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Nematology Department

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B. G. Peters (1955) *Nematology Department ; Report For 1954*, pp 96 - 102 - **DOI:**
<https://doi.org/10.23637/ERADOC-1-76>

NEMATOTOLOGY DEPARTMENT

B. G. PETERS

The following visitors have worked in the department during the year: Mr. N. D. N. Welford and Miss E. C. Mason from the National Agricultural Advisory Service, Cambridge (5 to 16 January); Mr. G. E. Thomas from the States Experimental Station, Jersey (25 January to 4 February); Mr. A. R. Al Adhami from Iraq (5 days in May); Mons. M. Luc from the Côte d'Ivoire (14 June to 17 July). Mr. Thomas required help in handling a large mass of experimental data: the others were interested in acquiring our laboratory and/or experimental techniques.

A new development for this department was marked by the arrival in April of Mr. H. E. Welch from the Insect Parasite Laboratory at Belleville, Ontario, who intends to work for two years on nematode parasites of insects.

For the Commonwealth Bureau of Agricultural Parasitology, J. B. Goodey and M. T. Franklin are working on a new edition of T. Goodey's *Nematode parasites of plants catalogued under their hosts* (1940), now seriously out of date.

MATERIAL AND METHODS

Root-knot material, received from Tanganyika, Nigeria, West Bengal, Canaries and Italy, has been tentatively identified and, where possible, confirmation has been sought from test inoculations of four key-plant species. Interpretation of the main diagnostic feature, the cuticular perineal pattern, has been facilitated by a set of photomicrographs of typical patterns kindly sent by Dr. A. L. Taylor of Beltsville, Md. (M. T. Franklin.)

In counting eggs from cysts of potato root eelworm the hypochlorite technique has been largely superseded by a squashing method: soaked cysts on a flat metal plate are crushed by a glass roller supported on side-strips $\frac{5}{1000}$ inch high and the cyst contents are freed from the cyst wall in a homogenizer. (E. Widdowson.)

The standard technique in which potato root eelworm cysts are floated from dried soil and then stored dry, is not satisfactory for cereal root eelworm cysts. Many newly-formed cysts fail to float, and cysts stored dry fail to hatch. The flotation technique has had to be modified, and cysts are stored wet at low temperature. (J. J. Hesling.)

For work on the race of *Ditylenchus dipsaci* attacking mushroom spawn, a thermostatically controlled chamber for the pot-culture of mushrooms has been constructed. (J. B. Goodey.)

EELWORM POPULATION CHANGES

Heterodera schachtii

For the third time, all the Barnfield plots have been sampled to follow the population changes of beet eelworm. Larval counts are

not yet available, but the cyst counts show only a trivial increase in the mean for the whole field, compared with 1948 and 1946 :

Year	Cysts per g. of soil (geometric mean)
1946	0.388
1948	0.405
1954	0.427

Broadly speaking, manurial treatments and forms of nitrogen which favour high yields of tops and roots also favour high cyst densities. (J. J. Hesling and B. G. Peters.)

Heterodera major (J. J. Hesling)

Exploratory sampling across the fallow section of Broadbalk revealed some cereal root eelworm, mainly on the dunged plot; as a result, all treatments and sections have been sampled after ploughing. This eelworm is also known to be present on Hoosfield, which may be sampled in 1955.

Observations on the Winchester farm of Mr. P. R. J. McMorland have shown a five-fold population increase under Herta barley, a decrease to one-fifth under linseed and a comparable decrease under cocksfoot, which, although technically a host, is apparently not an efficient one. In fallow pots of infested soil the density of larvae within cysts fell (owing to hatching) by 75 per cent from March to July, and a further 4 per cent from July to October.

The experiment at Stoke-on-Tern involving sixty-four micro-plots has satisfactorily come through its first complete year; the object is to see the effect, on a fairly uniform and low eelworm population, of growing oats, wheat, barley and rye, both autumn and spring sown, with and without fertilizers. Also in Shropshire, several commercial fields are under observation, in co-operation with Mr. H. C. F. Newton, to follow the population fall when heavily infested soils are put down to leys or non-host crops.

