

PROCEEDINGS OF THE S-19 WORKSHOP IN PHYTONEMATOLOGY, 1957



Prepared and published by the Technical Committee of the Southern Regional Nematology Project (S-19) from lectures given at an advanced phytonematology workshop held at the University of Tennessee, July 1-6, 1957.

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PREFACE

This workshop in phytonematology was held at the University of Tennessee during the period of July 1-6, 1957 as another one of the activities of the Southern Regional Nematology Project (S-19). The costs incurred by the discussion leaders, by the representatives from the member states of the regional project, and for the publication of these notes were borne by a grant from the Rockefeller Foundation. Acknowledgement and thanks are also extended to the U. S. Department of Agriculture for permitting participation of certain personnel as discussion leaders.

Although some of the talks were prepared in a written form by the speakers, to spare the discussion leaders the burden of preparing formal papers, the talks and discussions were tape recorded at the workshop. As time permitted, the recordings were transcribed and snapped into a more or less uniform pattern for printing. These drafts were submitted to the speakers for any corrections and clarifications they wanted to make. Then the papers were prepared into the final form as presented here. The editor is responsible for the illustrations which had to be redrawn to a suitable size from original copy or from notes. He is also responsible for some rewriting and editing of portions of the taped material which could not be written verbatim, for example, discussions based on illustrations or where there were difficulties of clearly recording all that was said. Editorial intervention was held to a minimum and it is believed that the objective of accurate reportage of the workshop has been maintained.

There was one regrettable casualty; the recording of Dr. Overgaard Nielsen's talk and the extensive discussion that followed was accidentally erased. Dr. Nielsen kindly wrote out the highlights of his presentation but, as the participants at the workshop will recall, a great deal of interesting material has been left out of this talk and discussion as it is printed here.

The organization and pagination will be evident in the table of contents. It is hoped that this arrangement will be as useful to the reader as it was in preparing this material a piece at a time for the printer.

Eldon J. Cairns
Editor

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PATHOGENICITY OF NEMATODES IN RELATION TO KOCH'S POSTULATES

W. B. Mountain

One aspect of research which has intrigued me for a number of years and which is basic to the scientific method is that of establishing the precise conclusions we are justified in deriving on the basis of our research. It is my belief that over the years in Plant Nematology there has been a tendency, perhaps unintentional, to try to define the role of a nematode in a plant disease with more precision than one should attempt to do with the data presented.

Also, speaking with the background of a Plant Pathologist, I am convinced that many nematode papers which have appeared in print have not been held in particularly high regard by Plant Pathologists simply because the writers used, in too haphazard a manner, plant pathological terms which, in that discipline, have precise connotations and should be used only when certain definite facts have been established. I might say here that great credit and respect are due to the earlier Plant Pathologists who developed with clarity and precision many of the fundamental concepts in plant pathology and particularly for adapting to plant pathology the highly developed and critical standards of general pathology which originated in the late nineteenth and early twentieth centuries.

I want to refer particularly to the concepts of pathogen and pathogenicity. It is my belief that these words are being used much too loosely in plant nematology, and I might say in other phases of plant pathology, as well. In connection with the loose usage of such technical words as pathogen and pathogenicity, the report of the Committee on Technical Words of the American Phytopathological Society issued at the 31'st Annual Meeting is just as meaningful today as it was sixteen years ago. "We feel that something should be done to aid in reducing loose use of technical words. Loose usage is not the same as different usage. An author may use words in a sense quite different from the usual, but, if his concepts are clearly explained and his meanings explicitly defined, consistent with each other and consistently applied by him, he cannot be accused of loose usage."

I wonder if we might be able to eliminate loose usage of the words pathogen and pathogenicity by carefully evaluating our concept of these words, by placing restrictions on their use and meaning, and, if necessary, by erecting new concepts in order that we might be able to define with precision the implications of our research.

I would like to refer to a typical example of loose usage of the word pathogenicity in an otherwise excellent research paper. In a scientific paper which appeared recently, the writer concluded that he had proven the pathogenicity of a particular nematode with reference to a particular crop. The nematode, which is an ectoparasite, was reared in soil upon

the roots of a certain plant. For the experiment, the author screened this soil and the nematodes, including some saprophytic forms, I assume, were allowed to settle to the bottom of a beaker for a short time. It was reasoned that the supernatant liquid could serve as a suitable control, since the bacteria and fungi of that soil would be present, whereas the residue in the beaker could serve as the inoculum, since it contained the nematode, as well as the bacteria and fungi.

Now, the author obtained stunting only when the residue was poured around the roots of the test plants growing in steamed soil, and he concluded that the pathogenicity of this nematode had been established. Although the worker has made a valuable contribution, and I am not in any way trying to discredit his work, I maintain he has not proven the pathogenicity of this nematode at all. It is true that he has proven some type of relationship between the host and this nematode, but, to prove pathogenicity, one must fulfill certain prescribed criteria. If we are going to use the concepts of the plant pathologist, we must use his rules in developing these concepts.

Let us look at the word pathogen—the word which first appeared in 1876 originated during the so-called golden age of bacteriology. The literal meaning and original definition of a pathogen is simply an organism which causes a disease in another organism. However, as our knowledge of disease and host-parasite relations in general has grown tremendously during the twentieth century, it is now realized that a diseased plant frequently is the result of an extremely complex reaction, or series of interactions, involving several organisms; and, consequently, the concept of a pathogen, particularly in the case of root-rot, must become much more specific.

Perhaps in the case of a root-rot we should define a pathogen as a parasitic organism which, through its activities in relation to the host, produces the full disease syndrome. This definition would exclude those organisms which, although of great importance in the etiology, cannot by themselves reproduce the full disease syndrome. Also the parasitic organisms which have somewhat secondary roles in the etiology of disease complexes must be excluded.

We should also consider the role of the relatively unspecialized saprophytic or weakly parasitic organisms which occur frequently in the infection court. Can we define the role of such organisms in the etiology of a root-rot? What I would like to discuss is how one can establish the precise host-parasite relations of a nematode involved in a root-rot disease.

I think the place to start is to return to the original concepts of pathogenicity to see of what use they may be to us. Robert Koch, in 1882, postulated his four laws of pathogenicity and gave to the bacteriologists a sound and logical procedure for etiological investigations of the bacterial diseases of man and animals. Koch's so-called rules of proof of pathogenicity, as rather loosely translated by Garrett, are as follows:

- (1) constant association of the organism with the disease
- (2) isolation of the organism in pure culture
- (3) reproduction of the disease by inoculation with the pure culture of the organism
- (4) re-isolation of the organism from the inoculated diseased host, and identification of it with the original inoculant.

It is apparent that there are at least two difficulties which make a literal application of these postulates to root-rot diseases unrealistic and perhaps unattainable. The first problem is that of obtaining the organism in pure culture. To my knowledge, plant parasitic nematodes have not yet been cultured on a chemically defined medium. Nematologists can share this problem with virologists and workers investigating the role of obligately parasitic fungi. It is perfectly obvious that if one cannot culture the organism, it is impossible to fulfill the second of Koch's Laws.

A second problem is that the literal application of Koch's postulates to plant pathological research is not always as simple or as logical as was first believed. Koch was a bacteriologist, and the environment with which he was concerned was the mammalian bloodstream, which normally is essentially axenic. The host-parasite relation which Koch defined as pathogenicity was relatively simple. It was merely the result of introducing a parasitic bacterium into a normally sterile system where complications resulting from the interaction of other microorganisms were normally absent.

