ROTHAMSTED RESEARCH

Report for 1957

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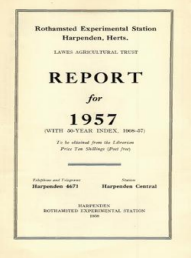

Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

F. G. W. JONES

R. D. Winslow joined the staff in June and has begun work on migratory soil nematodes. Joan M. W. Hurrell left in February. In July F. G. W. Jones paid a short visit to the U.S.A. and took part in a nematology " seminar-workshop " held at the Agricultural Experiment Station, Knoxville, Tennessee, later visiting three research centres in Alabama and Florida. In September C. C. Doncaster, Mary T. Franklin, J. J. Hesling, F. G. W. Jones and R. D. Winslow attended the 4th International Nematology Symposium in Hamburg. F. G. W. Jones was chairman of the opening session and also of a session in the 4th International Congress of Crop Protection that followed.

As in previous years, many specimens from home and abroad were received for identification. By far the greatest number from abroad were species of root-knot eelworm (*Meloidogyne*). Other nematodes of special interest received were *Sphaeronema* sp. from Indonesia on citrus roots and *Radopholus similis* from Jamaica on banana roots. A sample of galled tomato roots from a glasshouse at Wokingham, Berks, contained a species of *Nacobbus*, the first record of the occurrence of this genus outside the U.S.A. The species of *Sphaeronema* has been described, and further observations are being made on the host range and biology of the species of *Nacobbus*.

NON-CYST-FORMING AND OTHER SOIL NEMATODES

Investigations on migratory soil nematodes have been started, with a view to assessing their prevalence and economic importance. Initial work shows that species of *Tylenchorhynchus, Tylenchus, Pratylenchus, Rotylenchus* and *Paratylenchus* are widespread in permanent grassland and various cultivated crops. All five genera contain known or suspected root-feeding species. Other genera encountered and containing known or suspected plant parasites are *Aphelenchoides* (encountered frequently), *Longidorus* and *Aphelenchus* (occasionally), and *Paraphelenchus* and *Criconemoides* (rarely). Nematodes apparently identical with T. Goodey's *Anguillulina obtusa* (1932 and 1940) have been found in Rothamsted turf. They are believed to represent a new genus close to *Pratylenchus*. (R. D. Winslow.)

Work on the relationships of *Hoplolaimus uniformis* to Sitka spruce seedlings, in conjunction with the Chemistry Department, has finished. An experiment, in which individual seedlings were grown in sterilized Ringwood soil and inoculated with 0, 20 and 200 eelworms per plant, gave striking evidence of the damaging effect of the eelworms. All the work of the last few years has been written up, and will form a section of the general account of the Forest

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Nursery experiments now in course of preparation by the Chemistry Department as a Forestry Commission Bulletin. (J. B. Goodey.)

Amongst several populations of *Aphelenchoides* which have been cultured for the past year or two on agar medium with fungus (*Altenaria tenuis*), two species have been distinguished which did not agree in morphological characters with any of the known species. These have been described and named *A. composticola* and *A. saprophilus* respectively. The former occurs commonly in mushroom compost, where it feeds on the mycelium and causes reduced yields of mushrooms. The second is a species which is commonly encountered in soil and decaying plant tissues, where it is evidently a fungal feeder. These two species are morphologically very close to each other and to *A. parietinus*. (Mary T. Franklin.)

A new species of *Paraphelenchus* has also been found in mushroom compost and the mushroom spawn nematode, *Ditylenchus* sp., previously confused with *D. destructor*, has been distinguished on characteristics of the tail, post-vulval sac and spicule. Descriptions of both will be published shortly. (J. B. Goodey.)

Ditylenchus sp., *Paraphelenchus* sp., *Aphelenchus avenae*, *Aphelenchoides saprophilus* and three undescribed species of *Aphelenchoides* have been cultured on mushroom mycelium (*Agaricus hortensis* Cooke) grown on malt agar plates, but attempts to culture *Aphelenchoides blastophthorus* and *Tylenchus* sp. were unsuccessful. Mushroom is a new host record for *Aphelenchus avenae* and *Aphelenchoides saprophilus*. Descriptions of the three new species of *Aphelenchoides* are being prepared.

