ROTHAMSTED RESEARCH

Report for 1948

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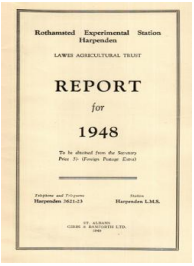

Nematology Department

T. Goodey

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NEMATOLOGY DEPARTMENT

By T. Goodey

Our first year as a department of Rothamsted has, of necessity, been a broken one as we continued in our old quarters at Winches Farm, St. Albans, until the transfer to Rothamsted Experimental Station which was carried out at the end of June 1948. Work was naturally impeded by the impending removal, the uncertainty of the date of the latter and also by the disturbance entailed in the move itself and the process of settling in. In spite of these adverse factors, valuable work has been carried out and some useful results obtained.

Early in the year Dr. Goodey prepared a report on his visit to the United States and Canada (May-September, 1947) and this was duly presented to the Ministry of Agriculture and to the Agricultural Research Council. As a British delegate appointed by the Royal Society, Dr. Goodey attended the 13th International Congress of Zoology held in Paris in July 1948, and delivered a lecture on Plant Parasitic Nematodes to the Section on Applied Zoology and Parasitology.

Although we were established in our new laboratories in July 1948, there was considerable delay before our greenhouses were erected and we could begin the digging and the laying-out of the area on which our experimental plots are to be established. By the middle of November, however, most of the ground had been dug, the lay-out of the plots determined and a few of them planted. These plots are to serve as the living museum of plant parasitic eelworms.

Research conducted in the department falls naturally into two main sections: (1) problems connected with plant infestations by species of the genera *Anguillulina* and *Aphelenchoides* and soil nematodes generally (Dr. T. Goodey, Dr. M. T. Franklin and Mr. J. B. Goodey); (2) those connected with *Heterodera* species (Dr. B. G. Peters and Mr. D. W. Fenwick).

Anguillulina and Aphelenchoides

Anguillulina

The stem eelworm, *Anguillulina dipsaci*, is probably best described as an aggregate species which can be roughly sub-divided into so-called biological races and a main line of work is directed towards the more precise definition and differentiation of these races. Fortunately a small quantity of infested material of Teasel, the type host, *Dipsacus fullonum*, from Oregon, was given to Dr. Goodey during his visit to the United States and this has provided a norm for comparative studies. It is now known that the eelworm disease of Teasel occurs in England and a native source of material has thus become available.

Some work has been done during the year on host transference of the teasel eelworm and certain other races such as the oat/onion/bean race, the narcissus, the red clover and the lucerne races. All of these appear able to transfer successfully to onion seedlings and to reproduce in them. On the other hand the oat/onion/bean race does not transfer to narcissus nor the red clover race to oats. The further implications of this work are being followed up.

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The eelworm causing tuber-rot of potatoes was shown by Thorne in 1945 to be a species distinct from the true stem eelworm, *A. dipsaci*, and the name *Ditylenchus destructor* was given to it. The same parasite attacks potato tubers in Great Britain and we have been studying it closely during the year. We have confirmed, by pot experiments, its ability to transfer to and cause necrotic lesions in the rhizomes of Corn Mint, *Mentha arvensis* L.; a host transference first found in Prince Edward Island. On visiting fields in the Fens where in 1947 potatoes were affected by the pest and in 1948 wheat was grown, Corn Mint was found as a common weed and in many plants examined necrosed areas were found on the rhizomes in which *Ditylenchus destructor* was found in all stages of development. The parasite was also found in similar necrotic areas on the rhizomes of another common weed, viz. Corn Sowthistle, *Sonchus arvensis* L., a new host record. A paper on these findings has been written and accepted for publication.

Several new weed hosts of the onion race of *A. dipsaci* have been discovered and an experiment attempting the control of the oat race on a field scale in its weed hosts is planned for next Spring.