Contrast with this the problem of defining the host-parasite relations in a root-rot disease where the activities of a primary parasite may be almost immediately obscured due to the colonization of the infection court by numbers of other microorganisms. A logical question is whether Koch's original concept of pathogenicity has any application in root-rot investigations. I believe that it has, but in a relatively restricted sense, as I shall try to develop a little later.

Returning to Koch's postulates, I should like you to notice how logically they were developed. Within the four postulates, one can discern three distinct stages or steps, and these are exactly the same three stages and occur in exactly the same order that a research project in plant pathology should follow. The stages are as follows:

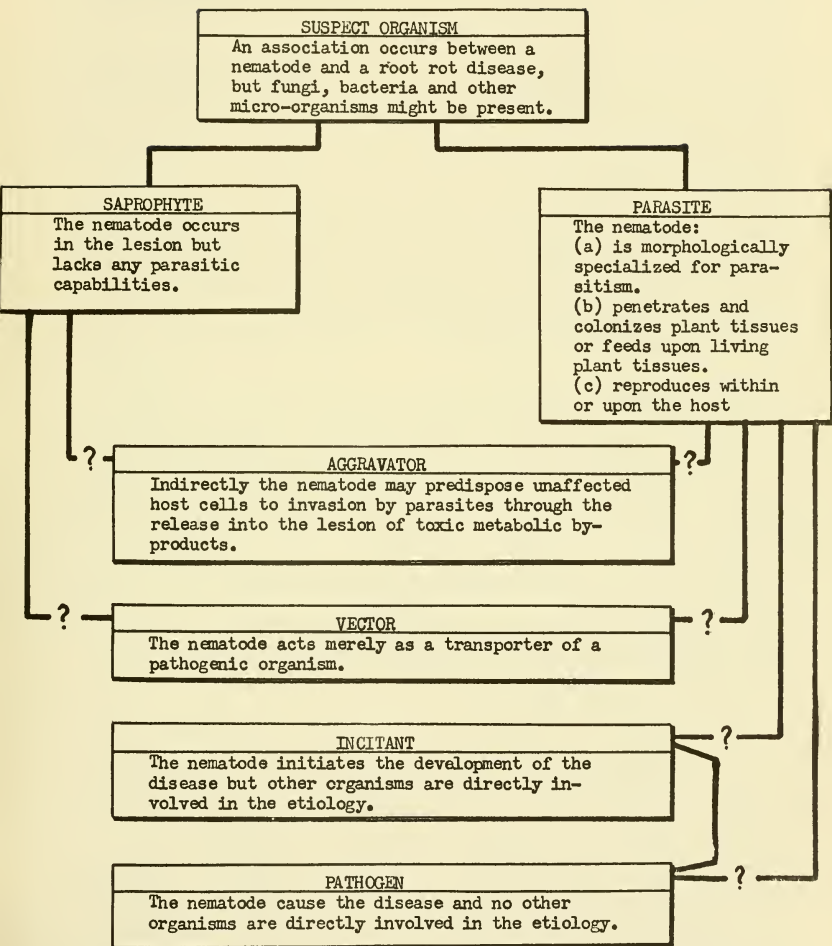
- (1) establishing the association of the organism to the disease
- (2) establishing the parasitic capabilities of the organism
- (3) establishing the host-parasite relations.

At this point we have separated parasitic capability from host-parasite relation. Such a separation is necessary because a plant parasite should never be considered of necessity a pathogen. For instance, a variety of tobacco tolerant to meadow nematodes may harbor within its roots many hundreds of the parasite without the development of any discernible host-

parasite relations - obviously the parasite is not a pathogen. On the other hand, if we substitute another, less tolerant, tobacco variety, brown root-rot develops, and the nematode is now acting as a pathogen. You see, parasitic capability is a more or less innate, absolute, characteristic of an organism, determined mainly by morphological characteristics and physiological requirements. In contrast, the host-parasite relations are quite relative, dependent upon the interaction of two organisms and subject to a full range of physiological and environmental factors. Parasite and pathogen are separate concepts and must always be considered in that way.

Within the framework of Koch's postulates and using the ideas and concepts of many workers besides myself, I would like to present for consideration a flow-sheet which I have prepared in an attempt to enable us, on the basis of our research data, to define with some precision the relationship between the host plant and the nematode. I am then going to take the liberty of illustrating some of the stages in this flow-sheet with some research data of my own.

HOST-PARASITE RELATIONSHIP



This represents a flow-sheet prepared with the idea of showing how we might develop the host-parasite relations by following Koch's original idea of moving from the association to the parasitic capabilities and finally to the host-parasite relations.

First then, a suspect organism is established on the basis of the association. To determine whether an organism is suspect is relatively simple. It is based mainly upon field studies; distribution of the organism in relation to distribution of the disease, the intensity of the disease in relation to the inoculum level, control of the disease by control of the suspect organism, and so on. Now this is a most important phase in any etiological study. It is at this stage that I believe we are justified in criticizing some of the other branches of plant pathology in addition to our own. There is a tendency to bring our studies of a disease into the laboratory at far too early a stage, to isolate any organism which might be present and to try to define the host-parasite relations. The results of this type of research often are meaningless. We should always be certain of the existence of a real and consistent association of the organism to the disease before attempting to define its role in the etiology.

The next step is to try to establish the parasitic capabilities of the suspect organism. We can refer therefore to the concepts of parasite and saprophyte. I admit that my basis of separating these is rather naive. We can perhaps define a parasite as I have done, but insofar as nematodes are concerned, we are on rather dangerous ground when we discuss saprophytes. Our concepts of saprophytism and our knowledge of nematode bionomics are too fragmentary at this stage, and many saprophytic nematodes are so-designated on practically no scientific evidence at all.

Assuming that we have established the suspect organism as a parasite, we should now make an attempt to define, with some precision, the host-parasite relations involved. As far as host-parasite relations are concerned, I have attempted to make the flow-sheet travel from completely unspecialized relations (involving even saprophytes theoretically) to the very highly developed host-parasite relationship of pathogenicity. For the first stage, we might postulate the concept of Aggravator.

Metabolic break-down products excreted by animals are said to be (by and large) phytotoxic. When one examines a root lesion, relatively large, so-called saprophytic nematodes frequently occur in the more advanced portion of the lesion which is characterized by cellular debris, fungi, bacteria, etc. Of course, these nematodes might be feeding on bacteria or fungi and are scarcely saprophytes, yet, insofar as the host-plant is concerned, they have no parasitic capabilities. Nevertheless, they are present in the lesion, feeding in some manner and excreting products which are very likely phytotoxic. The products are capable of diffusing across the lesion to its periphery and conceivably so affect uninvaded plant cells that they become predisposed to invasion by the parasite responsible for the disease. If this is true (and I have no evidence that it is) then these organisms do play a part in the host-parasite

relationship (regardless of whether the so-called primary parasite is a fungus, bacterium, virus, nematode, etc.). Some day the role of these aggravators will have to be investigated.

A slightly more precise relationship will be found in the role of a Vector. The definition of a vector in the flow-sheet is quite straightforward. Many workers do suspect that nematodes are acting as vectors in certain virus diseases, but such a relationship has yet to be proven. It is possible that saprophytes could act as vectors, but a highly specialized vector-virus relationship would almost certainly require a plant parasitic nematode.

As far as we are concerned, the more highly specialized relationships between a nematode-parasite and a host are more interesting and important at the present time. The definition of a pathogen on the flow-sheet is essentially Koch's concept of a pathogen, i.e., the parasitic activities of the organism are the direct and sole cause of the disease. On this basis, many of our nematode parasites have been proven to be pathogens, as for instance, with species of Ditylenchus, Meloidogyne, and a few others. We would certainly be wise to restrict the word pathogen to the meaning on the flow-sheet.