For further studies, large populations of various saprophytic nematodes are being cultured on Nigon's agar medium seeded with baker's yeast. Species successfully cultured include *Acrobeloides* sp., two species of *Caenorhabditis*, three species of *Diplogaster*, *Diploscapter* sp., *Eucephalobus* sp., *Panagrellus redivivus*, *Panagrolaimus rigidus*, *Pelodera* (*Cruznema*) *lambdiensis*, *Plectus* sp. and *Rhabditis* (*Rhabditella*) *axei* (D. J. Hooper).

In collaboration with Mr. F. C. Wood of Darlington & Sons Ltd., Worthing, experiments were conducted on the irradiation of infested mushroom compost with gamma-rays. Doses lying between 48,000 and 96,000 reps caused failure to reproduce in both *Ditylenchus* sp. and *Rhabditis* spp. (J. B. Goodey.)

Studies on the biology, morphology and host ranges of *Anguina* spp., *Ditylenchus radicicola* and various races of *Ditylenchus dipsaci* are continuing.

In conjunction with the National Institute of Agricultural Botany, Cambridge, seventeen winter oat varieties were tested for susceptibility to *D. dipsaci*. (J. B. Goodey and D. J. Hooper.)

Root-knot Nematodes

A start has been made on a survey of the species of *Meloidogyne* occurring in England and Wales. As a result of a request to the National Agricultural Advisory Service, specimens have been received from seventeen sources, both from glasshouses and out-of-doors. Seven of them from field crops and out-door nursery crops

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contained *M. hapla*, and this species occurred also in two glasshouses. Of the other glasshouse crops four were infested with *M. incognita* var. *acrita* and two with *M. incognita*. Two populations were not identified and are being kept going in the greenhouse for further examination.

Populations of root-knot nematodes derived from single females of three species of *Meloidogyne* have been started in order to make observations on the variability of certain morphological characters useful in identification of the species. The populations are now in their third generation. (Mary T. Franklin.)

A list of plants resistant to *Meloidogyne* spp. has been compiled from the literature published during and after 1949. (D. J. Hooper.)

Cyst-forming Nematodes of the Genus *Heterodera*

Potato-root eelworm (Heterodera rostochiensis *Woll.*)

The results of two rotational experiments, one on a farm near Outwell and the other at Rothamsted, are undergoing analysis. Observations on population changes of potato-root eelworm on the Kirton Experimental Husbandry Farm are continuing. The first trial investigated the effects of D-D injection at the rate of 400 lb./acre in the framework of a six-course rotation of potatoes. In this silt soil a significant reduction in the field population of potato-root eelworm in terms of encysted eggs after the last potato crop appears to have been achieved only by four and five successive annual injections of D-D. (C. C. Doncaster.)

Investigation of the errors associated with field sampling have been made in conjunction with M. H. Westmacott of the Statistics Department. The aim has been to assess the magnitude of within-field variability which the methods of sampling in current use ignore. The results indicate that variability between the sampling points from which cores are taken to make up a composite bulk sample is much greater than that within points. Theoretical minimum errors corresponding to bulk samples composed of different numbers of cores have been calculated from the data, and these indicate the limits of accuracy obtainable by this method.

Direct comparisons between oxygen uptake and carbon dioxide production of cysts, eggs and larvae indicate that both cyst walls and egg shells tend to limit the passage of these gases and thus inhibit respiration. Experiments on the effect of root diffusates on respiration are in progress, but are not sufficiently advanced for any conclusions to be drawn.

The effect of ethylene dibromide on the hatchability of encysted eggs has been investigated. Fumigation reduces hatchability and modifies the form of the hatching curve by increasing the time necessary to attain the point of inflection, so that direct comparison between the hatch from control and from fumigated cysts is difficult unless the hatching test is carried on to completion. The shift in the point of inflection is less if cysts are allowed to stand for several months before testing, but even then it is inadvisable to draw conclusions from tests continued for a short, fixed time. (D. W. Fenwick.)

