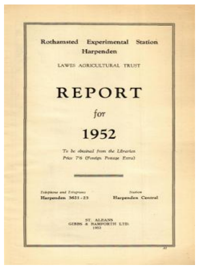
Report for 1952



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Nematology Department

B. G. Peters

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NEMATOLOGY DEPARTMENT

B. G. Peters

At the end of September T. Goodey retired from the headship of the department but is continuing his work on a full-time basis. J. J. Hesling has been transferred from an A.R.C. Studentship to the scientific staff and A. R. Forster has been appointed an Experimental Officer. D. W. Fenwick was awarded the D.Sc. degree of the University of Wales. B. G. Peters gave a course of 12 lectures on the Platyhelminthes for the parasitology course at Imperial College, London, on a *locum tenens* basis.

Dr. J. Gallego returned to Spain after working for six months in the department. Mr. C. A. Loos of the Talawakelle Tea Research Institute, Ceylon, also worked in the department for six months making a critical study of eelworms attacking tea roots. In addition, three workers have attended for periods of 1 to 3 weeks to acquire techniques used in experimental work with species of *Heterodera* : Miss J. Bywater of Birmingham University, Mr. J. Keeping of Glaxo Laboratories, Ltd., and Mr. A. J. A. Pearson of Shell Chemicals, Ltd.

Material and Methods

Most members of the staff have shared the considerable work of identifying eelworm material sent in. An interesting case is the peculiar eelworm *Tylenchus fungorum*, unknown since first described by Bütschli in 1837, which occurred with a second species new to science in culture jars set up by Professor P. A. Buxton for the collection of insects which breed in toadstools. It appears certain that the females of both species must become parasitic in insects for the completion of their life cycles. A detailed and fully illustrated description of the eelworms has been accepted for publication (T. Goodey).

Two further cases concern root-knot eelworms. *Meloidogyne incognita* var. *acrita* has been identified as the cause of severe galling of tomatoes under glass, the identification being confirmed by testing the range of hosts susceptible to the eelworm, and *M. hapla* has been found to be damaging *Scabiosa caucasica* growing out-of-doors in a Suffolk nursery. Root-knot is thought of mainly as a glasshouse disease in this country, but several outdoor records are known from cultivated plants in England, and this year it has been reported from Italian ryegrass in Wales and from maram grass on the coast of Northumberland. It seems very likely that *M. hapla* is the species involved, and that it is indigenous (M. T. Franklin).

Several members of the staff have also sent out identified eelworm material, on request. This work of identification, exchanging material, and building up a departmental collection of mounted specimens is an important part of our activities. As the subject extends, morphological work makes increasing demands on powers of observation and necessitates new microscopical methods and equipment. Vertical illumination, through the microscopic objective, is now revealing fine surface structure previously obscure

(J. B. Goodey). Also, new techniques for staining and processing eelworms have been tested and adopted as routine where suitable (M. T. Franklin).

The department requires for its work a readily available source of living eelworms. This need, partially met by the small outdoor plots known as the Living Museum, inevitably grows. The root-knot eelworm, formerly regarded as a single species, *Heterodera marioni*, has suddenly come to be regarded as several different species of the genus *Meloidogyne*. This year has seen the establishing of four of these species, plus a distinct variety, in electrically heated beds of suitable soil in a greenhouse where (it is hoped) they will multiply on suitable host plants (M. T. Franklin).

Cysts of the potato root eelworm are used in very large numbers each year and something like mass-production methods are necessary in recovering them from soil by flotation, freeing them from other floating material by rolling, and handling them expeditiously. Work is proceeding on a mechanical rolling device which should save many hours of tedious work. Also, a simple apparatus has been adapted for picking up cysts by suction (J. J. Hesling).

Resistance and Susceptibility

Four degrees of susceptibility of a plant to an endoparasitic eelworm can be conveniently distinguished: (1) the eelworm may fail to enter the plant at all; (2) it may enter but fail to grow and reproduce; (3) it may grow and reproduce but fail to produce obvious disease symptoms; (4) it may produce disease symptoms. The situation can be complicated by differing behaviour of different biological races of any one eelworm species in different varieties of any one host species. Moreover, the degrees are not rigid categories: the result may partly depend on the level of infestation. All these points are illustrated by experimental results obtained this year.

