. As the spear is moved forwards an evagination of the sheath occurs in front of the fixed, anterior ring, and the end of this evagination is seen as a second ring. Thus, whether one or two rings are seen depends on the position of the spear relative to the head and the state in which the nematode is fixed when being mounted. In *Aporcelaimus*, which is said to have no ring, the double ring may occur when the spear is protruded. (Goodey.)

A study of several *Longidorus* populations from Great Britain showed that at least five species occur. One of these, often found in association with virus-infected plants, is *Longidorus elongatus* (de Man 1876) Thorne and Swanger 1936. The others are new species which are being described. (Hooper.)

Work continued on the Mononchidae of New Zealand. Hitherto unobserved structures have been seen in the region of the oesophago-intestinal valve, some of which disappear shortly after fixation, and this may be why published accounts of this region do not agree with the morphology of live nematodes. The oesophago-intestinal valves

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of the Mononchidae are of two types; *Anatonchus*, *Iotonchus* and *Miconchus* have three conspicuous, hollow tubercles on the posterior end of the oesophageal lining near the funnel-shaped valve, whereas *Mononchus*, *Prionchulus*, *Mylonchulus* and *Cobbonchus* have no tubercles and the lining narrows near the posterior end. *M. truncatus*, *M. papillatus* and *P. muscorum* have been redescribed, the genera *Mononchus*, *Prionchulus*, *Iotonchus* and *Cobbonchus* have been reviewed, and several new species described. The Enoplida have been reclassified into seven suborders, mainly on the basis of the oesophageal glands and their ducts. The Alaimidae form a new suborder. The Mononchidae together with the new family Bathyodontidae (type genus *Bathyodontus* Fielding 1950) form the new superfamily Mononchoidea within the Dorylaimina. The life history of *Dioctophyme renale* indicates a relationship between the Dioctophymatina and the Gordiacea. (Clark.)

Work on *Meloidogyne* spp. dealt with the morphology and variability of natural and cultured populations. Some of the original type material used by Chitwood for his descriptions of *M. arenaria*, *M. arenaria* subsp. *thamesi* and *M. hapla* in his 1949 paper was obtained from America. The specimens are being compared with other populations of the same species. In conjunction with the study of field populations, artificially reared cultures were started to show the variability which can occur among individuals descended from the same female. For this work the foam agar technique developed by den Ouden at Wageningen has been adopted. The progeny from these inoculations are used to continue the particular line for further generations and to compare members of the same lines, different generations of the same line or different lines of the same population. The principal character used for identification is the cuticular pattern in the vulval–anal region. Photomicrographs were made of specimens from the type material and from one or two other populations for comparison with the types. By these various means it is hoped to improve descriptions of species.

A new root-knot nematode from Norfolk was described, the first indigenous species from Britain. *M. artiellia* n.sp. differs from the other known species in the posterior cuticular pattern, the stylet of the infective larva and the length and shape of its tail. It has been found in one locality only, on the roots of oats and brassicas. It also attacks barley, wheat, red clover, broad bean, pea, lucerne and *Medicago lupulina*. There has been no report of crop damage.

Root-knot diseased plants were received from Ghana, Nigeria, Sierra Leone, Libya and South Africa; from East Pakistan and India; from five counties in England and two in Scotland. The nematodes were usually either *M. javanica* or *M. incognita* (*sensu lato*). The British ones were all from glasshouses. One interesting sample of chrysanthemums from a glasshouse was infested with *M. hapla*, which is usually looked upon in this country as the "outdoor root-knot", although it has also been found occasionally under glass. (Franklin.)

The experimental work done some years ago on how three parasitic nematodes, *Ditylenchus myceliophagus*, *Aphelenchoides composticola* and *Paraphelenchus myceliophthorus*, affect the growth and yield of mushrooms, was published and is to be reprinted in the *Bulletin of the Mushroom Growers' Association.* (Goodey.)

Out of many samples of mushroom compost examined, a few contained *Aphelenchus avenae*, usually mixed with other nematodes parasitic on mushrooms. When *A. avenae*, raised in pure culture on mushroom mycelium grown on agar plates, were added to mushroom compost, the mycelium was destroyed and the nematodes multiplied, sometimes more than a thousandfold in 6 weeks on 25 g. of compost. Evidently *A. avenae*, a nematode often associated with diseased plants and known to be a fungal feeder, can be injurious to mushrooms.

