ROTHAMSTED RESEARCH

Report for 1958

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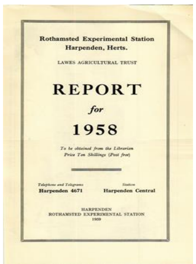
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Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

F. G. W. JONES

In April D. W. Fenwick left to take a post in Trinidad. Elizabeth Widdowson also left in September. Audrey M. Shepherd, Patricia M. Nelson and J. E. Peachey joined the staff in February, July and September respectively. F. G. W. Jones was awarded a travel grant by the Rockefeller Foundation and, after taking part in the Golden Jubilee Meeting of the American Phytopathological Society in Bloomington, Indiana, U.S.A., spent eight weeks visiting research centres in the U.S.A. and Canada. The department provided an exhibit entitled " Root-Knot Eelworms " at Chelsea Flower Show and took part in the Symposium in Soil Zoology at Rothamsted, which preceded the meeting of the Zoological Congress in London. The work of the department continues to be greatly hampered by the lack of proper glasshouses and soil-handling facilities.

As in previous years, many specimens have been received for identification, including *Pratylenchus goodeyi* causing considerable damage to banana roots in the Canary Islands and *Pratylenchus coffeae* on tea roots from Ceylon. A number of new species have been encountered and will be described in due course.

J. B. Goodey has devoted most of his time to a revision and extension of the late T. Goodey's book *Soil and Freshwater Nematodes* (1951), which, when completed, will be a valuable contribution to nematology. The preparation of a supplement to *Nematode Parasites of Plants Catalogued under their Hosts* (2nd ed., 1956) is under way, and a list of plants resistant to root-knot eelworms is also being prepared (Goodey, Franklin, Hooper). Work on the genera *Meloidogyne* and *Aphelenchoides* has continued (Franklin). During the summer a visitor, Dr. V. H. Dropkin, tested the reaction of some pure lines of soybean to a native population of *Meloidogyne*.

MIGRATORY SOIL NEMATODES

Population studies

The Rothamsted six-course rotation has been sampled at monthly intervals throughout most of 1958, and similar sampling of the Woburn six-course was started in September. Results to date suggest that the different crops and soil types have widely differing effects on the generic and specific constitution of the root-parasitic nematode fauna, but much more work is necessary before more positive conclusions can be drawn. Laboratory and greenhouse techniques to determine host efficacy and eelworm pathogenicity are being investigated, as a preliminary to possible outdoor tests in pots, microplots or field plots.

In collaboration with A. J. Gibbs (Plant Pathology Department), nematodes have been extracted from roots of sick and healthy plants and from soils from fields containing unthrifty patches of sugar beet attributed to " Docking disorder ". The results on the whole have been inconclusive, but the fact that soils from some affected patches contain relatively few plant-parasitic nematodes suggests that the nematodes are not the cause of the disorder. Other nematode extractions done in connection with the same problem indicate that the application of 300 lb./acre of " DD Mixture " to Breckland soil in the winter of 1957–58 controlled two genera of migratory root nematodes; in November 1958 numbers of *Paratylenchus* in samples of treated soil were about 10 per cent of those in untreated soils, while the level of *Pratylenchus* was reduced much more—in fact, below detectable levels.

In collaboration with B. D. Harrison (Plant Pathology Department), nematode extractions have been made from samples of healthy and affected roots and from soils from various fields containing patches infested with ringspot viruses. To date, the numbers of root nematodes and occurrence of the viruses have not been closely correlated.

Further samples of " yellow-tuft " turf have been received from the Sports Turf Research Institute, but as no plant-parasitic nematodes were detected in one sample with typical symptoms nematodes did not cause this trouble.

Extraction techniques

The effects of chemical additives on the movement of nematodes and their extractability from soil were studied, but none greatly improved extraction. The Seinhorst mistifier and various modifications of it have been tested for extracting nematodes from soil. Although superior to the Baermann funnel in quantitative recovery from artificial samples, made by adding *Heterodera* larvae to nematode-free loam, it was no better, quantitatively, than a modified Baermann (in which a Petri-dish replace the conventional funnel), and had the disadvantage of delivering the nematodes in an inconveniently large volume of water. (Winslow.)