Work is in progress on the eelworm infesting bulbous irises, the taxonomic and biological relationships of which have been very obscure and puzzling for many years. This work promises some most interesting results. In attempting the differentiation of the various biological races of *A. dipsaci* it became apparent that the conditions under which nematodes are killed and fixed needed standardization and work is in progress along these lines.

A. dipsaci in the living, quiescent condition can be seed-borne on onion, red clover and teasel seed and it was shown by Goodey in 1945 that infested onion seed could be successfully fumigated with methyl bromide with negligible effect on the germination of the seed. Further tests have been made with methyl bromide and we have found that in the case of red clover and teasel seed, fumigation is equally efficient and that there is no deleterious effect on the seed.

Work has been done on the detailed morphology of *Anguillulina* species which are often found associated with plant roots as parasites or partial parasites. A new species of the genus has been discovered and will shortly be described. A paper on the occurrence of phasmids (sensory papillae) on the male tails of three species of the genus has been written and will appear very shortly.

APHELENCHOIDES

Morphology and biology. Most of the work has been on strawberry nematodes, as more than one species seemed to be present. Strawberry plants with eelworms of the genus *Aphelenchoides* in the buds were received from about 10 places in England and Scotland. The nematodes were examined and in many cases drawn and measured and it became evident that two species were present, *Aphelenchoides fragariæ*, and what appeared to be *Aph. olesistus*; occasionally both were present on the same plant. They were differentiated by differences in width, position of the excretory pore and curvature of the male tail on killing by heat. Support for the identification of the second nematode as *Aph. olesistus* was given by an infection experiment: on four occasions the strawberry form was inoculated on to different fronds of *Pteris*, and in one case

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the nematodes successfully entered the tissues of the frond and typical 'leaf-blotch' symptoms appeared in 5–6 weeks. After 3 months the diseased patch was excised and teased up and about three times as many worms were found as had been used as an inoculum. No morphological differences could be found between *Aph. olesistus* from strawberry and those from violet and fern.

Culture of Aphelenchoides *species.* Making use of a maize-meal agar on which a growth of the fungus *Alternaria tenuis* is established certain species of *Aphelenchoides* can be successfully grown; usually at 24°C. Under these conditions *Aph. olesistus* from strawberry more than doubled its numbers in a month and many eggs were found. *Aph. fragariæ* on a similar medium also multiplied but not quite so vigorously. *Aph. ritzema-bosi* (the chrysanthemum foliar eelworm) maintained itself on agar but when inoculated into ripe tomato multiplied six-fold and spread throughout the fruit in 10 weeks. In a second tomato fruit the numbers doubled in 2 weeks. *Aph. olesistus* from infested leaves of *Lilium* sp. and *Aph. subtenuis* from narcissus were also established on agar plates along with the fungus *Alternaria tenuis*; the former multiplying to very large numbers. *Aph. olesistus* from violet was successfully transferred to *Pteris*, the nematodes increasing three-fold in about 9 weeks.

Using the same cultural technique the nematode, *Aphelenchus avenæ*, which may be a facultative parasite on plant roots, has been cultivated successfully; its feeding habits and the behaviour of the mouth spear have been studied under these conditions.

Staining technique. Dr. Franklin has developed a quick staining method for demonstrating eelworms in plant tissues. It is a modification of the acid fuchsin lactophenol technique. She has also done much work on a review of the genus *Heterodera* and the relevant literature, a genus on which she is an acknowledged authority.

Heterodera

That side of the department's work concerned with eelworms of the genus *Heterodera* has consisted largely in bringing to completion the Agricultural Research Council field trials on the nematocidal effects of D-D mixture, and in pursuing certain technical problems raised by those trials. The routine laboratory work on the final series of soil samples was completed in March, and a full report was then drawn up and submitted to the *ad hoc* Agricultural Research Council Committee on 30th April. On the recommendation of that Committee, the report has since been recast and expanded in a form suitable for publication and is now ready for the press.