Samples of vigorous and poor turf were received from the Sports Turf Research Institute. No particular nematode could be correlated with the disease, but both healthy and diseased lots had very high populations of *Tylenchorhynchus* and *Helicotylenchus*, and the roots of *Poa annua* were heavily galled by *Ditylenchus radicicola*. Terminal galls on the roots of *Agrostis* sp. contained *Pratylenchus pratensis*. In a replicated experiment fifty specimens of *Tylenchorhynchus dubius*, added to the grass, *Agrostis tenuis*, in $3\frac{1}{2}$-inch pots at the time of sowing, yielded 45,000 specimens after 12 months, but the growth of the grass was apparently unaffected. (Hooper.)

Feeding Mechanisms

The structure of the oesophageal " valve " of *Pelodera* (*Cruznema*) *lambdiensis* and *Rhabditis* (*Cephaloboides*) *oxycerca* was studied by serial sections and by whole mounts of excised oesophagi containing heavily pigmented particulate " food ". A cinematographic technique was used to demonstrate the inter-relations of serial transverse sections.

By feeding nematodes on Indian ink and taking ciné films it was shown that, in rhabditids, the oesophageal lumen immediately behind and in front of the so-called " valve " forms the true valve mechanism. The " valve " itself, which appears to behave as a pump, is responsible for conducting food from the posterior end of the isthmus to the intestine. The " valve flaps " do not come into such close contact with one another that they function either as a valve or as a piston within the pump, and their significance is not yet understood. The pumping action is achieved by the postero-lateral walls of the chamber opening outwards, with the oesophago-intestinal canal (the " outlet valve ") closed. This creates a suction which works on food accumulated at the posterior end of the isthmus and draws some of it into the chamber. The anterior wall of the chamber and the " valve flaps " then move backwards, while the oesophageal lumen (the " inlet valve "), which passes through them, closes. The postero-lateral walls of the chamber now close inwards, thus tending to build up pressure within, but simultaneously the " outlet valve " opens and the contents of the chamber are expelled into the intestine.

In *Diplogaster* the median oesophageal bulb is the region where food particles are accumulated. (In rhabditids it is the anterior half of the isthmus where this occurs.) Food moves very rapidly down the isthmus to the posterior half of the second oesophageal

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bulb, from which it is passed to the intestine by a wave of muscle action resembling that in the isthmus of rhabditids.

Attempts were made to study the feeding of several genera of spear-bearing nematodes. Film records at high magnifications were obtained of *Aphelenchoides ritzemabosi* feeding on mesophyll and epidermal cells of chickweed leaves. In comparison with *Aphelenchus avenae*, these showed a very gentle pulsation of the oesophageal bulb during the whole period that the spear was within the plant cell. Shortly after withdrawal of the spear, the bulb underwent three or four vigorous pulsations, possibly to clear the oesophagus of food.

Preliminary studies were made of the pH of the gut wall of two genera of nematodes, *Pelodera* and *Diplogaster*, which were cultured in nutrient agar mixed with neutral red or methyl red as pH indicators, both red at the lower end of their pH range and yellow at the upper. These dyes were absorbed into the gut wall in concentrations sufficient to show their colours distinctly in about 2 days. Neutral red always remained red, indicating that the pH never rose above 6·8. Methyl red showed that the region of the intestine of *Pelodera* immediately behind the front few cells usually had a pH below 5 while the rest of the gut was around 6. *Diplogaster* showed little consistency in the pattern of pH variation in the gut wall, although the range appeared similar to that of *Pelodera*. Where indicator dye was held in the oesophagus, as in one *Pelodera*, the pH was at least 6. (Doncaster.)

Predators of Nematodes

Further film records were obtained of predators of nematodes. Tardigrades, found feeding on free-living nematodes, were filmed, and these showed considerable tenacity when they attached themselves to large active prey. Small nematodes were sometimes drawn right into the mouth, others were held at some point along the body and gradually killed and fed upon.

Large predatory ciliate Protozoa (genus *Urostyla*?) which freely ingest nematodes were filmed and photographed with nematodes at various stages of ingestion, some showing the prey completely engulfed. (Doncaster and Hooper.)

Cyst-Forming Nematodes of the Genus *Heterodera*

Hatching factors

The joint work with A. J. Clarke (Biochemistry Department) on the chemical nature of hatching factors continued. Cyst stocks of the potato, beet and cabbage eelworms are being built up by extraction from field soils or by rearing on inoculated pot plants. Over 300 gallons of crude potato diffusate were produced in addition to 200 gallons of crude rape kale diffusate. Rape kale diffusate was also obtained from seedlings grown in 2-litre aspirators and leached by distilled water. This method produces a diffusate free from soil impurities, but quantities are small. (Nelson and Gander.)

The literature contains statements that leachings from grass roots stimulate a hatch of potato-root eelworm eggs, but tests with diffusates from fifteen species of grasses were all negative. (Pawelska.)