Feeding

During the last 2 years eight species of spear-bearing soil and plant nematodes have been successfully cultured on agar plates inoculated with *Agaricus hortensis* or *Alternaria tenuis*. For most species the former, although slow growing, is the better and gives a greater yield of larger worms. Twelve species of non-spear-bearing eelworms, including *Demaniella cibourgensis* and *Tricephalobus* sp., have also been successfully cultured on mixed bacterial colonies produced by seeding Nigon's agar with brewer's yeast. Successful culture on fungal plates of spear-bearing forms associated with plants, besides providing an abundance of specimens for description, also indicates that the species are not obligate parasites of the tissues of higher plants. Cultures also provide excellent material for the study of feeding habits and feeding mechanisms. (Hooper.)

Cyst-forming Nematodes of the Genus Heterodera

Potato-root eelworm (Heteroderea rostochiensis)

Joint work with A. J. Clarke (Biochemistry Department) has continued on the chemical nature of the hatching factor which

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diffuses from potato roots into soil and induces the hatching of encysted eggs of the potato-root eelworms. The Nematology Department produces quantities of dilute crude factor by the leaching of potted potato plants. It also assays the crude diffusate and the various stages of its concentration and purification which, it is hoped, will lead to enough pure factor for chemical characterization. Altogether over 1,000 gallons of crude diffusate have been collected during the growing season, and attention has been turned to the production of factor from germinating tomato seeds during winter. In bioassays, well over a million potato-root eelworm cysts have been used. Two difficulties are encountered from time to time in the bioassay work: variability in the hatching rate of stocks of cysts and a decline in the potency of stocks of diffusate used as standards. Whether seasonal variability in the hatch from cysts is inherent, induced by external conditions, or a function of age is still uncertain. When stock samples of diffusate are kept for a year in the refrigerator at 2–4° C. their potency declines considerably, possibly from bacterial activity. However, decline is not prevented by the addition of a bacteriostat such as sulphur dioxide. In preliminary tests a purified form of the factor obtained by absorption on charcoal and elution with acetone has maintained activity over 26 weeks when stored in the refrigerator. Slight losses of activity occurred when stored at room temperature, especially when exposed to light. Attempts have been made to determine in greater detail than heretofore the form of the time-concentration curves for the hatch from cysts immersed in root diffusates and also to compare the results with the hatching responses of eggs freed from cysts. (Widdowson and Nelson.)

Cereal-root eelworm (Heterodera major)

Interesting results have been obtained from a long-term experiment on sandy soil in Shropshire in which oats, barley, wheat and rye were grown continuously on the same plots for 3 years. The experiment also compared autumn with spring sowing and fertilizer with no fertilizer. Starting with a reasonably uniform soil population level of cereal-root eelworm, autumn-sown oats raised the population most, whereas autumn-sown wheat and rye sown in autumn or spring reduced the population. The order of host efficiency was oats (best), barley, wheat, rye. Fertilizer tended to increase soil populations.

At Woburn an experiment has been started to find out whether the eelworm population can be reduced by ploughing-in trap crops of oats and rye before the cysts which develop on them mature. Both oats and rye are followed by a green manure crop (mustard) which is ploughed-in in the hope that increased organic matter will encourage eelworm predators.

To follow population changes under grasses, cocksfoot, timothy, Italian and Perennial ryegrass, and a mixture containing these grasses and late flowering red clover have been sown on small field plots also at Woburn. (Hesling.)

Pea-root eelworm (Heterodera göttingiana)

Work on pea-root eelworm has been increased. In an attempt to find when larvae leave the cysts, a pot experiment has been set up to follow weekly changes in the soil population level. Cysts from the roots of spring-sown peas produced a partial second generation when added to clean soil sown with peas. (Hesling.)