Heterodera rostochiensis

Solanum demissum has been used as a convenient (because small) host plant in a study of population increases in soils initially at five known levels, established with counted cysts of graded size. There was no obvious effect of level on size of plant. (J. J. Hesling.)

HOST-PARASITE RELATIONSHIPS

Host-ranges and resistance

Host-range tests in soils infested with five species of *Meloidogyne* are continuing in electrically-heated glasshouse beds; these beds get infested with parasitic fungi, and the electrical heating wires become corroded, necessitating frequent renewals. (M. T. Franklin and A. R. Forster.)

No morphological criteria being available for most of the biologic races of stem eelworm, host-range tests are essential. Such tests indicate that the East Anglian carrot race is a population of the oat race, for, although it produces symptoms in *Vicia faba* like those due to the so-called "giant race", it is not of that race. It has been confirmed that the only alternative host of many tested

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(mainly legumes) for the lucerne race is alsike clover. Of lucerne varieties Grimm is seriously attacked, while De Puits produced a good stand in spite of being attacked. The American resistant varieties are unsuited to our conditions. (J. B. Goodey.)

In collaboration with the National Institute of Agricultural Botany, investigations into the varietal susceptibility of oats to the stem eelworm have continued, both in pot tests and in field trials in co-operation with the National Agricultural Advisory Service. It is confirmed that the spring variety Milford, and the winter varieties Grey Winter, Avoine d'hiver, Picton and S.172 are highly resistant but not absolutely so. It is hoped that further routine testing of oat varieties will be undertaken by the National Institute of Agricultural Botany and oat breeders. (J. B. Goodey.)

The pot tests of resistance of oat and barley varieties to *Heterodera major* mentioned last year were inconclusive. These pots had been inoculated with dried cysts, from which larvae are now known to hatch with difficulty. The tests have therefore been repeated using naturally-infested soil. (J. J. Hesling.)

Solanum nigrum shows a partial resistance to *Heterodera rostochiensis* in that the roots are readily invaded by larvae, which, however, fail to become mature. Repetition of an experiment reported in 1951 and 1952 has shown interesting results from exposing seedlings of *S. nigrum* and tomato (a normal host) to soil containing 0, 5, 30 and 180 larvae/g., testing root-diffusate (R.D.) production at 1, 2, 4 and 8 weeks, then weighing root-systems and examining them for contained eelworms. R.D. production in the tomatoes was more seriously impeded by high densities of inoculum than was that in *S. nigrum*, in which infestation caused the peak of R.D. production to be postponed. Stunting of plants at high inocula, was more severe in tomatoes. (C. C. Doncaster.)

It seems likely that the different degrees of resistance shown by solanaceous species to *H. rostochiensis* are biochemical in nature, either by roots of susceptible species containing a factor essential to the parasite or by roots of resistant species containing an inimical factor. In either case it is possible that the factor is elaborated in the aerial parts of the plant. If so, grafting before infestation might contribute information, if the graft conferred on the stock-root system the factor normally lacking. A preliminary series of grafts has been made using tomato, potato and *S. nigrum* and including self-grafts (tomato on tomato; *S. nigrum* on *S. nigrum*), to control any physiological disturbance due to the grafting process, and transplanting to soil infested with *H. rostochiensis*, after the grafts were established. The result so far is that each root-system behaved normally, without showing any noticeable effects from the scion; thus, tomato roots became infested and *S. nigrum* roots did not, irrespective of scion. (A. R. Forster.)

Pathogenicity

In 1953 a pot experiment was set up at the Connecticut Agricultural Experiment Station to ascertain whether *Heterodera tabacum* had detectable pathogenic effects on tobacco plants. Plants were exposed to a geometric series of inoculum densities, with and without fertilizers. Using as criteria the heights of plants at various times and the final weight of tops, there was an inverse correlation between

log density and height or weight, which in no case departed significantly from rectilinearity. An interesting feature was that, at the lowest density (50 larvae/g. of soil) among the fertilized plants, plants were slightly taller and heavier than in the controls. (Dr. B. F. Lownsbery and B. G. Peters.) Strictly comparable data for *H. rostochiensis* not being available, a similar experiment with potted potatoes has been carried out this year, but at lower densities (lowest about 2 larvae/g.). A comparable inverse relationship has been found between log density and height. Numbers and weights of tubers produced, though complicated by an attack of blight, showed a similar relationship, and for all criteria the lower inoculum densities gave taller plants and more and heavier tubers than did the uninfested controls. (B. G. Peters.)

