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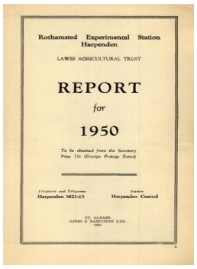

Nematology Department

T. Goodey

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NEMATOLOGY DEPARTMENT

By T. GOODEY

GENERAL

Dr. T. Goodey attended the 7th International Botanical Congress, Stockholm, 12-20 July, 1950, and delivered a paper in the Agronomy/Phytopathology Section on " Oats and varietal susceptibility to stem eelworm infestation." In passing through Denmark on the way to Sweden a visit was paid to the State Plant Pathological Station at Lyngby to see the work in progress on plant-infesting nematodes under the care of Dr. Prosper Bovien. During the course of the Congress, contact was made with Dr. Olaf Ahlberg, of the Statens Växtskyddanstalt, who has done much work on the occurrence and distribution of the potato root eelworm, *Heterodera rostochiensis*, in Sweden. On the occasion of the Congress Excursion to Uppsala special attention was given to the work being done at the Sveriges Utsädesförening on the selection and testing of races of red clover for resistance to clover eelworm, *Ditylenchus dipsaci*. These studies are largely carried out by Mr. Sven Bingefors who worked for two months in our department last year.

Members of the staff of the department are frequently consulted by the National Agricultural Advisory Service and other workers for advice on matters connected with nematode infestations and much time has been given during the year to the identification of eelworms, the diagnosis of eelworm infestations and to help in the planning of field and plot experiments where nematodes are involved.

During the year three temporary workers from abroad each spent periods of about one month in the department learning our nematological methods. These were Dr. J. W. Seinhorst of the Institute for Phytopathological Research, Wageningen, Holland; Monsieur M. Ritter of the Station Centrale de Zoologie Agricole, Route de Saint-Cyr, Versailles, France, and Ir. R. H. Kips of the Rijkslandbouwhogeschool, Ghent, Belgium.

An exhibit illustrating stem eelworm infestation of onion, known as " bloat," was staged for the Chelsea Flower Show in May, 1950, in the preparation of this much valuable work was done by Mr. C. C. Doncaster and Mr. J. B. Goodey.

These two have also collaborated in the production of a general-purpose photographic apparatus which has been mainly constructed from second-hand equipment already in the department. The apparatus is used for lower-power photomicrography where a wide evenly illuminated field is required and it is also of use for obtaining pictures of known greater magnifications. An enlarger attachment for use with it is also under construction.

Research carried out by the members of the staff falls naturally, as in previous years, into two main sections: (1) problems connected with plant infestations by eelworms belonging to the families Tylenchidae and Aphelenchidae and soil nematodes generally (Dr. T. Goodey, Dr. Mary T. Franklin and Mr. J. B. Goodey), (2) problems connected with root-infesting nematodes belonging to the family Heteroderidae (Dr. B. G. Peters, Mr. D. W. Fenwick and Mr. C. C. Doncaster).

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Tylenchidae and Aphelenchidae

Tylenchidae

Dr. Goodey has continued his investigations on the stem eelworm, *Ditylenchus dipsaci*, particularly on the races infesting teasel, oats and red clover. Many inoculation experiments were carried out to test the range of hosts susceptible to attack from these and other races of the parasite. Particular attention was devoted to studies on the oat race of *D. dipsaci* with a view to finding Spring oat varieties resistant to attack, and some progress was made though difficulty was experienced in the influence of soil type and temperature on infestation and manifestation of symptoms.

In the course of this work it was found that the oat race of the parasite can attack and seriously injure rye. This finding from pot experiments confirms observations made by a National Agricultural Advisory Service Officer in North Wales where rye was badly affected in a field known to be infested with the oat race and indicates that the oat and rye races of the stem eelworm are most probably identical ; a fact which seems to be recognized in Germany and Holland. The oat race also infests vetches, and pot experiments carried out during the year have proved its ability seriously to injure the vetches *Vicia villosa* and *V. sativa*. A paper embodying these results is in course of publication.

Mr. J. B. Goodey reports that work has continued on the plant parasitic species of *Ditylenchus*. The potato-tuber eelworm, *D. destructor* has been found in the field causing light brown lesions, somewhat similar to those on Mint, on the rhizomes of a new host, *Stachys palustris*, which is a common weed of some Fenland potato fields. The experimental infestation of this plant has also been successfully carried out. Observations in the field have shown that the presence of *Mentha arvensis* in particular, is probably to be correlated with the continuing infestation of *D. destructor*. These observations have been further supported by the failure to maintain an infestation on a plot of bulbous Iris, which has no Mint growing on it. The attempt is being made again with the addition of a cover of *Mentha arvensis*.