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Investigation of the chemical nature of the hatching factor has continued in collaboration with A. J. Clarke of the Biochemistry Department. Five hundred gallons of root diffusate were collected between March and November from five plantings of potatoes in pots. Most samples were highly active, having L.A. values in excess of 3·5. The active factor was extracted after acidification with hydrochloric acid and adsorption on charcoal. Elution from the charcoal with hot acetone gave recoveries of between 30 and 60 per cent of the active principle if done within a few days of collection. Some loss of activity occurred, if elution from the charcoal was delayed, which indicates breakdown of the factor on the stored charcoal samples. This, and breakdown in acetone solutions, is under investigation.

The monitoring of diffusate production and the bioassay of chemical fractions in connection with the above work necessitated the conduct of hatching tests on more than 10,000 batches of 100 cysts each, each test lasting three weeks. (Elizabeth Widdowson.)

Potato-root eelworm has been successfully cultured in excised tomato roots growing in tissue culture. Tomato seeds, sterilized with bleaching-powder solution, are germinated in culture tubes on White's nutrient agar medium, and the root tip is removed and grown in flasks containing White's plant-culture solution. Growth is rapid, and many new root tips are produced each week. Although it is possible to hatch sterilized eggs aseptically to produce sterile larvae for inoculation, equally satisfactory results are obtained by introducing eggs sterilized with hydrogen peroxide directly into root cultures in tubes of nutrient agar. Evidently the hatching factor is produced by excised root tips, for hatching occurs very rapidly. Larval penetration of the roots begins after about 7 days, but only a small proportion of the available larvae succeed in gaining entry. Possibly this is due to local anaerobiosis, since larvae collect in dense clusters around the most attractive parts of the roots, particularly near root tips and the points of origin of lateral rootlets. Within the roots development proceeds normally and adult females burst through the surface of the cortex about 21 days after inoculation. Males are formed, but the right conditions for mating may not exist. Attempts to overcome this difficulty and to breed the parasites through several generations are being made in sterile sand cultures. (D. W. Fenwick and Elizabeth Widdowson.)

Work on biotypes of potato-root eelworm capable of breaking the resistance of crosses between *Solanum tuberosum* ssp. *tuberosum* and *S. tuberosum* ssp. *andigena* has been continued. A further twenty-five populations have been tested, of which only eight were non-aggressive. *S. vernei* proved resistant to all these populations and to twelve others as well. Cysts produced on resistant plants in 1956 were used to inoculate pots in 1957, which were again planted with resistant tubers, Arran Banner tubers being used as controls. The proportion of resistance-breaking females in the various populations increased markedly, even in the group which in 1956 were classified as non-aggressive. This indicates that when new potato varieties with resistance based on the dominant gene H from *S. andigena* are grown in the field, they may be heavily attacked in some fields and more or less immune in others. In the latter fields

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they will cause a reduction in the population level at first, but repeated cropping with a resistant variety of the same type may lead to a change in the nature of the population such that immunity will be lost. No estimate can yet be given of the number of plantings necessary to bring about this change. (F. G. W. Jones.)

Cereal-root eelworm (Heterodera major)

One hundred and twenty species and varieties of oats from Europe and Africa were tested in soil infested with cereal-root eelworm for the presence of cysts on the roots. Thirty-five plants showed promise of resistance, in that very few or no cysts were seen on them.

A comparison of the rate of hatching from cysts in fallow soil with cysts in soil growing oat plants showed that cysts in fallow soil did not empty as quickly as the cysts in soil with plants. The difference was not great, but it suggests that oat roots may liberate a hatching factor into the soil.

The long-term experiment at Stoke-on-Tern has been completed. A test crop of oats was grown over the trial area that had been sown (for three years previously) with plots of oats, wheat, barley and rye. Only the plots previously down to autumn-sown rye and autumn-sown wheat which had received an annual application of fertilizer gave a good crop of oats; all other plots were poor, those after oats being complete failures. (J. J. Hesling.)

Pea-root eelworm (Heterodera göttingiana)

A detailed population experiment was set up using six infestation levels, ranged in a geometric series rising to 98 larvae/g. of soil, and six hosts, viz., field bean, " Windsor " broad bean, " Longpod " broad bean, " Kelvedon Wonder " pea, " Harrison's Glory " pea, " Lincoln " pea. The eelworm had little effect on the beans, judged by measurement of the plant weight, plant height and yield of seed, but its effect on the peas was marked, especially on their yield, which was reduced to less than 25 per cent of the control. Soil population counts have not yet been attempted, because it has been found that a special technique was required to ensure complete extraction of cysts from soil. The cysts remain embedded in the host-plant roots, which must all be recovered. At present the percentage loss of cysts in rootlets retained in the various stages of cyst extraction is being investigated, as is a method of digesting the rootlets in a cellulose enzyme 19AP, to release the embedded cysts.

