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Nematology Department

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NEMATOTOLOGY DEPARTMENT

By T. GOODEY

GENERAL

Nowhere at the present time is it possible for the worker in applied biology to obtain instruction in the handling of plant and soil nematodes. The need for a course in nematology and nematological methods and techniques has been urgent for several years. At the request of the Plant Pests and Diseases Committee of the Association of Applied Biologists the staff of this department agreed to be responsible for conducting a course during the past summer. By permission of Sir Frank Engledow it was held at the School of Agriculture, Cambridge, from July 7-16, 1949. Mr. F. G. W. Jones, Entomologist in the School of Agriculture, made the necessary local arrangements and secured accommodation at Jesus College for the 30 students who attended. Selection of the students (20 of whom were from the National Agricultural Advisory Service) was carried out by the staff of the Ministry of Agriculture Plant Pathology Laboratory, Harpenden and Mr. E. Dunn, Assistant Entomologist of that laboratory acted as secretary/treasurer. A strenuous 10 days of intensive lectures and practical work was spent by those conducting and taking the course, the first of its kind to be arranged in this country. The many letters of appreciation and thanks received from those who took the course show that it was successful.

Dr. T. Goodey paid a short visit to Holland from April 26 to May 6, 1949 and spent most of the time at Wageningen in Professor H. M. Quanjer's Laboratory for Mycology and Potato Investigations. Here Mr. J. W. Seinhorst has been working for some years on problems connected with stem eelworm disease ("stock") of rye and has devised new methods for extracting the infective larvæ of the parasite, in large numbers, from dried infested rye plants and for cleaning and concentrating them. Details of these methods have not yet been published but Mr. Seinhorst kindly supplied particulars for the construction of the apparatus and gave details of the methods he has successfully developed. Similar apparatus has now been erected in this department and is in full working order. It has provided us not only with large numbers of infective larvæ of several races of the stem eelworm from teasel, oats, red clover, narcissus, and broad beans, which will be used in due course for inoculation experiments, but also with *Aphelenchoides* species from strawberry buds, violet plants and chrysanthemum leaves. On leaving Wageningen a short stay was made at the Bulb Research Station, Lisse where a profitable opportunity was taken for discussions on nematode infestations of bulbs with Professor E. van Slogteren and Dr. M. P. de Bruyn Ouboter.

Dr. Peters attended an Arable Conference at St. Helens in January and gave a talk on potato root eelworm, illustrated by the C.O.I. documentary film.

During the year two temporary workers have spent periods in the department learning nematological methods, namely Mr. R. S. Pitcher of East Malling Research Station and Mr. Sven Bingefors of the Swedish Seeds Association, Uppsala.

On the area adjacent to the laboratory our living museum of plant parasitic species and races of eelworms is now largely established and cinder paths have been made between several small plots. The two greenhouses have been fitted with an electric heating system, concrete paths have been laid, staging erected in one of them and ventilators fixed in the side walls.

Research carried out by the members of the staff falls naturally into two main sections: (1) problems connected with plant infestations by eelworms belonging to the families Tylenchidæ and Aphelenchidæ 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 Heteroderidæ, (Dr. B. G. Peters and Mr. D. W. Fenwick).

TYLENCHIDÆ AND APHELENCHIDÆ

Tylenchidæ

Dr. Goodey has carried out investigations on stem eelworm (*Ditylenchus dipsaci*) attacks on the fuller's thistle or teasel (*Dipsacus fullonum* L.) with a view to determining the route taken by the parasite in reaching the flower head and thus becoming dispersed in and on the seed in a dry quiescent but viable state.

He has written a booklet entitled "Laboratory Methods for Work with Plant and Soil Nematodes" which has been published by the Ministry of Agriculture and Fisheries as Technical Bulletin No. 2.

He has also revised much of the text and prepared further illustrations for his new book, "Soil and Freshwater Nematodes" which is now passing through the press and should be issued about mid 1950.

Mr. J. B. Goodey reports that work has been continued on the plant-parasitic species of *Ditylenchus*. The host-list of the Stem eelworm, *D. dipsaci*, contains the names of some 350 plants and a beginning has now been made in trying to find out which biologic race is responsible in each particular infestation. *D. destructor* the potato tuber-rot nematode has been shown to be the cause of eelworm disease of bulbous iris, hitherto ascribed to *D. dipsaci*, and further experiments are now in progress which show that *D. destructor* attacks several other hosts as well as the few already known. Some new biological aspects of the life cycle of *D. destructor* have been discovered. A paper embodying the results of these investigations has been prepared.

Reports of attacks by *D. dipsaci* on strawberry have been numerous recently and work has been carried out on the subject. It has been shown that the nematode will transfer from strawberry to oats, but it is suspected that the problem is more complex and that races of *D. dipsaci* other than the oat race may be involved.