In many cases, especially with root-attacking nematodes, we will find that the host-parasite relations are not sufficiently precise to call them pathogens. In many instances, we will find that fungi, bacteria, and perhaps other nematodes have a necessary role in the etiology, and I think perhaps the concept of Incitant should be used here. If an axenic culture of the nematode cannot reproduce the full disease syndrome, then we should not speak of a pathogen. Perhaps in many cases, we will find that endoparasitic, root-attacking nematodes, although capable of acting as pathogens, invariably act as incitants, and the same organism could, therefore, act in either capacity.

To conclude, I would like to stress that the various host-parasite relationships which I have suggested by no means illustrated all of the possible relationships which might develop between a plant and a parasite. However, in the case of root-rots I am not certain that we shall ever be able to define such relationships with great precision especially, as they likely will over-lap.

You might ask of what value is all of this theory in the development of a nematode control program. This is a valid question, because it is likely that so long as there is any type of host-parasite relationship, there will be some growth response to the control of the nematode. This is why soil fumigation alone can never be used experimentally to determine the exact host-parasite relationship. However, in defense of this discussion, there are at least two important reasons why a nematologist should try to establish the host-parasite relations as precisely as possible.

First, it will lead to a much clearer understanding of the disease, especially of its epidemiology and potentiality. Also, it appears to me

that a sound breeding program must be founded upon a clear understanding of the role of the parasite.

Secondly, no one can deny that our obligation as scientists is to interpret our findings with precision and, especially in our publications, to ensure that we define exactly what we mean; and, most important of all, that our conclusions are rigidly supported by our data.

PRINCIPLES OF POPULATION MEASUREMENT

F. G. W. Jones

The remarks below apply primarily to cyst-forming nematodes, but the principles raised have a much wider application to many types of non-mobile or semi-mobile organisms found in soil.

Cyst-forming species of nematodes (Genus *Heterodera*) are important pests of many field crops in Europe, North America, and elsewhere. Quantitative estimation of soil nematodes dates from Cobb (1918); and the first attempts to estimate the cyst-forming species in Great Britain were those of Morgan (1925), during investigation of the potato-root eelworm in Lincolnshire. Soil population estimates of cyst-forming nematodes are now extensively used for the determination of presence or absence, in connexion with the so-called certificates of freedom from infestation (Peters 1951) that are required for the export of produce, such as seed potatoes.

Soil population estimates are also used as the basis of advice to farmers, on the risks of planting susceptible crops on infested land, and in research into population dynamics. Table 1 gives some practical figures for judging the situation. The standard of accuracy demanded in research is generally higher than for advisory work. There is no fundamental reason why this should be so; but in advisory work, time is often pressing, and accuracy may have to be sacrificed for speed.

Soil Extraction

The extraction of cysts from soil is the basis of all population estimates. For this purpose, water flotation is universally employed. Morgan (1925) used ordinary flasks, and these are still of value where the soil contains much floatable debris (Jones 1945). Most frequently, however, the Fenwick can is used (Fenwick 1940). In many advisory centres throughout Europe, whole batteries of Fenwick cans can be seen in action; and the yearly through-put of samples is very great. The chief modification of Fenwick's original model is the provision of a sloping floor and sludge drain to facilitate clearance of waste soil between samples.

Other methods of cyst extraction worth mention include those of Hellinga (1942) and Buhr (1954). In the former, the soil containing cysts is first washed through two sieves of appropriate mesh, and then the contents of the lower sieve are transferred to a porcelain dish in which flotation occurs. In the latter, the soil is placed in a glass cylinder, and a strip of filter paper is fitted around the wall. When water is added and the contents stirred, cysts and debris rise to the surface and adhere to the filter paper, which, when unrolled, gives a continuous band from which cyst collection is relatively easy.

On the Continent, the float is usually searched in an enamel or porcelain bowl containing water, the cysts being collected from around the edge with a camel hair brush by the unaided eye. With such a technique there is considerable scope for personal errors. In Great Britain, collection of cysts is usually accomplished from filter papers or from the Fenwick tray with the aid of a binocular microscope.

Control of Laboratory Errors

Repeated counts of cysts from samples drawn from the same mixed bulk of soil should fit the Poisson distribution, so that, whatever the extraction technique employed, its efficiency may be tested by a χ^2 test (Fisher 1938). In practice, the error variance generally exceeds the expected Poisson variance because of imperfections in technique. Provided this difference is not too great, the technique may be regarded as satisfactory. Because of the approximate fit to the Poisson distribution, certain important consequences follow:

- (1) The accuracy of a count is determined solely by its magnitude. Thus if x = the count, then \sqrt{x} = its standard error, and the degree of accuracy may be expressed by the standard error as a percentage of the count.
- (2) For counts of 10 or more, the Poisson distribution approaches the normal distribution so that the 95% range is given by $x \pm 2SE$.
- (3) Nothing is gained by estimating a series of small samples and taking the mean. In fact, there are definite pitfalls to such a procedure. If a = the first count, there may be a tendency to search until subsequent counts reach the same magnitude and to cease searching thereafter. This introduces bias and gives error variances significantly less than expected. A case may be made, however, for the estimation of two samples, the one as a check on the other and intended to avoid gross mistakes.

The implications of (1) and (2) above are illustrated in Tables 2 and 3.

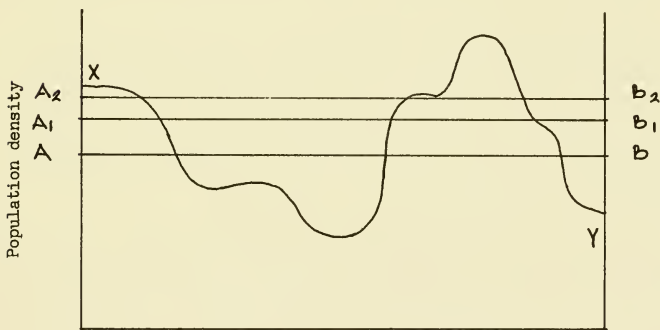
Table 2 shows the relative inaccuracy of small counts, the rapid increase in accuracy up to counts of 100, and the slower improvement thereafter. Table 3 illustrates further the uncertainty connected with small counts. On a basis of 100 g. samples, certainty of detection is not reached until the equivalent field population reaches a concentration of 50 to 60 millions per acre. This is of considerable importance in connexion with the so-called certificates of freedom from infestation already mentioned. The conclusion to be drawn from the information shown in Tables 2 and 3 is that, for research, it is desirable to work with counts of the order of 100 or more and to vary the sample size accordingly, rather than to work with samples of fixed size.

Field Sampling

In field sampling, the usual procedure is to take a number of small cores from an area of land and to mix them together to form a composite bulk-sample. A true picture of the eelworm population could only be obtained by the separate estimation of an infinite number of small samples. Some idea of the relationship of bulk sampling to the true population of land can be obtained by considering a transect (Figure 1). XY shows the variation in eelworm population per unit area along the transect; AB, the true mean of these values; A_1B_1 , the deviation of the bulk-sample mean from the true mean; and A_2B_2 , the laboratory estimate of the bulk-sample mean. The last may depart considerably from the true mean of the population and gives no indication of the point to point variability of the population.

Figure 1

Relationship between within-field, or plot variability,
bulk sampling and laboratory estimation of bulk-sample.



Position or transect:

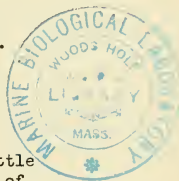
XY = variations in population density along transect.

AB = true mean of XY.

A_1B_1 = true bulk-sample mean.

A_2B_2 = laboratory estimate of true mean.