In 1895 and 1939, the Hop plant, *Humulus lupulus*, was recorded as a host of *D. dipsaci*, a peculiarity of both reports being that the infestations were confined to the roots. Re-examination of the 1939 material which was in this department showed that the eelworms had all the characteristics of *D. destructor*. An experimental transfer was successfully made from potato to pieces of hop roots with the setting up of typical root lesions. Later, when diseased hop sets from the site of the 1939 material, near Sittingbourne, Kent, were obtained, transfers to mint, bulbous iris and potato were successful as well as further transfers from mint to hop.

Lilac, *Syringa vulgaris*, has also been recorded as having its roots attacked by *D. dipsaci*. Again, it has now been shown that *D. destructor* is the eelworm responsible and transfer has been effected from potato to lilac roots and back again to patato.

Experimental infestation of *Begonia tuberhybrida*, *Tigridia pavonia* and *Gladiolus hybridus* by *D. destructor* and the Narcissus race of *D. dipsaci* have shown that both eelworms can attack these plants. On *Begonia*, *D. dipsaci* caused lesions and ultimately shrivelling of the leaves whilst *D. destructor* had no apparent effec-

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on the growing plant. Small brownish blotches were produced on the leaves and leaf bases of *Tigridia* by both parasites but those caused by *D. destructor* were confined to the underground parts of the plant. *D. dipsaci* caused considerable malformation of *Gladiolus* leaves and eelworms were plentiful in them; eelworms were also found in some of the seed pods. *D. destructor*, on the other hand, caused no apparent damage but could live and reproduce in the leaf bases so that *Gladiolus* might be termed a harbourer of *D. destructor*. A paper on these investigations is being prepared. During the work on *D. destructor* it became apparent that the host plant was influencing the size of eelworms comprising the parasitic population. Numerous individuals from different populations were measured and it was found, for instance, that mean lengths of males or females of different populations differed significantly from one another. This work will shortly be written up.

Observations on the attack of *D. dipsaci* on strawberry have continued and it has been shown experimentally that populations from teasel, oats, onion, red clover, narcissus and rye will all infest most of the popular varieties of strawberry in cultivation to-day. Investigations into the ways in which infestation is maintained have been carried out, and it is suggested that the discontinuity of infestation seen in some plants can be related to the way in which infestation originates. A paper embodying these findings has been prepared.

Further observations have been made on a Hyphomycete referred to the genus *Cephalosporium* acting as a hyper-parasite of *D. dipsaci* infesting *Calceolaria*. A paper on these findings has been accepted for publication.

A new species of *Rotylenchus* attacking and aiding in the destruction of the roots of a species of hothouse *Hippeastrum* (Amaryllis) has been discovered and will be described shortly. The eelworm appears to be a parthenogenetic species since only females were found and no evidence of the presence of males was apparent. An interesting feature of the infestation was the occurrence of a secondary piliferous layer on the *Hippeastrum* roots, a condition not previously reported, as far as can be ascertained. A specimen of this eelworm was found attacked by a nematode-catching fungus of the genus *Harposporium*.

Investigations on the relationships of *Hoplolaimus uniformis* and its effects on the young seedlings of Sitka spruce, *Picea sitchensis*, are still continuing. Several eelworms attacked by fungi have been found. In one case attack was by a species of *Arthrobotrys* and in other cases by at least two different fungi which have not so far been identified; attempts to culture them on artificial media have been unsuccessful. Another nematode, an as yet unidentified species of *Trichodorus*, has also been found associated with the Sitka spruce seedling roots.

Aphelenchidae

Dr. Mary T. Franklin reports that work has been continued on the three species of *Aphelenchoides* well known as parasites of cultivated plants, namely *Aph. fragariae*, *Aph. ritzema-bosi* and *Aph. ribes*. A fourth species has been studied which, during the past wet season, has apparently been responsible for damage to

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Caucasian scabious on at least three nurseries. This is provisionally identified as *Aph. parietinus*, but it differs morphologically in some respects from that species as originally described by Bastian in 1865.

