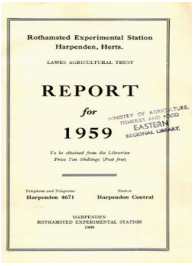
ROTHAMSTED RESEARCH

Report for 1959

Full Table of Content



Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

F. G. W. JONES

In July R. D. Winslow gave a course of lectures at North Carolina State College on the ecology of free-living and plant parasitic nematodes. H. R. Wallace attended the Botanical Congress in Montreal, as the guest of the organisers; M. T. Franklin, J. B. Goodey, F. G. W. Jones and A. M. Shepherd attended the Fifth International Nematology Symposium in Uppsala, Sweden; J. B. Goodey was elected to the editorial board of *Nematologica*. In November an exhibit on chrysanthemum eelworm was staged at the National Chrysanthemum Society's show in London. The work of the department continues to be hampered by overcrowding in the laboratory and by lack of proper glasshouses and soil-handling facilities.

SYSTEMATICS

Early in the year Dr. R. S. Pitcher of East Malling Research Station and Mr. F. C. Peacock of Imperial Chemical Industries, Jealott's Hill Laboratory, brought us specimens of *Xiphinema* from separate localities. These appear to be *X. diversicaudatum* as originally described by Micoletzky, not the species described in the standard monograph on the genus by Thorne. The range of variation of the male, female and immature stages was studied in detail on specimens from many sites. The tail usually bears a short, finger-like peg, but a few specimens from one site have smooth tails. Had these been found by themselves, they would have been considered a distinct species. The numbers of laterial pores on the tail and the sub-ventral series in the male anterior to the anus, which have been used as diagnostic characters in other species, vary widely. This species of *Xiphinema* has been shown separately by Harrison, Plant Pathology Department, Rothamsted, Dr. C. H. Cadman, Scottish Horticultural Research Institute, and Dr. A. F. Posnette, East Malling Research Station, to be a plant virus vector. (Goodey, Hooper.)

As usual, many specimens were received for identification, among them a series of samples associated with virus diseased sugar cane which yielded several interesting nematodes, including *Xiphinema pratensis*, *Tylenchorhynchus martini*, *Pratylenchus zeae*, *Rotylenchoides* sp. and *Trophurus* sp. Samples of manila hemp roots (*Musa textilis*) from North Borneo yielded *Pratylenchus coffeae*, new species of *Pratylenchus* and *Trophurus*, and *Tylenchulus semipenetrans*, the citrus nematode, for which manila hemp is a new host record. Work also continues on the genera *Meloidogyne*, *Aphelenchus* and *Aphelenchoides*. (Franklin, Goodey, Hooper.)

A visiting worker, Mr. W. C. Clark of New Zealand, began a study of the nematode fauna of New Zealand based on nematodes extracted from a considerable range of soils whose histories and physical properties are well known. Many are from volcanic ash soils of known

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age. Nematodes can often be run down to known genera but cannot be placed in known species, and there are interesting differences in the dimensions of one species that occurs in different localities.

Feeding Mechanisms of Nematodes

Many of the difficulties of observing nematodes spring from their microscopic size and the obscurity of the environments in which they are found. Special techniques are therefore necessary to make observations which, with most insects or other larger animals, could be made with the naked eye or with low-power magnification. Ciné and electronic flash photography coupled with the development of suitable observation chambers has made possible the recording of nematode activity, movement and feeding, and has thrown some light on the function of oesophageal muscles and valves during the act of feeding. Films were made of representative fluid feeders with mouth stylets and particulate feeders with and without oesophageal valves. Two rhabditids have so far been studied in detail. Films and observation of *Aphelenchus avenae* feeding on fungal hyphae indicate that hyphal walls are punctured solely by thrusting the stylet; the anterior of the nematode does not adhere to the hypha during penetration. Films were also made of predators feeding on white, immature females of the clover cyst nematode, *Heterodera trifolii*. These included the enchytraeids (*Fridericia*), large dorylaims and the spring-tails, *Onychiurus*, *Achorutes* and *Folsomia*. (Doncaster.)