Although larvae escape from cysts readily in the field at certain times, attempts to induce hatch and emergence in the laboratory have met with little success. This seriously handicaps various types of experimental work upon this important plant parasite. Laboratory hatching tests done in the standard way on batches of 100 cysts, immersed in various dilutions of pea-root diffusate in solid watch-glasses, give the same hatch as water, namely about 0·5 per cent of the egg content of the cysts in 3 weeks. Diffusate obtained in distilled water from germinated peas, and therefore free from ions and other contaminations contained in soil leachings that might inhibit hatch, was no better. Attempts to stimulate hatch by mixing cysts in sand or soil, by subjecting them to various temperatures, both constant and fluctuating, to chilling, drying and to the action of $10^{-2}M$ solutions of various simple organic and inorganic compounds have also failed to evoke response, as also have the root diffusates of several leguminous and non-leguminous plants. A slight increase in hatch (5 per cent in 9 weeks) occurred with improved aeration obtained by raising the cysts to the surface of pea-root diffusate on nylon sieves. Comparable tests with beet-eelworm cysts indicate that this method, and a similar one in which the sieve is moistened via a wick, gives cumulative hatches in 2 weeks that are four times those obtained by the standard watch-glass technique. (Shepherd.)

Beet eelworm (Heterodera schachtii)

In good hosts of the beet eelworm such as sugar beet and its allies and some species of Cruciferae, up to 45 per cent of the larvae used for inoculation entered the roots. The proportion of males and females that developed varied considerably. On the roots of the non-hosts *Chenopodium album* and *Beta patellaris* a few males developed and on *B. webbiana* very few: no mature females were observed on these plants. After invasion the highest death-rate occurred among second- and third-stage larvae. Those larvae that reached the fourth stage usually survived to maturity.

The hatching responses of *Heterodera* spp. to substances diffusing out from the roots of their host plants are often highly specific. During the course of the work mentioned above evidence was obtained which suggests that host finding by the larvae liberated from cysts is much less specific. Thus, many beet eelworm larvae invaded the roots of a range of non-host plants. The proportion invading non-hosts remained high even when planted in the same pot as the host, sugar beet. Similarly results were obtained in experiments with potato-root eelworm and pea-root eelworm. Besides indicating a low level of specificity in host finding, these experiments suggest that the attractant factor or factors, if such exist, which lead the larvae to plant roots are different from the hatching factors. (Shepherd.)

Other work on *Heterodera* spp. mentioned in previous reports, which is continuing, includes soil population studies (Doncaster), arthropod predators (Doncaster), biotypes breaking resistance of potato varieties bred from *Solanum tuberosum* ssp. *andigena* (Jones), methods for culturing *in vitro* (Doncaster and Widdowson), chromosome numbers (Hesling) and *in vitro* tests of Vapam and Phosdrin. (Fenwick and Widdowson.)

Movement of Eelworms

Research on the movement of eelworms in soil and over plants has continued. In sand, movement depends on such factors as the rate of percolation of water, the particle size, the rate of movement of the eelworm and also upon its length and activity. Formulae linking the various factors have been derived. For a study of the movement of eelworms over plants, *Aphelenchoides ritzemabosi* on chrysanthemums was chosen. The theory that the eelworms are stimulated to move upwards against downward water currents in wet weather is untenable. Active movement upwards occurs in the absence of such currents, provided the plant bears sufficient moisture either as a continuous or discontinuous film. Movement is facilitated by hairs, which are more numerous on the upper parts of the plant. Invasion of leaves is via the stomata, and occurs during the drying-out phase. Under wet conditions adults and larvae emerge from the leaves via the stomata, often in great numbers. The implications of these observations on control by cultural or chemical means are to be followed up.

Work on the wave formation observed during the undulatory movement of different species has begun. By taking ciné films of eelworms moving in water films of varying thickness on agar, the properties of the waves formed by different species can be analysed and the characteristic movements of different species described quantitatively. Further work on the mobility of eelworms in soil confirms the view that mobility is influenced chiefly by the pore-size distribution and moisture content irrespective of soil type. Work on the relationship between hydrostatic pressure deficiency (pF) in the soil and the emergence of larvae from cysts of the beet eelworm has been resumed, the object being to determine the factors which influence emergence at different suctions. Low levels of emergence at high suctions appear to be associated with inability of larvae to escape out of cyst openings into the very thin water films in the soil. (Wallace.)

Special Photographic Techniques

Considerable use has been made during the year of the improvised photographic equipment mentioned in last year's Rothamsted report. Additional equipment for time-lapse filming of nematodes is being made. The ciné equipment has already proved valuable in studying feeding and feeding mechanisms, and in the analysis of movement of nematodes. Various types of culture chamber and perfusion chamber are being investigated for use in photographic observation of the various stages in nematode life cycles. (Doncaster.)