Biotypes of potato-root eelworm (Heterodera rostochiensis)

Tests with thirty-nine populations of potato-root eelworm from the United Kingdom showed that 43% of the populations were mainly biotype A, 15% were mainly biotype B and the remaining 42% were mixtures of biotypes A and B. Both biotypes reproduce successfully on commercial potato varieties; A reproduces on resistant varieties bred from *Solanum multidissectum* but not on those bred from *S. tuberosum* and sp. *andigena*, whereas biotype B reverses this behaviour. A long-term experiment is under way on a field at Woburn, mainly infested with biotype A, to test the effect on the soil population of growing resistant *S. andigena* hybrids. Continuous cultivation of a resistant variety is being compared with continuous cultivation of some commerical varieties and with rotations which include the resistant variety and the variety Majestic in different proportions. (Jones and Pawelska.)

Cytology of Heterodera galeopsidis

Attempts were made to count the chromosomes of the Galeopsis eelworm, *H. galeopsidis*. The number is high, and may be the same as the somatic number, 27, in *H. trifolii*. No sperms were seen in the reproductive tract. Evidently the species is distinct from *H. schachtii*, which has the somatic number of 18 and usually has sperm in the reproductive tract. *H. galeopsidis* is probably triploid and parthenogenetic, like *H. trifolii*. (Hesling.)

Pea-root eelworm Heterodera göttingiana

Work is in progress on the bionomics of the pea-root eelworm, *Heterodera göttingiana*, in the field. After failing to stimulate larvae of this eelworm to hatch *in vitro*, hatch in soil under a pea crop was studied to find whether this species differed from others. The numbers of free larvae in the soil and in the roots of the pea crop grown in a small plot of infested soil were estimated at weekly intervals throughout the spring and summer of 1959, and from this the percentage hatch estimated. This was about 10% of the original egg content of the cysts at the beginning of the season, a small hatch compared with the calculated annual fall of 30%. This discrepancy is unexplained, but it may be because larvae die in the soil and in roots, or because of sampling errors and losses during extraction. In 1960, instead of attempting to assess hatch absolutely, the estimated hatch of pea, beet and potato-root eelworms were compared in soil under host crops, non-host crops and fallow. Pea-root eelworm and potato-root eelworm behave similarly, whereas beet eelworm under beet hatched less than the other two species under their respective host crops, perhaps because the beet grew poorly. In fallow soil beet eelworm hatched better than the others, as might be expected from the high hatch of the species in water.

This work confirmed that in field crops pea-root eelworm is stimulated to hatch by its host-plant roots. Moreover, it hatches under a host crop at much the same rate as other species whose larvae are readily stimulated to emerge in the root diffusate of host plants *in vitro*.

In an experiment in microplots, a single variety of pea (Onward) was grown in soil containing different population levels of pea-root eelworm, and the effect of these differences on yield was examined. Onward is highly susceptible and produces a high population of eelworms. The yield of peas decreased strikingly with increase in initial population level; the sharpest decrease was at levels between 20 and 40 eggs/g. soil. At 80 eggs/g. soil both the yield of peas and the total yield of haulms and peas were only 50% of that at 10 eggs/g. soil. This suggests that the level above which it would be unsafe to grow peas is near 10 eggs/g., the same level the National Advisory Service has found to apply to beet and beet eelworm and to oats and cereal-root eelworm, whereas potatoes can tolerate more potato-root eelworm. (Shepherd.)

Weekly counts of the encysted egg population in pots of fallow soil, containing early- and late-sown peas, variety Kelvedon Wonder, showed that the population declined by about 30% between early March and mid-July under fallow. Decline was more rapid in the early part of the season under peas, confirming that host roots stimulate hatching. Population increase under peas first showed 13 weeks after sowing. Late-sown peas suffered greater injury than the early-sown ones. (Hesling.)

Another pot experiment was completed in which the effect of five inoculum levels was tested on three types of bean (*Vicia*) and three varieties of pea. (Hesling.) Further population studies are under way in collaboration with the Pea Growing Research Organisation. (Jones.)

Other work on cyst-forming nematodes included tests of the resistance of twelve breeding lines of sugar beet to the beet eelworm (Pawelska), and rotational experiments with potato-root eelworm (Doncaster).