Migratory Soil Nematodes

Routine sampling of rotational experiments on the heavy soil at Rothamsted and the light soil at Woburn continued and sampling of the classical experiments started. Samples were also examined in connexion with the work on nematodes as virus vectors (Harrison, Plant Pathology) and with that on forest-tree nurseries (Benzian, Chemistry). There is no evidence that plant parasites are numerous in the nurseries, but most have large populations of *Tylenchus* spp.; the highest numbers (over 700/25 g. soil) were found in compost-treated plots, where they may be feeding on fungi.

Various chemicals were tested as accelerators in attempts to improve extraction of soil nematodes from Baermann funnels. Potassium dichromate was the best, but none was very effective. Improved extraction from samples artificially infested with beet eelworm larvae was obtained by suspending electric-light bulbs above the funnels to give a temperature gradient from the soil surface downwards. A modification of the funnel technique, in which small quantities of naturally infested soil were placed on paper tissue and supported by means of wire gauze approximately $\frac{1}{2}$ cm. above the bottom of a petri dish containing $\frac{1}{2}$ cm. of water, yielded five times as many nematodes as unmodified Baermann funnels and three times as many as funnels heated above by an electric lamp. The petri-dish technique, however, is not equally suitable for all nematodes; *Xiphinema*, *Longidorus* and possibly other large Dorylaimoidea are inefficiently extracted. Placing the petri-dishes in an atmosphere of oxygen did not increase the recovery of nematodes. (Winslow.)

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Cyst-forming Nematodes of the Genus *Heterodera*

Potato-root eelworm (Heterodera rostochiensis)

Joint work on the chemical nature of the hatching factor which diffuses from potato roots into soil continued in collaboration with A. J. Clarke (Biochemistry Department). Over 500 gallons of crude potato-root diffusate from potted plants were produced during the year, together with smaller quantities of tomato and rape diffusates from germinated seed and pot plants. (Nelson.)

More is now known about the distribution in England and Wales of biotypes of potato-root eelworm capable of infecting crosses between *Solanum tuberosum* spp. *tuberosum* and *S. tuberosum* spp. *andigena* that contain the resistant gene H. Additional populations were tested at Rothamsted and in collaboration with the National Agricultural Advisory Service. More than half the populations tested so far, whether drawn from fields, gardens or glasshouses, contained appreciable numbers of females able to attack the resistant plants. Most populations tested from East Anglia and Northern Ireland did not break resistance, whereas population from the silt lands around the Wash, from the East Midlands, from Lancashire and from the Channel Islands mostly did. In most fields in these areas commercial varieties containing the gene H would be valueless immediately or after growing one or two crops. Elsewhere such varieties, which may be available shortly, should have a longer useful life. Pot tests indicate that the proportion of resistance-breaking females increases each time resistant potatoes are grown. The rate of increase observed suggested that resistance-breaking of this type might depend on a simple recessive gene (aa). Attempts were made to cross resistance-breaking (aa) and non-resistance-breaking populations (AA) in such a way that males of the latter type (A) would be in great excess and so give progeny (i.e., eggs within cysts) of the heterozygous type (Aa). If the hypothesis that only the double recessive (aa) breaks resistance were correct, heterozygous progeny (Aa) should not reproduce on resistant plants. In fact, reproduction occurred which suggests that the hypothesis is false. Mass crossing, however, is not a satisfactory procedure, because mating is uncontrolled. Controlled mating *in vitro* is necessary before the genetics of resistance breaking can be determined. (Jones.)