During the last twenty years' work upon soil populations, little information has been obtained upon which to decide the depth of sampling, the number of cores per unit area, or the size of cores



(Anscombe 1950). These factors determine the size of the bulk-sample, and the greater this is, the nearer its mean concentration approaches the true mean of the parcel of land under investigation. Local variability is created by the cultivation of host plants, both laterally and at depth, especially where wide row spacing is used. Cultivations and ploughing in subsequent years tend to even up these local variations and render the surface soil more uniform. Gross variability is encountered, especially in the early stages of infestation, and tends to become less as the field becomes more uniformly infested.

Recently Fenwich (work about to be published) has attempted to get estimates of within-field variability and to deduce from this the influence of (a) size of count and (b) number of borings taken. His results are very briefly summarized in Table 4. The picture given is more favourable than would be obtained in the field for the following reasons: (1) the plots used were experimental plots and have had more uniform treatment than fields; (2) their eelworm populations were all relatively high; (3) their size was small so that the sampling rate per unit area (even at low levels) was higher than is usually possible in fields; and (4) the assumption that within spot variability is distributed according to the Poisson distribution. This is not so.

The success of sampling for advice to farmers on the desirability of growing susceptible crops on infested land, which has been practiced in Europe for many years, may be attributed to the fact that such population estimates are required mainly for fields in the heavily infested class and also to the high absolute level of the 'economic zeros' for cultivated crops. For potato root eelworm, this level has been estimated by Johnson & Thompson (1945) at 0.5 cysts with contents/g. soil or 500×10^6 cysts with contents/acre. Similar levels apply also for the beet and cereal root eelworms.

In advisory work, spatial variability has to be accepted as one of the hazards of soil sampling. In research, control of spatial variability is highly desirable. A measure of control can be obtained by use of "fixed plot," "fixed grid" sampling, or by employment of "microplots" or pots. In the last two, infested soil can first be homogenised by mixing. Small pots, however, are unnatural in their water, temperature, and aeration relationships; and it is difficult to translate results obtained in them in terms of field conditions. Larger glazed pots, as used by Peters (1952), are more satisfactory; but a closer approach to field conditions can be got with 'microplots' (Jones 1956).

Assay of Cyst Contents

Morgan (1925) and other early workers in this field attempted to use cysts as a measure of the soil population, but it soon became clear that cyst counts were invalid for this purpose. Figure 2 (Jones 1945) shows the lack of correlation between cyst and egg counts for the beet eelworm. A better measure is the number of cysts with contents

('viable cysts'), Figure 3 (Jones 1945); and here there is a strong correlation with numbers of eggs.

Figure 2

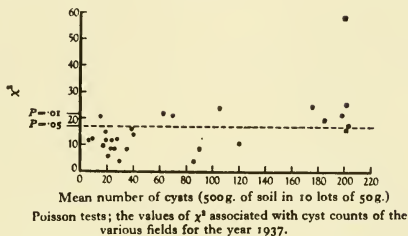
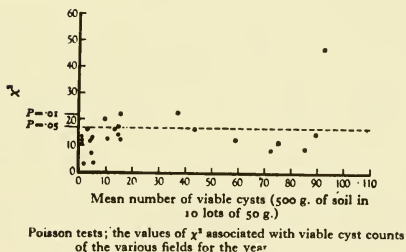


Figure 3



Numbers of cysts, with contents, is the measure usually employed in advisory work, and it is often coupled with some arbitrary standard of fullness (e.g. Triffitt 1934). These arbitrary standards may introduce errors of a personal nature, and, in any case, render it difficult to relate the results of different groups of workers. Petherbridge (1938) and Oostenbrink (1950) crushed cysts under a cover glass and estimated the 'halos' of eggs surrounding the ruptured cysts. Such methods give variable results that are generally much too low. A better method is to make up the liberated eggs (and larvae) into a suspension from which an aliquot may be withdrawn and counted. The estimation of aliquots from a bulk suspension presents the same

problem as does the estimation of cyst from samples of soil drawn from bulk-sample, and exactly the same statistical principles apply (see Tables 2 and 3). Two methods may be adopted for the estimation of the suspension: either the dropping apparatus of Jones (1945) or the modified McMaster slide of Fenwick. The second method is the simpler, and both are preferable to the hypochlorite method of Fenwick (1942). One disturbing feature of squash techniques is the failure of egg masses to separate completely. This may be overcome by whizzing the suspension in a food homogeniser (Bijloo 1954) to which various "tools" have been added, e.g. plastic "pestle," rubber flail.

None of the above methods give an indication of the viability of cyst contents. This may be assessed by plying batches of cysts (not less than 100) in suitable replication with root diffusates appropriate to the species. This is usually done in solid watch glasses at constant temperature. The method may be extended to give a useful separation of those species which respond to root diffusates (Winslow 1955), but it does not work well with newly produced cysts.

Estimation of hatchable larvae represents an additional step in the technique; and the more steps there are, the greater the variability introduced. This can only be overcome by working with greater numbers of cysts. The labour involved is, therefore, rather great. Moreover, the hatching process is affected by the pre-treatment of cysts (Fenwick 1949, et seq.; Wallace 1955). Homeyer (1953) tried to overcome this by using acridine dyes and ultraviolet light to obtain differential fluorescence of living and dead eggs and larvae. It has also been noted by several workers that if eggs are "hatched" by pressure, the living larvae emerging immediately adopt the natural straight or sinuous outline of healthy larvae, while dead larvae remain kinked. Details of this method with proffs of reliability have not yet been published.

If greater detail is required about the cyst population, recourse must be had to the single cyst techniques of Gemell (1940), Ellenby (1943), or den Ouden (1953). These, however, are sometimes difficult to interpret, since the larval hatches include many 0's and require transformation before they can be treated statistically.

The above account follows the similar lines of an address given to the Jubilee Conference of the Association of Applied Biologists (Jones 1955).

REFERENCES

- Ansdcombe, F. J. (1950). Soil sampling for potato root eelworm cysts. A report presented to the conference of advisory entomologists. *Ann. appl. Biol.*, 37, 286-95.
- Bijloo, J. D. (1954). A new method for estimating the cyst contents of the potato-root eelworm, *Heterodera rostochiensis* Mollenw. *J. Helminth.*, 28, 123-28.