Two new host plants of *Aph. fragariae*, namely mint (*Mentha spicata*) and scabious (*Scabiosa caucasica*), have been added to the collection of host plants of this species on the museum plot. Clean bulbs of *Lilium regale* and *L. henryi*, planted amongst the infested violets on this plot, became infested with the same nematode.

A number of infection tests with *Aph. ritzema-bosi* and *Aph. ribes* on various hosts has strengthened the evidence for the identity of these two nematode species. A new method of inoculation, used on chrysanthemums, has been developed and gives positive results in 7-14 days. Examinations are being made of weeds associated with both chrysanthemums and black currants infested with eelworms to find out to what extent the weeds can harbour the parasite in the absence of the cultivated hosts.

Throughout the year infested blackcurrant bushes have been examined and invasion of the next year's buds has been found to take place at a very early stage. Adult *Aph. ribes* have been found capable in the laboratory of travelling at least 11 inches up a damp cotton wick down which there is a slow trickle of water. Larvae will travel, or are carried, down a similar wick. Migration over infested bushes in damp weather thus offers no difficulties. The warm-water treatments of unrooted blackcurrant cuttings started last year had no apparent detrimental effects on the cuttings, which produced satisfactory plants in the greenhouse. The buds, however, were stimulated to develop early, which might prove dangerous to cuttings grown out-of-doors where they are not protected from frost. More warm-water treatments were therefore carried out this year in November and the cuttings have been planted outside. Eight different varieties of blackcurrant have been treated at 46°C. for 25 minutes, 48°C. for 15 minutes, and 50°C. for 10 minutes.

A visit was paid to one of the nurseries where Scabious has been damaged by eelworm and material brought back for study of the parasites. These did not entirely agree with the original description of *Aph. parietinus* given by Bastian in 1865 of nematodes which he found in the lichen *Parmelia* (now *Xanthoria*) *parietina* growing on walls at Broadmoor, Berks. *Aph. parietinus*, also, has not generally been considered to be parasitic. In order to get a clearer idea of this species specimens of *Xanthoria parietina* were brought from Broadmoor and the nematodes in them are being examined.

Heteroderidae

Dr. Peters reports as follows :

Concerning population studies on *Heterodera rostochiensis*, a long-term experiment on the effect of edaphic factors on the growth of eelworm populations (and the effect of the latter on the potato plant) is in its second year, in 8-litre, glazed, cylindrical pots, in which a heavy clay is factorially modified by the addition of sand, peat, compost and artificials (the last two renewed annually). Starting with a light infestation of the eelworm, the first season showed a 20-fold increase in cysts per gm. of dry soil, and a 35-fold

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increase in eggs per gm., with significant effects from sand (negative) and peat (positive). The potatoes, on the other hand, responded significantly to compost and artificials. This experiment is expected to throw light on soil conditions favourable and unfavourable to the eelworm, and on the diversion of plant foods from tuber production to eelworm production.

The movements of potato root eelworm larvae through soil are being investigated, the vertical and horizontal components separately. First results show that movements are probably limited to a few inches in any direction, in one season, and that the larvae move upwards (against top watering) more readily than downwards (against bottom watering). Two factors limiting movement, pore space and water content of soil are being investigated in greater detail before proceeding with further migration problems.

Counts of potato root eelworm larvae hatching from cysts, recovered from soils used in the 1949 series of pot tests of nematicides, are of some interest. Two chlorophenol compounds, D-D mixture and ethylene dibromide had been injected into duplicate 8-litre pots of soil at 0, 1, 4 and 16 ml. per pot. Larvae per gramme of soil were estimated 7 weeks after injection (Y samples) and again after growing a potato in each pot (Z samples). The Y sample showed no effect from the cholorophenols, the vapour pressure of which is presumably too low for use as fumigants ; $L.D._{50}$ for D-D was about 0·4 ml. and for ethylene dibromide about 2·3 ml. per pot. A comparison of Z with Y samples showed a multiplication factor of 5·4 for larvae in the 8 control pots. The factor was reduced for D-D and ethylene dibromide at the 4 ml. dosage, negligibly small at 16 ml., but *increased* at the 1 ml. dosage : 8·0 for ethylene dibromide and 17·6 for D-D. This was correlated with an increased yield of tubers at this dosage.