During work on the fine details of the anatomy of *D. destructor*, a new structure was discovered on the ventral surface of the eelworm. Its function is not known but it has been found on several other members of the super-family Tylenchoidea. A short paper describing its structure and distribution has been written in which the term 'ventral gland' is proposed as its name.

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An interesting case of *Calceolaria integrifolia* attacked by *D. dipsaci* was received during the summer in which the eelworms were themselves parasitized by a nematode-attacking fungus. Its mode of attack and development on *D. dipsaci* has been observed and the fungus isolated in pure culture. The fungus has been identified provisionally as a species of *Acrostalagmus*, possibly an undescribed one. Work on this is being continued.

By courtesy of the Chemistry Department many specimens of Conifers, particularly Sitka spruce, have been examined for the presence of certain nematodes which have been reported attacking roots of Conifers in U.S.A. A species of *Hoplolaimus*, identified as *H. uniformis* Thorne, 1949 the first record of this eelworm in Great Britain, has been found in considerable numbers. A few specimens had their heads buried in the root tissue. The matter is being pursued further, but it is considered doubtful whether the eelworms cause any but trivial damage.

Work designed to find a fixative causing little or no shrinkage of eelworms has been continued. The result, a new formal-acetic mixture, has been incorporated into a joint paper with Dr. Mary T. Franklin in which the use of dilute Cotton Blue in Lactophenol as a medium for the rapid mounting of plant parasitic nematodes, has been described. Further work on mounting media is proceeding.

Aphelenchidæ

Dr. Mary T. Franklin reports that further investigations on the two species of *Aphelenchoides* found in strawberry plants have shown them to be *A. fragariæ* and *A. ritzema-bosi*. Reference to Ritzema-Bos's original descriptions of *A. fragariæ* (1891) and *A. olesistus* (1893) have failed to show sufficient difference between them to justify the separation of the second species, moreover the successful inoculation of a fern with *A. fragariæ* from strawberry, with the production of symptoms typical of infection with *A. olesistus*, has shown that the same nematode can parasitize both plants, therefore *A. olesistus* is regarded as a synonym of *A. fragariæ*. The other species found in strawberry resembles the chrysanthemum eelworm in morphological characters and is therefore considered to be *A. ritzema-bosi*. Cross-inoculations of these worms have been made from strawberry to chrysanthemum and *vice versa*, but they have not so far yielded results. The determination of the identity of these two species in strawberry was helped by the exchange of specimens with Dr. Merlin Allen of California.

Work has continued on the blackcurrant eelworm: blackcurrant bushes infested with *Aphelenchoides ribes* have been established on the plot and a beginning has been made in studying the biology of the parasite with a view to finding out the periods of maximum activity and of migration. It has been established that the nematodes may be found wandering on the branches in wet weather in summer and also that they will move upward against a downward trickle of water. Six varieties of blackcurrant and also one each of red currant and gooseberry have been planted next to the infested blackcurrant bushes to determine their susceptibility to infection with *A. ribes*. The past hot dry summer hindered the establishment of the bushes, but it is hoped that they will make good growth during the coming year.

Small-scale warm-water treatments having established the lethal temperatures for *A. ribes*, warm-water treatments of un-rooted blackcurrant cuttings have been started with a view to finding a suitable treatment which could be used in the starting of a clean stock. The treatments used (at the beginning of December), namely, 46°C. for 25 min., 47°C. for 20 min., 48°C. for 15 min., and 49°C. for 10 min., have caused sprouting of the buds, but it is too early yet to say whether roots will be produced normally.

Cross-inoculation experiments have shown that *A. ribes* from blackcurrant can become established on strawberry plants and *A. ritzema-bosi* from strawberries on blackcurrant seedlings with the production of lesions typical of eelworm damage. Further studies of morphology and host range are being made to determine whether *A. ribes* should be regarded as a synonym of *A. ritzema-bosi*.

Violet plants infested with what must now be called *A. fragariae* have been established on one of the plots. It has been found that the worms may be present in damaged flower buds and that the dried seed may also be infested with eelworms which become active on soaking in water.

Cultures of species of *Aphelenchoides* on agar together with fungus have been continued. *A. fragariae* from fern has become established and also *A. parietinus*, a species commonly found in decaying plant material, which has been thought to be parasitic in some cases.

In collaboration with J. B. Goodey a new technique for processing, staining and making permanent mounts of the smaller nematodes has been developed. By transferring nematodes from fixative to lactophenol containing cotton blue at a temperature of 65-75°C. they can be cleared, stained and mounted in lactophenol in a few minutes instead of requiring several weeks in slowly concentrating glycerine. The new method is particularly useful with *Aphelenchoides* which are very easily distorted when processed by the older method.