- Buhr, von H. (1954). Untersuchungen über den Kartoffelnematoden.
1. Die "Papierstreifen-Methode, einvereinfachtes Verfahren zur Untersuchung von Bodenproben auf ihren Besatz mit Nematodezysten." NachrBl dtsh. PflSchDienst, Berl.
- Cobb, N. A. (1918). Estimating the nema population of soil. Tech. Circ. U.S. Dep. Agric., no. 1, 1048.
- Ellenby, C. (1943). A modification of the Gemell single cyst technique for the potato strain of the eelworm Heterodera schachtii Schmidt. Nature Lond., 152, 133.
- Fenwick, D. W. (1940). Methods for the recovery and counting of cysts of Heterodera schachtii from soil. J. Helminth., 18, 155-72.
- _____ (1942). The degree of Heterodera infectivity of soil and its determination. J. Helminth., 20, 50-66.
- _____ (1949). Investigation of the emergence of larvae from cyst of the potato-root eelworm Heterodera rostochiensis. 1. Technique and variability. J. Helminth., 23, 157-70.
- _____ (1951). A new modification of the McMaster slide for use in potato-root eelworm investigations. J. Helminth., 25, 173-6.
- _____ (1952a). Sampling techniques and the limits of their applicability. Proc. Int. Memat. Suposium F.A.O. Rome, pp. 8-12.
- _____ (1952b). The estimation of the cyst contents of the potato-root eelworm H. rostochiensis. J. Helminth., 26, 55-68.
- Fisher, R. A. (1938). Statistical methods for research workers, 7th ed. Oliver and Boyd, Edinburgh.
- Gemell, A. R. (1940). Studies on the biology and control of Heterodera schachtii (Schmidt). 1. Comparison of H. schachtii cyst populations from two potato growing districts. Bull. W. Scot. Agric. Coll., no. 139, 1-15.
- Hellings, J. J. A. (1942). De invloed van het bietensyltje op de opbrengst en de samenstelling van suikerbieten. Meded. Inst. Suikerbiet, Bergen-o-Z., 12, 163-182.
- Homeyer, B. (1953). Die Prüfung von Nematodenmittlen auf flourenzenoptischen Wege. Mit. Biol. ZentAnst. Berl., 76, 232-5.
- Johnson, L. R. & Thompson, H. W. (1945). Potato root eelworm in Yorkshire. J. Minist. Agric., 52, 226-70.
- Jones, F. G. W. (1945). Soil populations of beet eelworm (Heterodera schachtii Schm.) in relation to cropping. Ann. appl. Biol., 32, 351-80.
- _____ (1955). Quantitative methods in nematology. Ann. appl. Biol., 42, (in press).
- _____ (1956). Soil populations of beet eelworm (Heterodera schachtii) in relation to cropping II. Microplot and field results. Ann. appl. Biol. 44, 25.
- Morgan, D. O. (1925). Investigations on eelworm in potatoes in South Lincolnshire. J. Helminth., 3, 185-92.

- Oostenbrink, M. (1950). Het aardappelaaltje (Heterodera rostochiensis Wollenweber) een gevaarlijke parasiet voor de eenzijdige aardappel-cultuur. Versl. PlZiekt. Dienst Wageningen, 155, 1-230.
- Ouden, Henden (1954). Het bietencystenaaltje en zijn bestrijding.
1. Methoden to gebruiken bij het onderzoek naar kunstmatige en natuurlijke lokstoffen. Meded. Inst. Suikerbiet. Bergen-o-Z., 24, 101-21.
- Peters, B. G. (1941). Dilution egg counts and the Poisson Series. J. Helminth., 19, 59-62.
- _____ (1951). The term "eelworm-free" soil in plant quarantine regulations. Nature, Lond., 167, 368.
- _____ (1952). Pot tests of nematocides against potato-root eelworm. I. Pilot test and methods. Ann. appl. Biol., 39, 447-56.
- Petherbridge, F. R., Stapley, J. H. & Thomas, I. (1938). The best eelworm (Heterodera schachtii Schm.) J. Minist. Agric., 45, 226-36.
- Triffitt, M. J. (1934). Experiments with the root secretion of grasses as a possible means of eliminating Heterodera schachtii from infected soil. J. Helminth., 12, 1-12.
- Wallace, H. R. (). Factors influencing the emergence of larvae of beet eelworm Heterodera schachtii Schmidt from cysts. J. Helminth., 29, (in press).
- Winslow, R. D. (1955). The hatching responses of some root eelworms of the genus Heterodera. Ann. appl. Biol., 43, 19-36.

TABLE 1

Soil populations of Beet Eelworm
in relation to "beet-sickness"
and the detection of infested fields.

V I A B L E C Y S T S
(i.e. cysts with contents)

	<u>Per acre</u>	<u>Per 2 lb. of soil</u>	
Completely "sick"	1,000,000,000	1,000)) Readily detect- able
"Sick" patches appearing	100,000,000	100)	
	(10,000,000	10) Detect- able
	(1,000,000	1)	
	(100,000)	
	(10,000)	
No crop symptoms	(1,000)) Virtually indetect- able
	(100)	
	(10)	
	(0)	
Uninfested	(0)	

For populations in terms of eelworm eggs, multiply all figures by 100, the average number of eggs per viable cyst.

TABLE 2

Accuracy and range of counts from samples fitting Poisson Distribution.

Count	Standard Error	SE % Count	95% Range
X	\sqrt{X}	$\frac{\sqrt{X} \times 100}{X}$	$X \pm 2 \sqrt{X}$
1	1	100	(0 - 3)*
4	2	50	(1 - 7)*
9	3	33	3 - 15
16	4	25	8 - 24
25	5	20	15 - 35
36	6	17	24 - 48
49	7	14	35 - 63
64	8	13	48 - 80
81	9	11	63 - 99
100	10	10	80 - 120
121	11	9	99 - 143
144	12	8	120 - 168
169	13	8	143 - 195
195	14	7	185 - 224
225	15	7	193 - 255
500	22	4	456 - 544
1000	32	3	936 - 1064

* The Poisson Distribution is asymmetrical but becomes more nearly symmetrical as values increase, so that twice the Standard Error may be taken as the 95% fiducial limits, as in the Normal Distribution for counts of 9 upwards.

TABLE 3

Chances of detecting eelworms in soil @ "low" population levels (100g. samples)

Expected count per sample 100g.	Percentage changes:		Equivalent concentration:	
	Of finding one or more cysts <u>Detection</u>	Of failing to find cysts <u>Failure</u>	Per Kilo	*In millions per acre
0.01	1	99	0.1	0.1
0.1	10	90	1	1
0.7	50	30	7	7
1	63	37	10	10
2	85	15	20	20
3	95	5	30	30
4	98	2	40	40
5	99	1	50	50
6	99.9	0.1	60	60

* Assuming 1000 tons or 10^9 g. of top soil per acre.

TABLE 4

Relationship between % error of estimates,
the number of borings bulked together,
and the number of cysts counted.

Number of borings bulked	Number of cysts counted				
	4	25	100	400	α
1	178	--	155	154	153
4	88	64	61	59	59
16	71	45	41	38	39
25	59	29	23	21	21
50	55	25	18	14	13
100	54	22	14	10	10
400	53	20	11	6	5
α	53	19	10	3	0

PRINCIPLES OF NEMATODE TAXONOMY

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This discussion of the subject of taxonomy of nematodes is undertaken with some hesitation primarily because of the difficulty of getting specialists to agree on the taxonomy of even a small genus. The main difficulty encountered is the fact that most nematode groups are not well enough known to supply the basic information necessary to establish satisfactory classifications. One should keep in mind that in most instances we are dealing with opinions and not precise data collected from carefully controlled experiments. It is unfortunate that the taxonomist cannot always undertake controlled experiments with the populations with which he is concerned. He must deal in general with dead specimens and these usually represent an extremely small sample of any particular population. If we consider that each individual taxonomist has the right to express his opinion concerning facts ascertained from the study of a few specimens that he assumes to be representative of a population, it is surprising that there does exist a relatively high degree of agreement concerning most nematode groups.

One should, of course, begin a discussion of taxonomy by pointing out the importance of taxonomy to all fields of biology. It must be obvious that it would be impossible to communicate knowledge about organisms without a system of naming. More important, it is necessary to have permanent names for organisms, and these names must be supplemented by information that will enable all workers to recognize a named species each time it is encountered. This concept is as important in the field of applied nematology as it is in ecology, evolution, morphology, or physiology of nematodes. In all of these fields we must have accurate identifications and a stable system of nomenclature if we are to make lasting contributions to our knowledge of the biology and control of nematodes.