Pot experiments have continued in 1950, using D-D mixture, ethylene dibromide, methallyl, chloride, dichloroethyl ether, chlorophenols and cresols (Sterizal). The D-D series covers the comparison of the same dosage used neat and emulsified with Triton N.100. Here also, effects on subsequently grown potatoes are being observed ; D-D at high rates and ethylene dibromide are especially phytocidal. Tests of the recent bromochloropropylene mixture will be carried out next season.

Mr. Fenwick has again co-operated in the joint experiment with the West Farmers' Co-operative and Shell Chemicals Ltd., on the effects of annually repeated injections of D-D on potato root eelworm, at Moulton and Prickwillow. This experiment has now terminated. Eelworm counts from the final samples are not yet available, but the final yields show a significant improvement from D-D at 800 lb. and 400 lb. per acre at Moulton. At Prickwillow the improvement was significant statistically but not economically.

Work on the use of the vinegar eelworm as a test animal in screening tests of nematicides has continued. Analysis of counts of some 60,000 worms has shown that too frequently the variance between parallel counts is higher than would be expected on Poisson theory, and the reason for this has been found. Some 20 experiments on methods of culturing are being summarized for publication. In connection with the sorption by the worms of nematicidal substances, a method has been found for estimating the

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surface, volume and mass of a single worm, and (by correlating these parameters with worm-length) of a culture of worms.

Analysis of Mr. Fenwick's larval counts of the 1948 soil samples from Barnfield (*Heterodera schachtii*) shows the following. The general mean, at 4·88 larvae per gm. of soil, is lower (but not significantly so) than that of the 1946 samples; thus there is no evidence that the population is increasing. The infestation has now fairly well covered the field, but is far from random. Counts on the " no manures " lots and " no nitrogen " series are significantly lower than all others. Counts on the " nitrate of soda " series are significantly higher than on the " sulphate of ammonia " series, and those on the " superphosphate only " plots lower than on the plots with complete minerals, P and K, and P with $MgSO_4$ and NaCl. There is again a significant correlation (r = ·6155) between larval count and the mean yield (1904-1940). Using the yields to forecast the counts, the treatment effects are similar to those just mentioned; in addition, the counts from the dunged plots are slightly below expectation.

Assistance has been given to Mr. Doncaster in carrying out tests with Pestox III against *Heterodera marioni* and *Heterodera rostochiensis*; these are still in progress.

Mr. D. W. Fenwick reports that the use of hypochlorite as a hatching agent for distinguishing between living and dead larvae of *Heterodera rostochiensis* has proved to be valueless. There appears to be little hope at present of using it, or any other short method, in place of the root diffusate technique, and further research into the method has been abandoned.

A new technique has consequently been evolved for the conduct of a shortened form of hatching test. Use is made of the fact that that cumulative hatching curve plotted against log-time is a sigmoid and is therefore symmetrical about its own point of inflection. The method has been written up and accepted for publication.

The effect of physical conditions on larval emergence has been investigated in detail and a record of this work has also been accepted for publication. Presoaking of cysts for about 12 days prior to immersion in diffusate has the effect of speeding up hatching very considerably but little, if any, effect is discernible on the total number of larvae which ultimately emerge. Temperatures over 30°C. inhibit hatching but below this temperature variations between 15 and 25°C. are without effect on total emergence, although rise of temperature is accompanied by an increase in hatching rate. Volume of diffusate per cyst is without apparent effect on either total hatch or rate of hatching. pH fluctuations between pH 4·0 and pH 8·0 are also without effect. The effect of dilution of diffusate is interesting in that when hatch, expressed as total larvae emerging, is plotted against dilution, expressed logarithmically, a linear relationship is obtained and it is reasonable to draw the tentative conclusion that the number of larvae emerging from a given sample of a single stock of cysts is directly proportional to the logarithm of the concentration of the diffusate. Use is being made of this linear relationship, for the biossay of diffusate.

The promising results obtained by using purely biological methods of biossay are in marked contrast to the disappointing

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results obtained using anhydrotetronic acid. This chemical appears to be markedly inconstant from sample to sample, moreover the activity of any given sample is variable and no further investigations into its activity are contemplated.

Preliminary experiments have been conducted on the breakdown of root diffusate in soil. It has been found that after four days following on the application of a single dose of potato root diffusate, 90 per cent of it had undergone breakdown. Moreover, examination of cysts recovered from the soil showed that larval emergence was restricted to the four days prior to this breakdown, and amounted to approximately 20 per cent. Further experiments are in progress to investigate the factors influencing this breakdown.