Pea-root eelworm (Heterodera göttingiana)

Attempts to induce the hatch and emergence of larvae from cysts continued. Subjecting the cysts to various temperature and moisture regimes after extracting them from soil and before putting them to hatch in diffusate had no effect, nor did changing the relative concentrations of oxygen and carbon dioxide. The possibility that pea-root diffusate is unstable was considered in another experiment in which diffusate, freshly produced by leaching germinating peas with distilled water, was added to cysts three times daily, but it did not stimulate hatching. Culture filtrates of six of the most prevalent rhizosphere fungi associated with peas added to cysts in pea-root diffusate and in water likewise gave no hatch.

Experiments in sand with various moisture contents gave a

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maximum emergence of 2% in the presence of peas. Comparable tests with beet eelworm and rape plants gave a maximum emergence of 50%, but of the few pea-root eelworm larvae that hatched, far more succeeded in invading the roots of the host plant. In a plot of peas infested with pea-root eelworm the maximum emergence in weekly soil samplings was 8% of the eggs within cysts, which suggests that the rate of hatching in the field is also low, but more work is necessary to confirm this. (Shepherd.)

Other work in progress on cyst-nematodes includes population studies on potato-root eelworm, cereal-root eelworm and pea-root eelworm. (Doncaster, Hesling and Shepherd.)

Chrysanthemum Eelworm *Aphelenchoides ritzema bosi*

Work was started on chrysanthemum eelworm, and a simple technique devised for infecting plants with known numbers of worms. Cuttings of the variety Orange Peach Blossom disinfested by hot-water treatment were infested with eight inoculum levels ranging from 15 to 400 eelworms/cutting in ninefold replication. As the inoculum level rose, the number of plants showing symptoms and the severity of the symptoms increased. At 400 eelworms/cutting the mean height of the plants was only 46% of the controls. In a trial of 13 chrysanthemum varieties, 15 disinfested cuttings of each variety were inoculated with about 100 eelworms each. Five cuttings of each variety were left uninoculated as controls. Varietal differences in susceptibility and in the expression of symptoms were observed, and all reported symptoms were produced on one variety or another (blind plants, plants with distorted or needle-like leaves, etc.). Sections cut through infested leaves showed that the nematodes invade the palisade tissue as well as the mesophyll. The most obvious symptom was the disappearance of chloroplasts when the nematodes began to feed.

Soil from beneath heavily infested chrysanthemums was divided into two portions, one of which was sterilized, and both were stored in bunkers during winter. All chrysanthemum plants grown in both soils in the following year grew well and showed no symptoms, which suggests that the eelworm cannot overwinter without weeds. Spread of the eelworm from plant to plant by leaf contact or through the soil was slight, but during the year the soil population immediately beneath infested plants increased and reached a level of about 50–100 eelworms/100 ml. of soil in late October.

Infesting the surface of single, rooted leaves produced definite and consistent varietal reactions. Some varieties turn brown within 2 days whereas others show little or no discoloration for more than 8 days. The eelworm does not multiply in rapidly browning tissue, which is the reaction of resistant varieties in the field and may be regarded as a form of hypersensitivity to feeding. Varieties that show little or no browning are susceptible in the field, and the eelworm multiplies on them. (Hesling and Wallace.)

Parasitism in Aseptic Plant Tissue Cultures

Dr. L. R. Krusberg, a visiting worker from the United States, successfully cultured *Ditylenchus dipsaci* and *Aphelenchoides rit-*

https://doi.org/10.23637/ERADOC-1-92

worm larvae through sandy loam, heavy clay and peat soils all lay between 150 and 400 μ diameter. There was little difference in mobility in sandy loam or clay. In peat, however, mobility increased with suction and was still increasing at the highest suction used, so that the optimum suction was not established. This suggests that the larvae can move in relatively dry peat. In a moisture gradient in sand, larvae tended to move to the wet end. The rate of spread of larvae in sand with particles from 150 and 250 μ diameter varied between 2 and 3 cm. a day, depending on suction. The relationship between the cross-section of the larval body and pore size appears to be important in deciding whether the larvae respond to a moisture gradient. Direct observations on larvae newly emerged from cysts in the presence of host roots suggest that larvae orientate themselves at a distance from the root and do not reach the root by random movement. (Wallace.)