In order to illustrate some of the difficulties that are inherent in taxonomic studies I will use, so far as is possible, some of my own work as examples. In this way I can be as critical as is necessary and also avoid offending any of my fellow taxonomists. Most discussions of taxonomy are usually concerned with the species, and this is rightfully recognized as the most important category in classification by most taxonomists. Above the species level we have a varying number of collective categories, the subgenus, genus, subfamily, family, etc. Also there are, in most groups of animals and particularly those that are well known, infraspecific categories. The best known and most widely used of the officially recognized infraspecific groups is the subspecies. There are a relatively few described subspecies in the nematodes in comparison to other animal groups such as insects, mammals, and birds. We frequently forget the proper relationships that are intended to be

expressed by the species concept in zoology. There is sometimes a tendency to regard the subspecies as being equal to the species. For example, in the genus Meloidogyne there are a number of described subspecies, and if one examines the literature it will be noted that various authors have used the names M. incognita (Kofoid and White, 1919) and M. incognita acrita Chitwood, 1949, as though they were dealing with a species and a subspecies. Properly used, M. incognita is the species and there are two subspecies, M. incognita incognita and M. incognita acrita. To be precise, one should indicate the subspecies with which he is dealing, because the use of the species name alone indicates that one or both subspecies were present.

The present day species concept has been developed over a rather long period of time by scientists working in well known groups, e.g. insects, birds, mammals, etc. It is generally conceded by most workers in taxonomy that the first stage in achieving a satisfactory classification is one concerned almost exclusively with the description of new species. Descriptions in the less well known groups, such as nematodes, are based almost exclusively upon morphology. In some respects it can be considered that a purely morphological classification is based, to a large extent, upon gaps between species. As these gaps are gradually filled in by the description of new species in a group, there should be a trend toward using other biological evidence to support the taxonomy based upon morphology. Eventually, as the process of classification is continued, it should be anticipated that genetics, ecology, and physiology will be needed to insure that a system of classification is sound. If we can base our assumptions concerning taxonomy upon the trends that have taken place in a group such as birds, where more than 98% of the existing species are believed to have been described, we can draw some conclusions concerning the future of nematode taxonomy.

We are certainly in the first stage of taxonomy where we are primarily concerned with the descriptions of new species. Eventually, we should reach a point where it will become obvious that there are more species' names in the literature that there are valid species in nature. This should result in extensive revisions of genera in which more use will be made of the subspecies. This is the trend in well known groups, and we have no reason to assume that such will not be the case with the nematodes. On this basis, we can then assume that at least some of the present day described species will prove to be subspecies. These subspecies will be forms of a polytypic species.

Polytypic Species - Let us take as an example the genus Trichodorus and consider the species californicus, obscurus, and proximus; each of these species is known from widely separated geographical areas, and they were described from relatively few specimens in each case. The descriptions are entirely based upon morphological characters; that is, the gaps between these species appeared to me to be sufficient to support the opinion that they were indeed new species. We will now suppose that intensive collecting in areas where these species are not known at present reveals forms that are intermediate and the original gaps between species become occupied by forms that have intermediate

morphological characters. Ultimately, it may be decided that these forms are simply geographical variants of the same species and that, although the majority of each form are recognizable on a morphological basis, they should be considered to be subspecies of a single species. This, then, would fit into the category of the polytypic species which consists of a number of geographically isolated populations, exhibiting sufficient differences to be called subspecies.

On the above basis, you can understand my feeling that one has to have a certain amount of courage to describe new species. This is particularly so if the worker recognized that, in addition to the possibility of re-describing as new a previously named species, he also may be describing merely subspecies and not valid species. You will recall that Sher and I synonymized some species in Pratylenchus; but if someone presented evidence that Pratylenchus coffeae, P. musicola, and P. mahogani were indeed valid species, I would be the first to agree, providing, of course, the evidence was conclusive. Similarly, if it could be shown that Trichodorus christiei was clearly a synonym of T. minor, this would be entirely acceptable because it would make the taxonomy of the group somewhat easier to handle. Taxonomy should not be solely concerned with descriptions of new species but should be directed toward providing a better understanding of the present taxonomic categories.

In addition to the polytypic species concept, there are two additional concepts that are useful to the taxonomist. These are designated by the terms "sympatric" and "allopatric." These words are used to describe two types of species distribution on a geographical basis. They are useful to the taxonomist because the terms have application in all work concerning the species.

Sympatric species - If you extract two species of Tylenchorhynchus from a single soil sample, they would be referred to as sympatric species, meaning that they occur together in the same geographical area. In such a case, we can be reasonably certain that we are dealing with two species, since it is very unlikely that two subspecies of the same species could maintain their identity in such a situation. One may assume from the fact that two species occur together that the differences between them are indeed valid and the taxonomist would not hesitate to indicate they were distinct species.

Allopatric Species - This type of species distribution presents an entirely different problem. Here we are dealing with a number of related species that are geographically isolated from each other. They appear to be different on the basis of morphology, but we know nothing about their possible behavior if they were brought together in the same geographical area. In the case of the sympatric species, we can be fairly certain that we are not dealing with subspecies, because they maintain their identity when they are not geographically isolated. In the case of allopatric distribution, we know nothing of the reproductive potentials of the various species; their separation is spacial, but we cannot be certain that they would not interbreed if brought together. This type of species distribution

should immediately indicate to the taxonomist that there is the possibility of the presence of the polytypic species group, and he should be prepared to accept this possibility if he describes the geographically isolated populations as species. In practice, we hope that the morphological differences between allopatric species are substantial; that is, the gaps should be large so that there is little possibility that they will be filled by intermediates. It is anticipated that in the future more use will be made of ability of populations to interbreed or not to interbreed as one of the criteria for the validity of a species. But, as inticated earlier, the genetist, physiologist, and economic nematologist must contribute information to supplement the purely morphological species concept that must, of necessity, be the concept most used by the taxonomist of nematodes at the present time.

When Dr. Cairns asked me to discuss taxonomy, I immediately asked him what sort of information the group would be most interested in considering. He suggested that we might discuss the degree of morphological difference required in order to describe a new species and how to arrive at a decision regarding the morphological characters used to distinguish subspecies. As I have indicated previously, at least by implication, the species concept is subjective. By this we mean that it is impossible to see all of the individuals that comprise a species, so we must make certain assumptions based upon small samples. There are no set rules or criteria that are precise enough to allow us to say that morphological differences of a certain magnitude must be used to separate species. In actual practice, it is the opinion of the individual taxonomist that determines the composition of the various categories. For this reason, I consider it would be foolhardy on my part to attempt setting up criteria to be met before a nematode could be described as a new species. At the subspecies level it has been suggested that subspecies are justified when it is possible to distinguish 75% of the individuals of one population from another on a morphological basis. My present opinion is that our classifications must be based upon morphological characters, which may or may not be associated with differences in host plant range, etc. I would certainly suggest that the individual contemplating describing a new species should devote considerable time to the study of other species in the genus. He should examine the group and attempt to form an opinion as to the characters used by other authors in distinguishing the species. He should look for additional characters that have not previously been used. He should finally ask for opinions of others interested in the same groups. If they agree with his opinions concerning the species, he should be most happy and describe the new species without hesitation. If there is a difference of opinion, he may proceed with the description, but possibility only after additional study.

I believe that it would be appropriate to briefly mention types. It is recommended in the Zoological Rules of Nomenclature that when a new species is described, one specimen should be designated as the type. This specimen becomes the name bearer, and, so long as it is in existence, it represents the species. The type specimen is objective; it can be seen, measured, studied, etc. This specimen is the Holotype,

and it may be either sex. Some authors also designate an Allotype which is a specimen of the opposite sex from the holotype. In addition, it is customary and desirable to designate Paratypes at the time of the original description. If possible, the paratypes should be from the same host, locality, etc., as the holotype. In each instance, these types should carry with them precise information on locality, date, host, and collector. This information should also be included in the published description because of its value if the types are destroyed. In the case of the holotype, it should be the only specimen on the slide. I would also recommend this for the paratypes. Instances are known where two specimens were on a type slide, and the author, thinking they were the same species, based his description on both specimens. A subsequent worker, finding that these specimens represent two species, must select one of them as representing the species described by the previous worker. His selection may or may not agree with the opinions of other workers, and this can cause considerable confusion. I would advise all nematode taxonomists to conform to the Zoological Rules of Nomenclature in all taxonomic procedures. These rules are available in their original form, and there are also several text books where the rules are discussed in simple language.