In a somewhat similar experiment with *H. cruciferae* and potted plants of Brussels sprouts, starting at a minimum density of 5 larvae/g. of soil, there was again a stunting effect from high densities and again a slight stimulating effect from low densities. The fact that stunting was significant at 125 and 625 larvae/g. suggests that this eelworm may have pathological effects on the host under agricultural conditions, a matter on which there has been some doubt. (C. C. Doncaster.)

Another host-parasite situation in which it has never been established that the host suffers significant damage is root-knot on lettuce. In a complex pot experiment three varieties of lettuce were infested with three species of *Meloidogyne*, each at two levels. A pilot test had indicated suitable levels of inoculation and times of observation. Plants were inoculated at 2 weeks of age and were lifted at five different times, when the criteria measured were: weight of tops and roots, number of galls and number of eelworms within the roots (giving also eelworms/g. of root). The data have not been fully analysed, but it is obvious that pathological effects, if any, are very slight, even at inoculation densities of 1,000 larvae per seedling. The varieties tested are of low susceptibility, at least at the relatively low temperatures at which lettuces are grown. (M. T. Franklin and A. R. Forster.)

Preliminary investigations have been made into a serious and rapid decline in mushrooms, frequently associated with the presence of species of *Rhabditis*. These eelworms are usually saprophagous rather than parasitic, and the nature of the association is of interest. Work is continuing on the association between *Hoplolaimus uniformis* and conifer seedlings, in conjunction with the Chemistry Department's investigations on nutritional problems in forest nurseries. (J. B. Goodey.) A curious intervenal chlorotic marbling has been observed in the leaves of cabbage plants which were both infested with *H. cruciferae* and inadequately fertilized. (C. C. Doncaster.)

ROOT DIFFUSATE PROBLEMS

The production and storage of large quantities of active potato root diffusate present problems. It has been found satisfactory to grow Arran Banner in 6½-inch pots of unsterilized loam plus one-quarter of sand in a cool greenhouse. Leaching so as to yield 200 ml./pot can proceed twice a week from break-through for 6 weeks, and by successive plantings collection is maintained for 5 months

from early May. Loss of activity by storing at 2–5° C. is very variable, and for experimental purposes the bulk storage of one main stock is being attempted. (E. Widdowson.)

The tripartite Agricultural Research Council project continues. Professor R. H. Stoughton (Reading) produces the root diffusate; Professor A. R. Todd (Cambridge) works at its chemical analysis; D. W. Fenwick monitors the Reading samples and bio-assays various Cambridge fractions. This year has seen two major developments at Reading. First, it has been found that potatoes grown in soil in the new outside frames produce a far more active diffusate than potatoes or tomatoes grown in soil-less culture under glass. Secondly, the resulting diffusate is much more efficiently concentrated by partial evaporation under reduced pressure than by charcoal extraction. It seems likely that Cambridge can now be given adequate quantities of the active material. (D. W. Fenwick.)

The action of root diffusate from a growing crop and of other factors regulating the hatching of *H. rostochiensis* larvae is being investigated. After charging shallow 10-inch pans of infested sand with root diffusate at a central charging point and leaving the pans for varying times, cysts were recovered from annular samples of sand. The rate of cyst-emptying, revealed by counts of residual eggs and larvae still within the cysts, varied directly with time and inversely with radial distance from charging-point. This basic technique will be used to investigate the effects of temperature, soil type, etc. (E. Widdowson.)