The trials were carried out at seven 2-acre sites on sandy, silty, and blackland soils. Apart from a pilot trial where soil was injected in Spring, injections were carried out in the autumn, and potatoes were grown the following year. Factors investigated were rate of application (0, 200, 400 and 800 lb. D-D per acre), depth of injection (4 or 8 inches) and the effect of rolling after injection. At the most responsive of the sites (Wainfleet), yield, kill and the final eelworm population were all roughly proportional to the rate of application. Under favourable circumstances a 50 per cent. increase in yield and something like a 50 per cent. reduction in eelworm

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population can be expected from 800 lb. per acre, but the latter reduction is more than made good during the growth of the subsequent crop; accelerated multiplication of eelworm on the treated plots leads to their finally having a larger population than the untreated controls. Of the sites tested, the blackland soils gave a lower eelworm kill and a much lower yield increase from D-D than silts or sands. After autumn injection the nematocidal, and probably the phytocidal, effects of D-D persist in the soil for many weeks.

The counting of over a million cysts, eggs and larvæ, from upwards of 1400 soil samples collected during the trials, has left a large body of data, the statistical analysis of which was undertaken by Mr. G. V. Dyke of the Statistical Department. These data show that there are pronounced anomalies in the counts of larvæ "hatched" by the calcium hypochlorite technique. This technique has failed not merely to differentiate between living and dead larvæ, but even to give a reliable total count of the content of cysts. Accordingly, a detailed investigation of the technique has been undertaken, as a result of which it is now possible to secure a reliable total count. Some progress has also been made towards differentiating living from dead larvæ by the same technique, but at present it is doubtful whether results can ever compare with those of the lengthy root-diffusate technique. The latter is also under investigation with a view to improving the consistency of counts. Sigmoid curves have been obtained from plotting "percentage hatch" against "time" and these may prove amenable to probit analysis. Progress is necessarily slow since hatching requires some 8 weeks, and inherent variability necessitates large samples, but to date the hatch from 1,500 individual cysts has been separately counted at least weekly. Work on the dilution of root-diffusate is also in hand, and on its concentration by adsorption on animal charcoal followed by solution with 20 per cent. acetone and evaporation.

The nematocidal action of D-D mixture is also under investigation, both used neat in small pot experiments and in aqueous solutions. Solutions for nematocidal tests have also been prepared from fractions of D-D mixture volatilized at room temperature by passing measured volumes of air through it. The fractions are not homogeneous; the complete D-D is soluble in spirit, but, after volatilizing 9/10, the residual 1/10 is insoluble in spirit but soluble in acetone.

At the invitation of Mr. L. N. Staniland, who has found considerable nematocidal power in trichlorophenol (T.C.P.), tests are in hand on dilutions of this material. In these, and the previously mentioned tests, use is also being made of the vinegar eelworm as a convenient laboratory animal. The hypothesis, awaiting confirmation, is that substances with no effect on vinegar eelworm will also be without effect on encysted *Heterodera* larvæ. If this is confirmed it will give a rapid method for eliminating useless substances and/or concentrations; at present, many weeks are wasted in such elimination when using the root-diffusate technique. A modification of the McMaster counting slide enables eelworms in 1 ml. of liquid to be counted rapidly and with greater convenience than in the previously used solid watch glasses.

Experiments on the thermal death point of *H. rostochiensis* have been carried out in greater detail than hitherto; it is confirmed

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that damp cysts entail a lower lethal temperature than dry cysts. Extensive previously-collected data on larval lengths of different *Heterodera species* have been analysed and results are being prepared for publication.

The following joint investigations and contacts should be reported:

(1) The department is co-operating with the West Norfolk Farmers Co-operative and Messrs. Shell to investigate the effects of annually repeated injections of D-D mixture in infested potato soils. Data for this (the first) year have been analysed.

(2) The department plans co-operating with Dr. H. C. Gough of Cambridge in long-term investigations on eelworm population changes during different crop rotations in different soil types.

(3) Messrs. Seymour Cobley Ltd. have been advised on the warm-water treatment of seed potatoes to kill adherent *Heterodera* cysts: at present they prefer to carry out their own tests.

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