When we place several species in one genus, we assume that these species are more closely related to each other than they are to species in another genus. This same pattern applies in the groups above the genus. It is one of the functions of taxonomy to attempt to show phylogenetic relationships and contribute to an understanding of evolution in addition to providing a means of separating and identifying populations. The generic concept is entirely subjective, and we only hope that we can show proper relationships by grouping species. It is natural to ask what characters are usually considered to be generic. I am not certain that there is any satisfactory answer to this question. There are some examples that can be cited, and once again it will be obvious that the opinion of the individual specializing in the group is of extreme importance.

The genus Trichodorus can be used to illustrate at least two points. The presence or absence of caudal alae in the male is in most groups regarded as being of at least a generic character. However, in Trichodorus we presently have males with a caudal alae in some species and without caudal alae in others. I must admit that there was considerable temptation to make two genera of the present genus. However, I could find no characters that would enable a generic separation based upon females and so discarded the idea. Subsequent workers may not agree with this and may base such a separation upon male characters. It would, however, mean that several of the species could not be placed because males are not yet known.

The number of ovaries is used as a generic character in some groups, but this may be primarily because both one and two ovaried species are not yet known. When one considers that two opposed ovaries is regarded as the primitive condition and that species with one ovary probably had ancestors with two ovaries, as is indicated by the presence of rudiments

of the gonad, it is not surprising that species with one and two ovaries are placed in the same genus. It is interesting to compare the genus Pratylenchus with Radopholus. Morphologically and biologically, they are very similar, particularly when a species of Radopholus has males that do not exhibit sexual dimorphism. In this case, it is the number of ovaries that becomes the primary character. This is particularly the case if the observer is not experienced. By way of contrast, you are all familiar with a number of present day genera that contain species with one gonad and others with two gonads. Examples may be found in Psilenchus amongst the tylenchs and in many genera in the Dorylaimoidea. I think this means we recognize there must be other characters that are as important or more important than number of ovaries and that these characters are judged to show relationships that are of a generic nature.

Taxonomy is by no means static, new evidence is being introduced continuously, some of it serves to confirm previous groupings and some indicates needed changes and revisions. We frequently are reluctant to accept changes proposed by taxonomists. Sometimes this is due to the fact that we must use new names for long established names and other times we simply do not agree with the opinions of the particular worker. One of the suggestions that has been made relative to new names, generic changes, and revisions is, I believe, worth considering. This concerns the acceptance of the opinions of the latest revisor as being valid until proven otherwise. There is no need to point out the confusion that is caused by the use of species names after the species has been synonymized. Such usage indicates that an author has evidence to indicate that the concerned species is not a synonym. He should present this evidence at the time he removes the species from synonymy.

If we are to make lasting progress in the field of nematode taxonomy, we should exchange information and opinions freely. We should and must exchange specimens, and, most important of all, we should have a satisfactory place to deposit type material where it would be available for study. Type material belongs to science and not to individuals, and it should be properly housed and cared for. A worthy objective for this group and other similar groups concerned with nematodes would be to work toward obtaining better facilities for a central nematode collection which would also house type material.

NEMATODE ECOLOGY

C. Overgaard Nielsen

The title provides a very spacious framework, and since I cannot possibly cover the whole field of nematode ecology within the time at my disposal, I propose to restrict myself to a few selected aspects.

Most of the papers which have been given so far at this meeting have been rather detailed and technical, which is in agreement with the purpose of the meeting. I shall follow a somewhat different line and only talk about generalities.

What I am attempting to do here is to present the general physiological background against which I would like the practical nematologist to consider the activity of nematodes. When I say nematodes, I mean primarily free-living soil nematodes, but I hope to present some generalities at a level which also applies to many problems connected with plant parasites.

First, I would like to emphasize that in essence soil nematodes are fresh-water organisms, although they show many adaptations to the conditions prevailing in the water phase of the soil. These adaptations show up when we consider the distribution of nematode species throughout an imaginary transect from river bottom through water meadow, up the slope of the river valley to the high ground, and ending in a moss cushion on a tree trunk. The distribution of species may be as follows, where the figures replace the names of species:

	<u>species numbers</u>
In river bottom.....	1-40
In water meadow.....	5-50
In soil on high ground.....	35-75
In moss cushion on tree trunk.....	75-76

When we consider habitats with very different moisture regimes, the species spectrum is seen to change. In going from the bottom layers of the river to the water meadow, four species have disappeared but ten newcomers have appeared (the actual figures are, of course, chosen quite arbitrarily in order to illustrate a principle).

Why this changing spectrum? Do some nematodes prefer wet stations and others, dry? One often comes across such statements. The explanation is entirely different: All nematodes require the presence of available water in order to be active, but some have developed the ability to survive periodical desiccations. That is what makes them true soil inhabitants; they are not seriously affected by droughts but spend such periods in a state of dormancy. This category of nematodes is represented by species numbers 35-76, and they are the ones I am going to speak of here. The nematodes predominant in soils with a stable and rich water supply have been studied by Dr. Hirschmann, and I can refer

to her fine work. These nematodes most often are unable to survive desiccations, although some of the drought resistant species may also occur in wet situations.

The ability to survive desiccation is not possessed to the same extent by all species; in some it is only feebly developed, in others, very strongly so. In some it is restricted to certain developmental stages; in others it occurs in all stages. The most extreme case I know of is Plectus rhizophilus. In the transect, it is number 76, the only species really competent to build up a dense population in habitats where water is rarely present and, when present, likely to disappear again within a few hours. Incidentally, this nematode is better at utilizing the few drops of dew than is the moss with which it lives.

It would, of course, be nice to know exactly how much water must be present in order to enable nematodes to be active, but the question cannot be answered in terms of absolute amounts. In one particular soil containing about 60% of organic matter, large nematodes stopped activity at a water content of about 33%. In a sand, practically without organic matter, the nematodes' activity ceased at about 2% of water. As perceived by the nematodes, the two percentages represent an identical moisture status, and they determine the limit between available and non-available water. Although we cannot express availability of water in terms of volume or weight, we can—and I would add that we always should—express the water status in terms of water tension or some derived unit of measure. In doing so, it comes out that nematode activity stops a little below the wilting range of plants. Several micro-organisms are able to exploit water that is more firmly bound to soil particles; Dr. Bartholomew sets their limit at about pF 5.6. I know little about this, but it is quite reasonable that they are more efficient in extracting water held at high tensions, partly because the organisms are smaller and, thus, able to exploit minute soil crevices and partly because some fungi and bacteria are reported to live at very high osmotic pressures.

We know far too little about the details to enable us to be precise about the water requirements of nematodes. However, very promising results have been obtained by Dr. Wallace at Rothamstead in connection with the hatching of cysts. We can say with a fair degree of certainty that nematode activity stops before, perhaps well before, the wilting range of higher plants is reached. Some nematodes will only stop activity at this critical value while others will die.

It may now be asked if it is all that important to know how different nematode species behave with respect to critical water tension and desiccation. I can only answer that it is. Two examples may illustrate the point:

- (1) We want to determine the density of active, free-living nematodes or free-living stages of a plant parasite. We can do that by several methods, but since the techniques involve water treatment, the number we arrive at has

reference to the moment of separating the nematodes from the soil and not to the moment of sampling. Nematodes might have been dormant in all of them.