Chrysanthemum Eelworm *Aphelenchoides ritzemabosi*

In experiments on varietal susceptibility, artificial infection of chrysanthemum cuttings destroyed the plants. Although late inoculation of clean plants in early August soon produced symptoms on the leaf inoculated, a month elapsed before the disease spread, and it was never severe. Thus, when plants are destroyed by eelworms in the autumn, the infestation was probably present at an early stage in the plant's life. Late infestations on resistant or less susceptible varieties may be overlooked and the stock may seem clean. These results stress the need to control eelworm in the chrysanthemum stool, but unfortunately experiments suggested that hot-water treatment is difficult to apply, and effective treatment of stools on a commercial scale is impracticable. (Hesling and Wallace.)

Three features of the infestation of leaves by chrysanthemum eelworm were studied: (1) the behaviour of the eelworms in the leaf; (2) the cause of browning in infested leaves; and (3) the differential resistance of chrysanthemum varieties to eelworm attack.

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The length of the life cycle of the chrysanthemum eelworm is about 10–13 days. Each female lays about 25–35 eggs in compact groups, which take about 4 days to hatch; larvae become adult in 9–10 days. Egg laying is mainly confined to the boundary between the green and discoloured tissues, and adults are chiefly responsible for spreading infestations. Infested leaves turn brown more quickly as relative humidity and number of eelworms increase. Eelworms are the primary cause of browning, although discoloured tissues have a distinctive population of saprophytic fungi. Chlorogenic acid, isochlorogenic acid and a glycoside of luteolin are the main polyphenolic constituents of chrysanthemum leaves, and the first two are the major substrates for enzymic oxidation which gives the brown end-products. The polyphenols and the enzyme polyphenol oxidase in the leaf tissues meet when cells are pierced by the eelworms during feeding.

Infested leaves of resistant varieties brown quickly, and eelworm multiplication is inhibited. This hypersensitive reaction prevents further eelworm spread. There is no correlation between rate of browning in different varieties and polyphenolic content or polyphenol oxidase concentration. Infested leaves of resistant varieties turn brown sooner because the females move about more in the leaf, piercing hundreds of cells which subsequently turn brown, but the females lay few eggs. In susceptible varieties the females move very little, pierce few cells and lay many eggs. Neither polyphenols nor their oxidation products affect movement or egg-laying in the leaf. The primary cause of resistance in chrysanthemums to eelworm is undetermined, but it may be the lack of something essential to the nutrition of the eelworm. (Wallace.)

Ecology of Soil Nematodes

Rothamsted and Woburn Six-Course Rotations were sampled frequently. Nematodes were fewest from May to July, and spiral nematodes were very rare at Woburn, confirming results in 1958–59.

Broadbalk, Hoosfield, Barnfield and Parkgrass were sampled every three months. Barnfield, fallowed in 1960 after continuous mangolds, had the poorest nematode fauna, but yielded some interesting species. The different fertiliser treatments applied to the classical fields did not greatly affect nematodes, but pH differences from liming on Park Grass were associated with changes in the nematode fauna: *Pratylenchus* was more numerous on the unlimed half of each plot, *Aphelenchus* and Diphtherophoridae on the limed half. Seasonal variations in numbers of nematodes on Broadbalk and Hoosfield were similar to those on the corresponding plots on the Rothamsted Six-course Rotation. The classical wheat and barley plots on Stackyard, Woburn, were sampled in November 1960. Nematodes were few, and no unusual species was detected.

The permanent fallow plots on Fosters and Highfield were sampled at intervals to observe how fallowing affected the nematode fauna. Unfortunately these two sites can only be regarded as partial fallows because weeds were inadequately suppressed in 1960. Nematodes on these sites, as on Barnfield, decreased in spring as in comparable cropped fields, but the numbers in Fosters and Barnfield did not increase later in the year. On Highfield, however, there was steady increase over the year, because saprozoic nematodes increased; presumably they found the matt of decaying turf a rich source of micro-organisms.

Grass plots on the Woburn Irrigation Experiment were sampled in June, August and October, and a few plots from the barley and bean strips were sampled in October, to study the effects of additional moisture on the nematode population. The irrigated grass in June contained more than twice as many nematodes as the non-irrigated. In August and October this difference was not evident, probably because the excessive rain eliminated most of the moisture differences. The outstanding features of the August and October samplings were that the non-irrigated grass contained more *Pratylenchus* than the irrigated and that there were consistently high differences in numbers of *Pratylenchus* between adjacent blocks. This suggests that there may be big site differences in numbers of nematodes even in land with no recent cultural and manurial differences. As with the Rothamsted classical fields, fertiliser differences seemed to have little effect on nematodes.

Several sites at Rothamsted and elsewhere were sampled at different depths as a preliminary to a larger programme in 1961, when variations in nematode depth distribution with soil type, vegetation, season, etc., will be studied. Nematodes were numerous throughout the top 9–12 inches, and became fewer down to 3–3½ feet, the maximum sampling depth. Different categories of nematodes (root parasites, fungal feeders, saprozoic nematodes), were distributed in different patterns.