Ditylenchus dipsaci

Old and new varieties of oats have been tested for susceptibility, for the National Institute of Agricultural Botany and the Welsh Plant Breeding Station, using three different rigorous techniques, which gave parallel results. Some oat varieties may possess a high degree of resistance which, however, is not always absolute (T. and J. B. Goodey). Various host range experiments on other races of *D. dipsaci* have been carried out including some on a race found attacking carrots in East Anglia (J. B. Goodey).

Ditylenchus destructor

Two populations, from potatoes and from mushroom mycelium, were tested in 8-inch pots on 10 varieties of potatoes, using the three inoculation rates: 9,000, 45,000 and 225,000 eelworms per pot, plus controls. No potatoes in the mushroom series were attacked, the haulms remaining normal and the new tubers quite clean, whereas all pots in the potato series produced infected tubers, with some indication that severity of attack was dependent on initial rate of inoculation. In spite of this universal attack no symptoms were shown by any variety at the lowest rate. At the middle rate

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Arran Banner, Arran Pilot and Duke of York showed some stunting: the others were symptomless. At the top rate these two Arran varieties with Gladstone, Great Scot and Homeguard showed marked leaf symptoms, whereas the other five (Duke of York, Epicure, King Edward, Majestic and Ulster Chieftain) were symptomless (J. B. Goodey).

Heterodera rostochiensis

Cysts from 8 localities in the British Isles were used in an attempt to infect the variety of tobacco which was recently found, in Connecticut, attacked by a species of *Heterodera* very like *H. rostochiensis*. While eelworm larvae penetrated into the young seedlings (some local " races " more readily than others), none was found to have grown to maturity on older plants and no symptoms were seen (J. J. Hesling).

Interesting results came from a comparison of black nightshade, *Solanum nigrum* (into the roots of which larvae readily penetrate but fail to become adult) and tomato, a normal host. In heavily inoculated soil, although larvae penetrated tomato roots more quickly and initially in larger numbers, the tomato plants became severely stunted whereas *S. nigrum* did not, so that its larger root system finally took in a greater number of larvae. On the other hand, in lightly inoculated soil tomato took in the greater number. There appears to be a peak density of larvae per weight of root within 2 to 4 weeks, after which the density declines. This peak occurs earlier, and is more marked, in tomatoes than in *S. nigrum* (C. C. Doncaster).

Heterodera cruciferae

It is a matter of opinion whether this species causes symptoms in brassica crops. Transplantation of clean cabbage seedlings into soils containing up to 625 larvae per g. of soil showed that no obvious symptoms occurred at 125 l./g., while at 625 l./g. there was moderate stunting with intervenal chlorotic marbling after 9 weeks. So far as is known, such high population densities do not occur under field conditions, but a small field plot has been infected and is under observation (C. C. Doncaster).

Eelworm Population Changes

The rates at which populations of plant-parasitic eelworms build up and decline, and the various factors influencing such changes, are matters of importance especially among species of *Heterodera* which have few generations per season (often only one) but can survive many years in the soil in the absence of a host plant.

Heterodera rostochiensis

The rotation experiment, in which the effects of four different 3-course rotations are being measured in terms of eelworm population, has shown the expected rise in those plots carrying potatoes and a fall in the others but, so far, no differences are apparent between the various rotation crops other than potatoes (C. C. Doncaster).

Populations show interesting changes within one growing season. One way to reveal these is to grow potatoes in infested soil in

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wooden boxes made up from 6 separate layers 2 inches deep. Eight such boxes were used, a pair being brought in and dismantled, layer by layer, after 5, 9, 13 and 19 weeks. The soil from each layer was washed to provide estimates of the cyst density (c/g.) and the density of eggs or larvae per cyst (l/c.), the product of these two criteria being the population index " larvae per g. of soil ", (l/g.). The soil originally contained 0·49 c/g., 71·9 l/c., and 35·0 l/g. Averaged over the whole box the cyst density showed a slight rise at 9 weeks, a significant rise to 0·96 c/g. at 13 weeks, and the greatest rise to 6·91 c/g. at 19 weeks. Owing to hatching, the density of larvae per cyst fell to a quarter of its original value at 9 weeks recovering to 42·4 l/c. and finally 229 l/c. The population index (l/g.) reflected this fall and rise and at 13 weeks was only slightly higher (40·6) than at the start; the phenomenal increase to 1,582 l/g. took place in the last 6 weeks, in July and August. Farmers rely on the last few weeks to give them an extra crop yield; in this way they also reap a harvest of eelworms. The separate layers showed that these same changes are delayed by up to 4 weeks in the bottom and top layers but, whereas the bottom layer had almost caught up at 19 weeks the top layer remained significantly reduced; the final l/g. data were: top: 714, 2nd layer: 2,254, bottom layer: 1,544 l/g. (B. G. Peters).