Experiments and techniques based on the hatching of Heterodera larvae in root diffusates commonly make use of counted or weighed batches of cysts. Work is now in progress on hatching from eggs previously removed from cysts. The different rates of sedimentation of eggs and larvae in water may upset efficient sampling, which can be restored by agitation with a slow air-stream while the sample is being removed. Hatching from eggs, rather than cysts, yields the same type of curves for hatch/log time and hatch/log dilution of diffusate. Soaking of cysts in water, for up to 12 days, before adding diffusate, results in improved hatching; but a similar pre-soaking of eggs removed from dry cysts almost inhibits subsequent hatching unless the volume of water for pre-soaking is greatly reduced. Work is not yet sufficiently advanced to suggest an explanation of this curious phenomenon. (D. W. Fenwick.)

EELWORM PHYSIOLOGY

Several attempts to render *H. rostochiensis* bacteriologically sterile without killing them have failed. It has now been found that their eggs, sterilized in hydrogen peroxide (at 20 vol. concentration), can be subsequently hatched in root diffusate. A new Warburg respirometer has been calibrated, and a programme of work on the fundamental physiology of this species is now under way. (D. W. Fenwick.)

CHEMICAL (AND OTHER) CONTROL METHODS

In a small-scale warm-water treatment of violet plants infested with *Aphelenchoides fragariae*, bundles of eight plants were plunged

first into warm and then into cold water before re-planting. Temperatures and times were :

112.5° F. for 10 and 15 minutes.

115° F. for 8, 10 and 12 minutes.

117° F. for 6 and 9 minutes.

Living parasites were found only in the controls, and slight damage to the plants was found only after 112.5° F. for 15 minutes. (M. T. Franklin.)

Pot tests

A number of halogenated hydrocarbons have been tested in glazed pots holding 20 lb. of soil infested with *Heterodera rostochiensis*. Hatching tests on cysts recovered from samples taken 4 weeks after injection show that, of materials tested, only allyl bromide at 1 ml./pot gave results comparable with Shell "DD Mixture". In another series of tests several isothiocyanates were used, dispersed in water and sprinkled over the soil spread out in a tray; at least one of these was promising, but of doubtful practical value owing to the difficulty of application. Last year an experiment was referred to in which DD was injected into infested soil in boxes made up of 2-inch-deep sections, enabling the viability of larvae to be determined according to depth in the soil; this showed that DD lost efficacy in the upper (and lower) levels owing to excessive ventilation, a loss only partially mitigated by using a seal of water or loosely-applied foil. At the dosage used, only 1.5 per cent of larvae survived treatment in the middle layers, but 68 per cent survived in the top 2 inches. This loss, which is probably comparable with what occurs in field injections, goes far to waste the efficacy of an inherently good nematicide. This year a similar experiment has been run to test the effect of treating the upper soil layer by drenching with a dispersion in water of *para*- and *meta*-cresols, solubilized with "Teepol" according to the technique of Mr. L. N. Staniland. Results show that this drench gives an excellent kill in the surface layer and, when combined with a DD injection, there is no interaction between the two at any depth-level, the whole box of soil being adequately disinfested. Such a double treatment might well be practicable in glasshouse soils, where *H. rostochiensis* continues to be a problem. (B. G. Peters.)

Field test

Near Wisbech a 6-year field-scale, DD experiment, in co-operation with Shell Chemicals Ltd. and the West Norfolk Farmers' Manure Co., has continued. DD is being injected in some plots once only at different stages of a five-course rotation free from potatoes, and in other plots injected in the first 2, 3, 4 and 5 years. The behaviour of the *H. rostochiensis* population under these treatments is being followed. This autumn the usual soil samples have been taken (to test the results of the third-year injections) and the fourth-year injections have been given. Next autumn the final (fifth) injections will be given and, in 1956, an indicator crop of potatoes will be grown. As anticipated in the last Annual Report (p. 102), plots injected in 1952 only, for which a poor kill was

recorded from soil samples of spring 1953, nevertheless showed a good kill in the samples of autumn 1953. In view of the curious unreliability of spring samples from this silty soil, they have now been dropped. This long experiment is now beginning to yield interesting results. (B. G. Peters, C. C. Doncaster and J. J. Hesling.)