At five experimental sites in forest nurseries—Ringwood, Hants; Wareham, Dorset, and three at Oxford—the nematode fauna of plots treated with soil sterilants and sown with Sitka spruce is being studied. *Hoplolaimus uniformis* occurs at Ringwood and can cripple Sitka spruce seedlings, but nematodes seem not to be the primary cause of the poor seedling growth in these nurseries, although there may be an association between nematodes and another pathogen. This confirms work of J. B. Goodey some years ago. In experiments at Kennington, Oxford. and Wareham, Dorset, run by the Chemistry Department the naturally low pH of the soil is varied by additions of sulphur or calcium carbonate to give a range from pH 3 to 7. *Hoplolaimus* spp. and *Trichodorus* spp. were not found at Wareham, but were found at Kennington in the soils with pH artificially raised. *Aphelenchus avenae* occurs at both centres, and is most abundant at the artificially high pHs. In some way, directly or indirectly, pH affects distribution: the coniferous host plants are the same at both sites and over the whole pH range. Another spear-bearing nematode, probably belonging to a new genus of Tylenchida, was found at Kennington in soil where the pH had been artificially lowered. (Winslow.)

Nematicides

Nematicides applied before planting potatoes were tested on Long Mead, Woburn, against potato-root eelworm. Root invasion was used to measure their effect in addition to the usual estimates of the viable encysted egg population before and after treatment. Yields were doubled by " Vapam " at 1 pint/50 sq. ft., the recommended dose, and there was some increase at considerably lower dosages. Increase in yield was related to the decrease in larval invasion. " Tridipam " and DD had little or no effect. " Nemagon ", orthonitro-chlorobenzene and some other compounds were phytotoxic. (Peachey.)

Some experiments were made with nematicidal dips and with substances mixed with potting soil. Tomato plants dipped in a solution of SD4965 and later planted in root-knot infested soil did not remain free from nematodes. In pot tests " Mylone ", " Nemagon " and SD4014 considerably decreased root-knot infestation of tomatoes. Six chemical dips for the control of chrysanthemum eelworm in chrysanthemum stools were compared with untreated stools, and stools subjected to the standard hot-water treatment. The Parathion dip and hot-water treatment gave the best control. An iodine dip was also effective but too phytotoxic. (Peachey and Hesling.)

Attempts were made to find a means of disinfecting banana " seeds " suitable for adoption in the quarantine service provided by the Royal Botanic Gardens, Kew, for planting stock in transit between the Commonwealth and colonial territories. Iodine, *p*-chlorophenol, a cationic detergent and Parathion were used as chemical dips for " seeds " peeled free from discoloured tissue. These were also compared with " seeds " peeled but not dipped. None of the treatments stunted the subsequent growth of the banana plants; indeed, early growth was improved. Nematode assessment seven months after treatment showed that Parathion considerably, and iodine and the cationic detergent to a lesser extent, decreased infestation by *Radopholus similis*. In another experiment, growing plants were repotted complete with their existing roots into soil mixed with " Nemagon " soil fumigant or SD4014 experimental fumigant, and compared with untreated plants. " Nemagon " gave the best root systems and almost 100% freedom from *Helicotylenchus* spp., *Meloidogyne* spp. and *R. similis*. SD4014 was less effective. A programme of " seed " and soil treatment has now been adopted for quarantine purposes. (Peachey and Hooper.)

At the Royal Horticultural Society's Gardens, Wisley, *Pratylenchus penetrans* was still very rare in carrot plots a year after they were treated with " Vapam " or methyl bromide. Plots treated with ethylene dibromide also had populations well below the original level, whereas populations of the untreated plots had trebled. Although other Tylenchida were less effectively controlled, their numbers showed similar trends, but populations remained below the original level only on the " Vapam " plots. Saprozoic nematodes, after being first brought to low levels by " Vapam ", increased to high levels the following spring and declined to the same levels as in the untreated plots by autumn. Yields from the methyl bromide plots in the second year were again higher than those from other plots, but the differences were less. All treated plots yielded better than the untreated, which yielded less than in the first year.

In another experiment with carrots, " Vapam " at full and half rates, "Mylone" and a very small dose of a methyl bromide-chloropicrin mixture all almost completely controlled soil nematodes, and DD decreased populations by 90%. The methyl bromide-chloropicrin plots yielded significantly better than all other plots and produced carrots judged to have the best quality. The yield effects in this experiment appeared to be related to "soil amendment" following partial sterilisation rather than to control of plant parasitic nematodes. (Peachey and Winslow.)