In 1951 a new edaphic experiment was set up in 32 pots each holding 20 lb. of soil. Bones Close soil was factorially modified by the addition of 35 per cent coarse sand (S), and 10 l. coarse peat (P) to half the pots. Mixed fertilizers were at two levels 5 g. and 25 g. (F), and 100 eelworm cysts were added to half the pots, giving 0·01 c/g. (H). Criteria were height of haulms, number and weight of tubers produced and (in the infested pots) eelworm population data. In this first year there were no significant effects of any kind, but the eelworm population increased to 0·24 c/g. In the second year fertilizers at the low rate were omitted. Height of haulms showed a strong fertilizer effect but haulms in infected pots were only slightly shorter. Number and weight of tubers produced, and weight per tuber, all showed a highly significant fertilizer effect and, while infection did not change the number of tubers, the total weight and weight per tuber were signifiantly reduced by eelworm. Cyst density increased on the average from 0·24 to 3·34 c/g. during the season but there was a highly significant fertilizer effect, the fertilized plots yielding 5·00 c/g. compared with 1·39 c/g in the unfertilized: a ratio of 3·6. Going back to the tuber weights and excluding the uninfected pots, 25 g. of fertilizers have increased the yield of tubers per pot from 30 g. to 474 g. but they have also increased the yield of cysts per pot from 12,500 to 45,000 (B. G. Peters).

Root Diffusates

In most species of *Heterodera* the larvae are stimulated to hatch from their egg shells and to leave the cyst in which they have been protected by a chemical substance diffusing in high dilution from the roots of certain plants. Usually, for any given species of eelworm, it is the host plants which produce the active diffusate but sometimes an " active " plant species is not a host for that

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eelworm at all and sometimes a host is not active. For two species of *Heterodera*, the pea and cereal root eelworms, it is not yet clear that root diffusates are implicated. There are at least as many different diffusates as there are responsive species or races of *Heterodera*.

Diffusates are used as laboratory reagents to induce hatching, so that larvae can be counted. Moreover, since unhatched eggs within their cyst can survive in soil for many years while hatched larvae soon die without the host plant, there are theoretical possibilities of using active non-hosts, or artificially prepared diffusates, on a field scale to hatch out and starve out the larvae. The scope for research is very large.

Chemical nature

Research on the chemical nature of the active principle in potato (or tomato) root diffusate is being financed by the Agricultural Research Council and carried out at Reading, Cambridge and Rothamsted. At Reading Professor R. H. Stoughton is growing potatoes and tomatoes under glass by soil-less culture methods and extracting the active principle from the used culture medium with charcoal. At Cambridge Professor A. R. Todd is working on the chemical analysis of the active principle after removing it from the charcoal. At Rothamsted we are primarily concerned with the bio-assay of fractions produced by Cambridge, and the monitoring of the charcoal extraction carried out at Reading. In addition to this routine work four grades of charcoal have been tested for extracting natural samples of root diffusate, best results being obtained with a finely powdered grade. Used for 24 hours at 1 g/litre, 80 per cent of the original activity was removed. Experiments on the extraction of diffusates of different strengths suggest that saturation of the charcoal is one factor of importance (D. W. Fenwick).

Factors influencing production

Experiments were reported last year on the effects of age and variety of potato and of the presence of *H. rostochiensis* infestation, indicating that diffusate production is correlated with rate of growth of roots and (since infestation stimulates secondary root production) also with infestation. Further experiments this year along similar lines more or less confirm these findings, and especially the relationship between rate of growth and diffusate production. The results are being prepared for publication (D. W. Fenwick).

The same correlation has been found in uninfested tomatoes and *Solanum nigrum* (an "active" plant but not an efficient host), and in infested tomatoes. But in young infested *S. nigrum* plants, although root growth was not significantly affected, there was a marked inhibition of diffusate production. Conversely in older *S. nigrum* plants whose growth was retarded by larval invasion, diffusate production was comparable with that of uninfested controls. The reasons for this anomalous behaviour in *S. nigrum* are not yet apparent (C. C. Doncaster).