The hatching effect of root diffusates produced by *Solanum nigrum* is under investigation. The diffusate has been shown to be active in inducing larval emergence and it is hoped to investigate the effect of growing this plant as a trap crop.

Experiments have been conducted on the effect of temperature on the development of the potato root eelworm. It has been found that a rise in temperature above 21°C. is unfavourable to the continued multiplication of the parasite. At 32°C. development is almost inhibited, and penetration of the larvae into the roots is checked, as is further development within the roots. The latter effect is apparently the more important. These experiments form the subject of a paper accepted for publication.

A series of experiments is in progress on fluctuations in eelworm populations during a growing season. This problem has been approached from two points of view, (a) the estimation of gross changes occurring in the whole population ; (b) the rate of penetration of the larvae into the root. Preliminary experiments conducted on the gross changes in population indicate that the number of cysts in an infected soil remains constant for the first four or five weeks of the plant's growth, then rises steadily as the new cysts are formed. The fluctuations in the larval population seem to depend on the size of pot used. In 6 in. pots the number of larvae per cyst falls to approximately 20 per cent of their original value in the first few weeks, thereafter rising rapidly in response to the formation of new cysts. In larger pots this effect is much less marked, the initial fall being in the neighbourhood of 10-20 per cent and the subsequent rise in larval content following on the formation of new cysts is less marked. This effect seems to indicate that root diffusate produced by the plant is only very local in its effect, and that in the larger pots used the majority of the cysts present never come into contact with the root diffusate. If this indication is correct, then it would appear unlikely that trap-cropping with potatoes would be of very much use in reducing the level of infestation present in a plot of land.

The rate of penetration of the larvae into roots has been studied by growing potatoes in infested soil and then transplanting at fixed times to clean soil : the number of cysts produced at the end of the season in this soil should then be a measure of the number of larvae which originally entered the root up to the time of transplanting. A surprising feature of these experiments is the large amount of penetration which occurs in the few days between planting a chitted tuber and its emergence through the soil. Many plants when trans-

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planted at the time of breakthrough yielded over 3,000 cysts when later examined. It is interesting to record also that the maximum yield of new cysts occurred when potatoes were transplanted at breakthrough; plants which were transplanted later and which should presumably contain more larvae were found to yield fewer cysts.

In conjunction with Miss E. Reid, a new technique for measuring infestation on a growing plant by estimating the density of white cysts produced has been evolved. The method is capable of estimating this density to a known degree of accuracy, and results can be expressed as number of white cysts per gramme of root. The method has been written up and submitted in the form of a letter to *Nature*.

A new type of McMaster slide has been evolved which accommodates a 1cc. sample with greater security than does the original type. It is possible to mount up to six counting chambers on a single unit. The design of the slide and its method of preparation have been described and submitted for publication.

Co-operation with Dr. H. C. Gough, of the Cambridge National Agricultural Advisory Service, on long-term changes in eelworm population during different crop rotations on different soils is continuing.

Experiments in conjunction with Dr. B. G. Peters on the effect of repeated annual injections with D-D have now been completed and the final results are being analyzed. These experiments were rendered possible by the co-operation of the West Norfolk Farmers' Co-operative, and it is now intended to carry out a further series of trials with the object of investigating the effect of introducing single and repeated D-D injections into different phases of a four course rotation.

Mr. C. C. Doncaster reports that investigations have been begun on the morphology of tomato and potato roots which have been parasitized by *Heteridera rostochiensis* and by *Heterodera marioni*. Tomatoes and potatoes were each infested with the Root-knot Nematode and with the Potato-root Nematode and samples of infested plants were removed and fixed at frequent intervals until advanced effects of parasitism has developed.

A detailed study of whole and sectioned material is being carried out in order to observe the different histological and morphological reactions of the two plants to each parasite.

In collaboration with Dr. B. G. Peters investigations on the use of systemic insecticides as nematicides have been started. Pestox III (bis(bis dimethylamino phosphonous)anhydride) has been tested on tomatoes, brassicas and nasturtiums to find a sub-phytotoxic dose which may be employed in tests against *H. marioni* and *H. rostochiensis*. These tests are now in operation. Preliminary *in vitro* tests on the effect of Pestox III on the hatching of cysts of *H. rostochiensis* and of egg-masses of *H. marioni* indicate that the substance merely retards hatching and that eggs and cysts are not killed even by 24 hours immersion in a 2 per cent solution of Pestox III.

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