Test of root diffusates from trefoil, lucerne, alsike, red clover, giant sainfoin, wild white clover, cultivated white clover, common sainfoin and garden pea showed that only diffusate from garden pea produced hatching at a higher level than the soil water control. (J. J. Hesling.)

Cabbage-root eelworm (Heterodera cruciferae)

Previous study of the morphology of the cabbage-root eelworm by means of dead permanent mounts has been supplemented by examination of living specimens. A series of photographs has been obtained of the development of a young fourth-stage male within the third-stage cuticle. Coiling and uncoiling movements were observed

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followed by rapid increase in body length and decrease in diameter resulting 24 hours later in four-fold flexure within the ensheathing cuticle.

Outdoor experiments to determine the time taken to complete one cycle of development were made using rape as the host plant. Plants invaded in January gave rise to cysts in which embryonated eggs appeared in July, a period of 29 weeks. Invasion of plants in subsequent months led to the formation of embryonated eggs in successively shorter periods of time, the shortest period, 6–8 weeks, being required for plants invaded in June and July. From these later invasions embryonated eggs appeared in August. After July the period required for development began to lengthen. (C. C. Doncaster.)

Predators of root eelworms

Differences in the degree to which *Heterodera* populations increase when host crops are grown have been noted in different soils. These differences may be due to soil structure/moisture relationships or to the influence of various enemies, including soil Arthropods. A method by which relative densities of collembolid predators can be compared in bulk samples of different soils is being developed, and a comparison has been made of the *Onychiuris armatus* populations, from: (1) an Essex soil with *H. cruciferae* infestation which in 1956 caused a crop failure of spring cabbage; (2) the Rothamsted *H. cruciferae* plot where the nematode does not increase even under continuous cropping; and (3) a flourishing Rothamsted *H. rostochiensis* plot. From 3-gallon soil samples from each of these sources, *O. armatus* was recovered in the proportions of 14 : 232 : 441.

New predators found include three or more species of Staphylinid beetle, two species of Myriapod and two species of mite. A method is being developed whereby the proportions of *Heterodera* populations devoured by soil meiofauna can be assessed. In a preliminary trial, in which potatoes infested with potato-root eelworm were used, an average of 22 per cent of newly formed females were destroyed. (C. C. Doncaster.)

Movement and Activity of Nematodes in Soil

Various aspects of the movement of eelworms in soil have been studied, especially in relation to the physical environment. Experiments with larvae of the beet eelworm, *Heterodera schachtii*, have shown that there is an optimum pore size for movement. Studies on the movement of beet eelworm larvae in water films indicate that there is also an optimum film thickness for progression. A comparative study of the beet eelworm and the stem eelworm *D. dipsaci* suggest that there is a simple relationship between body length, particle size and speed. Later experiments with eelworms of different length and activity tend to support the hypothesis that for maximum mobility the longer eelworms require correspondingly larger soil particles. The influence of water percolation into the soil on the movement of eelworms is also being investigated. Experiments on the attraction of eelworms to roots indicate that both the beet eelworm and the oat eelworm (*H. major*) are attracted to roots

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and that the nature of the attraction is an orientation to a concentration gradient of some chemical liberated by the roots. Relationships between rate of emergence from cysts and attraction in the presence of host roots in sand at different hydrostatic pressure deficiencies suggests that concentration of this hypothetical chemical factor is important. (H. R. Wallace.)

Special Photographic Techniques

A simple reflex viewing device and an exposure metering device have been made in collaboration with the Maintenance Department for cinematography. A film has been made from which H. R. Wallace was able to study the mechanics of nematode locomotion in various media. Still and ciné records of predators feeding on nematodes and films of oesophageal movements of various nematodes, the movement of larvae within eggs and within the uterus of *Pelodera* sp. have also been made. (C. C. Doncaster.)

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