HETERODERIDÆ

Dr. B. G. Peters reports as follows: The distribution of potato root eelworm in England suggests that peaty soils, sands, and silts are favourable and heavy clay soils unfavourable to the parasite. An investigation of these edaphic factors has been started by adding a light infestation of the eelworm to an unpromising subsoil clay, factorially modified by the addition of sand, peat, compost and mixed artificials, in which potatoes were grown in 32 large glazed pots. In the first year plant responses were not significant in terms of height of haulms at 45 days (beyond a suggestive 2.8 cm. increase from compost), or of number of tubers harvested; but weight of tubers showed a significant response to compost, peat, and fertilizers in that order, with no evident interaction effects. Counts of eelworm cysts and larvæ are not yet completed. The experiment will be continued.

Nematocidal investigations have continued on a laboratory scale and also in pots of soil grown outdoors. In the outdoor experiment 40 8-litre glazed pots, filled with soil naturally infected with *H. rostochiensis*, were injected in pairs at rates of 0, 1, 4 and 16 ml. per pot of the following: (1 & 2) two chlorphenol products, (3)

ethylene dibromide 5 per cent. v/v. in solvent naphtha, (4 & 5) D-D mixture. Apart from one set of 8 D-D pots (required for other purposes), a potato was grown in each pot. The chlorphenol materials at the rates used gave rise to no plant responses of any kind. Both ethylene dibromide and D-D at the highest rate caused a marked reduction in the height of haulms after 21 days and the yield of tubers was significantly higher at 1 and 4 ml. than at 0 and 16 ml.

A start has been made on determining the rate and direction of migration of potato-root eelworm larvæ through soil, both vertically and horizontally. To measure vertical migration, potatoes were grown in wooden boxes made by superimposing six 2-inch-high sections, 6 in. square in plan. Eelworm cysts were added at the top of some boxes and at the bottom of others. Leaching effects were controlled by watering some from below and others from above. The soil from each 2-in. section was separately collected and awaits eelworm counts.

The use of vinegar eelworm as a test animal in the preliminary selection of possible nematocides has been investigated in some detail. These eelworms are readily cultured in vinegar, neat or diluted with water, or in solutions of sucrose or ethyl alcohol or both, at temperatures up to 37°C. Living worms show a marked negative geotaxis which is made use of in concentrating them from a culture into a clean medium.

With time and temperature fixed, they are exposed to different concentrations of a nematocide in aqueous solution or emulsion. They are counted by sampling in a 1-ml. counting chamber on the haemocytometer principle. After exposure the number dead is also estimated by sampling, but many nematocides cause lysis of the worms to an extent depending on the concentration. At low kills it is impracticable to count living worms and accordingly, after estimating dead worms, sufficient iodine is added to kill the living and a further estimate is made of the total. The difference between "dead" and "total" estimates the living, and the difference between "living" and "exposed" estimates the kill. At high kills the living worms are sluggish and can be counted.

In spite of the superimposed sampling errors involved in a single estimation it is possible to secure reproducible results giving a tolerable rectilinear relation between probit and log concentration. Thus, a sample of D-D mixture emulsified with Triton N.100 has given at 32°C. a 20-hour L.D.₅₀ of about 24 p.p.m. by volume.

This technique has been applied in an investigation, suggested by Dr. Thornton, of the nematocidal power of D-D repeatedly injected into soil at fortnightly intervals. The soil was subsequently leached with water, to which leachings vinegar eelworms were exposed. Preliminary trials indicate that, instead of a progressive accumulation of toxic material in the soil, leachings from successive injections give progressively lower kills.

Continuing the joint experiment (with the West Norfolk Farmers' Co-operative and Shell) on the effects of annual injections of D-D on a field scale, at Moulton and Prickwillow, the second-year yields of potatoes reveal the following results. At Moulton there was no significant difference between plots injected in 1947 only and

uninjected controls, but plots injected in 1948 gave a higher yield. At Prickwillow, on the other hand, there was no difference between 1948-injected plots and the controls, whereas 1947-injected plots gave a significantly lower yield. At both sites the response to D-D this year was very much less than it was last year.

Regarding other species of *Heterodera*, soil samples taken in the autumn of 1948 from all the Barnfield plots have been processed, but the counts of *H. schachtii* cysts per g. and of eggs and larvæ per g. of soil have not yet been analysed.

Mr. D. W. Fenwick reports that six principal lines of research on *Heterodera rostochiensis* have been followed during the last year.