Nematicides

Work on nematicides was re-started. Various methods of applying nematicides in the field and in the laboratory were tested, and methods of assessing kill were explored. Silver compounds, iodine, chlorophenols, cresol, hydroxy-benzoic acids and Gentian violet showed some nematicidal activity towards chrysanthemum eelworm (*Aphelenchoides ritzema bosi*). Caprylic and pelargonic acids were so phytotoxic that their activity was not tested. (Peachey.)

Trials with nematicides were made on a carrot crop at the Royal Horticultural Society's Gardens at Wisley. In the soil at this site carrots suffer from an undetermined " sickness ", which may or may not be associated with the nematode population of the soil (*Pratylenchus penetrans* and other Tylenchida). " Vapam " and methyl bromide gave excellent control of plant parasitic nematodes at planting time. Populations were still very low after harvest on the " Vapam " plots, but had risen on the methyl bromide plots. Ethylene dibromide, " VC13 " and dichloro diethyl ether were less effective. The best carrot yield followed treatment with methyl bromide. Yields from " Vapam " and " VC13 " plots were about equal, although the latter gave only a poor control of nematodes. (Peachey and Winslow.)

https://doi.org/10.23637/ERADOC-1-92

zema bosi on lucerne seedling and callus tissue grown aseptically on nutrient agar. *Meloidogyne incognita* also reproduced on roots of tomato and lucerne seedlings in culture but not on callus tissue. Although lucerne is not a host of *Pratylenchus zeae*, this nematode will reproduce on lucerne callus in the presence of coconut milk. With 15% of coconut milk in the medium 30,000 nematodes were extracted from ½ g. of callus (fresh weight) 6 months after inoculation with 50 worms. So some substance or substances in coconut milk enable the nematode to grow and reproduce on lucerne callus.

The gross character and the histopathology of galls caused by *D. dipsaci* on Du Puits lucerne seedlings grown in soil or aseptically on nutrient agar are essentially the same. Infected shoot apices exhibiting cortical and pith infections are very similar. Externally they appear puffy, and internally they have enlarged and collapsed cells, cavities filled with nematodes and eggs, and the cytoplasm and nuclei are distorted. Infected tissues a few mm. below the apex are not swollen or distorted, although cavities formed by the nematodes contain many worms and eggs. A few cells near the cavities are distorted, but there is no galling or cell enlargement such as occurs at the apex.

Callus formed from the hypocotyls of lucerne seedlings under the influence of 2:4-D provided "ready made" galls and resembled the cortical tissues in old infected shoot apices of plants growing in the field. Nematodes occurred in groups in cavities within the callus and scattered between its loose parenchymatous cells. Infected callus 20 days after inoculation looked similar to uninfected callus except for the cavities containing nematodes.

Tests on extracts from lucerne tissues grown in the field, from seedlings and callus grown aseptically on nutrient agar, and from the medium upon which plant tissues had been grown, showed no qualitative effects on sugars, but there were differences in amino-acids and organic acids between non-infected plants and plants infected with *D. dipsaci*. The components separated on the paper chromatograms have not yet been completely identified.

Soil Conditions Affecting Hatch and Movement of Nematodes

Further studies of larval emergence from cysts were undertaken to test previous hypotheses in more detail. Using sand at various suctions, the peak emergence corresponded roughly to the point on the moisture characteristic where little water remained in the pores. The results confirmed that the optimum suction for emergence was related to particle size but also indicated that, at the optimum suction, the rate of emergence decreased with particle size. Contrary to previous suggestions, there does not appear to be a limiting suction for emergence. Results when the medium in which the hatching tests were done was changed at different intervals of time suggest that carbon dioxide accumulates in the vicinity of cysts and inhibits further hatch. Larval emergence from cysts after hatching is probably governed by the same factors that affect movement through soil.

The optimum crumb sizes for the movement of potato-root eel-

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