Breakdown of diffusate

Following the development of the bio-assay technique for potato root diffusate, reported last year, experiments have started on the breakdown of diffusate *in vitro* and in soil. Preliminary tests indicate that at room temperatures root diffusates lose considerable activity over a period of 3 to 4 weeks and this breakdown is not substantially decreased through sterilization by filtration. To obtain information on breakdown in soil a preliminary series of experiments using sand, peat and clay factorially has been started. Results are not yet available but there is some evidence to indicate that rate of breakdown in all three components is comparable with that in soil (D. W. Fenwick).

Other investigations

Among active non-hosts of *H. rostochiensis* several Gramineae have been claimed in the past. An experiment is in hand testing the hatching power of diffusates from 14 species of Gramineae; results are not yet available (J. J. Hesling).

There are many records of a winter dormancy period for *H. rostochiensis*, larvae failing to hatch in diffusate during winter months even though the diffusate was collected in summer. This dormancy is not found at Rothamsted and experiments show that it is due to the effects of winter conditions on cysts; cysts collected before winter and stored in the laboratory show normal hatching all the year round (D. W. Fenwick).

Chemical Control Methods

Pot tests

With the completion of the fourth annual test of nematicides against *Heterodera rostochiensis* in glazed pots of 20 lb. of infested soil, the method and the first year's results have been published (104). Analysis of the 1951 results showed that chlorobromopropylene used as a soil fumigant gave high kills closely similar to those given by equal doses of D-D mixture. The other nematicides were used as drenches in 1 l. of water. The only promising ones were a commercial chlorinated phenol and a chloroisothiocyanate. Poor kills were given by two chlorinated nitrobenzenes at up to 0·4g. per pot and no kill by elemental iodine (up to 0·45 g. per pot) or by parathion (up to 2 g. per pot, of a 27 per cent wettable powder). The only interesting effects on potato plants subsequently grown in the pots were increased yields of tubers from low doses of the two fumigants, greatly decreased yields from the high dose of iodine, and a large increase in the number (but not weight) of tubers from the parathion pots. This year a number of compounds based on 8-hydroxy-quinoline have been tested, data have been secured for probit analysis in respect of D-D and chlorobromopropylene, and D-D has been used to investigate kills at different depths of soil and at varying moisture levels. The final counts are not yet completed.

A separate experiment to find the effect on *H. rostochiensis* of dressings of ammoniacal gas liquor, was carried out by request in view of the possible use of this waste product as a source of nitrogen, replacing ammonium sulphate which threatens to be in short supply. At the highest rate, corresponding to about 9,000 gallons per acre, there was a marked increase in yield of tubers and the eelworm kill was 35 per cent. However, so rapid was the recovery of the eelworm population on the extensive roots of plants in the treated soil, that the latter finally contained 3·5 times as many eggs as the untreated soil. This gas liquor can therefore not be recommended for use on eelworm land which is about to carry potatoes, in spite of its obvious fertilizer value (B. G. Peters).

Field tests

In conjunction with Mr. E. B. Brown of the National Agricultural Advisory Service (Cambridge), tests have been carried out on the control of *Aphelenchoides ritzema-bozi* (formerly known as *A. ribes*) on blackcurrants. Severe pruning and parathion spraying were tested, separately and combined, on a plantation of bushes. The results indicate that either method gives appreciable reduction of the infestation and that a combination of both is the most effective procedure (M. T. Franklin).

The co-operative experiment with Shell Chemicals, Ltd. and the West Norfolk Farmers' Manure Co., to test the effect on a potato root eelworm population of single and annually repeated D-D injections at various stages in a 5-course rotation, is now in its second year. Pre-treatment samples showed a satisfactorily high and uniform eelworm population over the 30 plots, but the 1951 D-D injections, at 400 lb. per acre, gave no significant kill, for reasons that are obscure. The experiment was sensitive to a kill of 15 per cent, but 6 per cent was measured. The soil is a fine silt and previous experience with this type of soil would have suggested a kill between 30 and 50 per cent (B. G. Peters and D. W. Fenwick).

Observations have continued on the relationship to Sitka spruce seedlings of *Hoplolaimus uniformis* and *Trichodorus* sp. These have been made at Ringwood forestry nursery on experiments carried out by the Chemistry Department. Formalin applied to the soil reduced the numbers of eelworms but did not eradicate them and they gradually came back to a normal level as time passed (J. B. Goodey).