Viability determinations

The investigations into the hypochlorite technique mentioned in the previous year's report are almost complete. The method of arriving at a reliable total count of eggs plus larvæ per cyst has been written and accepted for publication. A further refinement resulting in a saving of at least 60 per cent. in time has been introduced by Miss E. Reid and is published as an appendix to the paper.

Larval emergence

The problem of larval emergence has received considerable attention and has been investigated from the point of view of "in vitro" laboratory experiments as well as in pots of soil. A preliminary stage to "in vitro" experiments has been the investigation of variability and this forms the subject matter of a paper at present in the press. As a result of this work it is now possible to set up experiments and obtain results of a predictable accuracy and thus ensure that effects of any given magnitude can be detected with a reasonable degree of certainty.

Investigations into the form of the hatching curve are now complete and have been recorded and are accepted for publication. The sigmoid nature of the curve has been established as a sufficiently close approximation for probit analysis to be practicable. Using this, it is possible to define any hatching curve by means of three constants (1) the total number of larvæ liberated, (2) the time at which 50 per cent. of the larvæ have emerged, corresponding to a probit value of 5, (3) the standard deviation of the hatching time, corresponding to the slope of the probit line.

Experiments have been conducted with anhydrotetrone acid, which is very active in inducing larval emergence. Its effect under different conditions is being investigated with the object of determining an optimum. One complication regarding its use is that two different samples of the chemical appear to have behaved differently. The first manifested its maximum activity at 1:2000, at which it was only slightly less active than was the root diffusate. The second sample, however, appeared to be most active at 1:1000 and its maximum activity appeared to be considerably less than that of the previous sample. The first sample in solution in distilled water gave a pH of between 5 and 6 but the second in solution gave a pH of between 2 and 3, and this discrepancy is now being investigated. Great importance is attached to experiments on this chemical since its action appears to be very similar to that of root diffusate,

and it is hoped to utilize it finally as a standard against which samples of diffusate can be tested.

A considerable amount of preliminary work has been carried out on the problem of larval emergence in soil as well as on the degree of persistence of root diffusate in soil. In the former case a large scale preliminary experiment has been conducted in which potato plants of three varieties were grown in a 3:1 mixture of loam and sand. Leachings from these plants were drained into three types of naturally infested soil. It was found that leachings from soil alone resulted in a 50 per cent. hatch, whilst in the case of potato leachings about 86 per cent. of the larvæ emerged. Significant differences were found to exist between soils and there was evidence of differences in degree of emergence due to leachings from different host varieties. A paper has been written on this subject and has been accepted for publication. Further experiments are in progress on the persistence of root diffusates in soil.

Population fluctuations

Parallel with experiments on larval emergence a series of experiments has been conducted to investigate fluctuations in eelworm populations during the growing season. This has been approached from two angles (1) estimation of the gross changes occurring in the whole population and (2) the rate of penetration of larvæ into new hosts.

Factors influencing larval penetration

Repeated failure to obtain infections by inoculating larvæ into clean soil containing growing potato plants resulted in making a simple experiment on the effect of different conditions on the penetration of eelworm larvæ. It was found impossible to infect plants grown under greenhouse conditions and very few cysts developed on plants grown in full sun. The highest yield of cysts was obtained from plants grown in the shade and in gravel plunges. It is believed that this result is a temperature effect, the high temperatures attained in a greenhouse being detrimental to larval penetration. Further experiments on this are contemplated in the coming season.

D-D experiments

A number of small scale experiments have been carried out on the soil fumigant D-D. The results of experiments mentioned in last year's report on the apparent unreliability of the "buried bag" technique are now complete and a letter has been written to "Nature" on this matter.

"In vitro" experiments are in progress on the nematocidal effects of D-D under different physical and chemical conditions. The effect of humidity has been investigated; and it has been found that a high humidity is essential for maximum effect.

An experiment has been carried out on the nematocidal properties of four different samples of D-D. It has been found that the samples exhibited significant differences in nematocidal properties, these differences being most marked at high mortalities. It was found that 7 times as much of one sample as of another was needed to secure a 99 per cent. kill. The results of these experiments have been recorded and accepted for publication.

Fungi infecting Heterodera cysts

In conjunction with Mr. C. L. Duddington of Regent Street Polytechnic, an investigation is in progress on the identity and biology of fungi found infecting *Heterodera* cysts. A number of these have been identified and there can be little doubt that at least one is present as a natural infection within the cyst. Attempts are being made to find a fungistatic agent which will prevent the development of these fungi in hatching experiments. The possibility of using these fungi on a field scale as a method of biological control is being considered.

In conjunction with Dr. Mary T. Franklin the analysis of data on the larval lengths of different species of *Heterodera* is complete and has been accepted for publication.

Co-operation with Dr. H. C. Gough of Cambridge in long term investigations on eelworm population changes during crop rotations on different soils is also continuing.