- (2) Let us consider a field carrying a particular crop. The field is infested by two potential parasites, one of which is able to survive dry periods, while the second one is more easily killed through prolonged or repeated desiccation. This second type is not giving serious troubles, but then the farmer starts an irrigation project whereby he gives it a better chance, and he may end up by having two serious pests. Or the farmer may give the first type of nematode still better conditions, such that it is able to increase the number of generations per year and, hence, to do more damage.

Sandy soils have a low water holding capacity and dry up easily while there is more stabilizing effect to moisture fluctuations in clay soils. A priori I should therefore think that an infestation of a given size would be more serious in clay than in sandy soil.

Another aspect of nematode ecology which I would like to say something about is how nematodes actually live in the soil. We know that they live in soil crevices, that is, in already existing pore spaces. It follows from this that nematodes do not influence the soil structure mechanically as do, for example, the earthworms.

We also know a good deal about their food. Three groups can be distinguished: One group feeds on liquid substances; this holds true of all species with a stylet or spear. Nematodes of this group pierce the surface of cells and feed on the cell content, whether it be a plant, fungus, or animal cell. Another group—and one very rich in species—feeds on particulate food, especially bacteria and small soil algae. A third group, comprising e.g. Mononchus and a few others, seems to feed on larger organisms which they catch alive.

I would like to point out that all nematodes feed selectively. They do not ingest soil in order to digest what is digestible in it for them and then pass out the residual matter as do oligochaetes. In agar cultures it is quite easy to watch the particulate feeding type of nematodes pick up single or small batches of bacteria.

Having outlined what they do in the soil, it might be interesting to inquire into their metabolism. Nematodes range in weight from about 0.02 micrograms to 60 micrograms, the majority falling within the range of 0.5-5 micrograms. Their respiratory metabolism varies largely with their size:

<u>Monhystera</u> , <u>Prismatolaimus</u>	Approximately 1500 ml. O ₂ per kg. per hour
<u>Plectus</u>	1200 at 16° C.

Dorylaimus, Mononchus,
Cephalobus, Rhabditis,
Achromadora, Acrobeles, etc.

Approximately 800 ml. O₂ per kg.
per hour
at 16° C.

The metabolism is little influenced by their activity. Narcotized and immobile worms respire at a rate only about 5 per cent lower than active worms. Immature worms respire at a higher rate than sexually mature and larger individuals of the same species. Respiration increases with temperature up to a certain point.

With these data in hand, we can calculate the order of magnitude of oxygen consumption per unit area of soil due to nematodes when the population density is known together with its species composition.

I have obtained population densities in a number of Danish habitats. They vary from about 100,000 to 20 million per square meter, corresponding to about 1-20 grams per square meter according to the nature of the habitat. The oxygen consumption of nematodes is calculated to vary between 0.5 and 17 ml. O₂/m² at 16° C.

This leads me on to my last point. It might be interesting to try to express the activity of nematodes in a way which will be more informative. On the basis of population estimates, temperature measurements, and water tension data of one particular grass field in 1955, I have arrived at the following annual budget:

Nematode population	= 20,000,000/sq. m.
Weight	= 20 g.
O ₂ consumption	= 625 m ³ /year

In this particular habitat, 4/7 of the respiration was due to nematodes which largely feed on bacteria, i. e. 360 m³O₂/year. I shall only consider this part of the population. If we assume that bacterial dry matter consists of 50% protein, 25% fat, and 25% carbohydrates, it will be found that an uptake of 2.42 liters of O₂ corresponds to the combustion of 1.6 g. of bacterial dry matter. Hence, 360 m³O₂ corresponds to 240 kg. bacterial dry matter, which is nearly one ton of live bacteria. This gives us the order of food necessary to keep 4/7 of the nematode population of one hectare going for a year.

I think that such calculations indicate that ecological considerations of nematodes in the soil have a broad importance and, of course, there is much that we do not know about the ecological factors of the soil and their effects upon the various kinds of nematodes.

SPECIALIZED MICROSCOPY FOR THE STUDY OF NEMATODES

Eldon J. Cairns

It is not very long before the critical worker in phytonematology comes to realize that the standard research microscope and the binocular dissecting microscope sometimes leave much to be desired when it comes to close study and observations of nematodes. Have not all of us wished for some new and presumably better high-powered microscope in order to make certain routine but decisive determinations concerning structures of nematodes which are near the 0.2μ resolving limits of our instruments?

Have you ever thought of how very little we know of the actions of the nematodes in or on their hosts or in the soil because of our inability to directly observe the nematodes with sufficient magnification to see what is going on? The objective lenses of our research microscopes have so limited a working distance that at the necessary high magnifications they cannot be brought to focus on anything over a fraction of a millimeter distant. The stereoscopic dissecting microscopes, which are designed for ample working distance, do not have sufficient image quality at their highest magnifications, which are not high enough.

Another of the problems we face is not necessarily one of seeing very small structural sizes but is the transparent nature of the specimens we want to view. New ways have been developed to meet this common problem in the microscopy of biological materials. Some of you may be looking for new research tools having quantitative and qualitative applications in the study of the tiny nematodes and of their hosts' cells and tissues.

Without going into extensive details in any parts of this discussion, we shall consider a few of the specialized instruments and methods of microscopy indicating how they operate, their advantages and disadvantages. Perhaps, you may find from this discussion solutions to particular problems, or you may get an idea for new and rewarding lines of research in phytonematology.

Electron Microscopy

If the problem at hand is one requiring the greatest magnification and resolution, then it is natural to think of utilizing the electron microscope. Unfortunately for us, the scarcity of such instruments is not the only deterrent for their application to nematology, as I shall explain later. The electron microscope is without equal for its resolving power and useful magnification. Current models can resolve to 20 Angstrom Units ($.002 \mu$). Direct magnifications range from about 100 diameters up to 50,000 diameters. Fine grain panchromatic photographic films or plates exposed at such magnifications can be further enlarged optically with increased benefit yielding final specimen magnifications up to

300,000 diameters. A nematode one millimeter long, at this scale of magnification, would appear a little over a sixth of a mile long.

The limitations presently inherent in the use of these instruments are serious ones to contend with. Biological materials must be on the order of only 0.1-0.2 μ maximum thickness, if internal details are to be seen. New procedures and equipment developments in microtomy and freeze-drying now render this a much less difficult requirement to be met.

The ultra thin slice of the specimen is placed within the tube of the microscope for exposure to the beam of electrons. The interior of this tube then has to be highly evacuated to permit steady and prolonged operation and to avoid scattering of the electrons from the beam by chance collisions with residual gas molecules. Thus, the specimens are exposed to pressures not exceeding 10^{-4} mm. of mercury, at the most. Such low pressures are attained in about four minutes. Living tissue cannot survive this. There is said to be some prospect for eventual viewing of biological specimens in the wet state. The beam of electrons also is not without effect on the specimen. There is an electrical charge involved, although this has only a minor effect on the specimen. However, heat is conveyed to the specimen by the beam and this is a serious problem. At moderate illumination intensities, specimen temperatures of 150-200° C. are reached, although using special precautions, the specimen temperatures can be held to 50-60° C. Chemical changes in biological materials also occur as a result of the exposure to electrons.

In summary then, nematode specimens can not be observed in the living state by electron microscopy. They must be dehydrated and sectioned to about 0.1 μ . Considerable skill would be required in preparing the specimen mount for observation, particularly if cross-sections of the typical eel-shaped nematodes are wanted. However, the unsurpassed resolution and magnification of the electron microscope can be brought to bear on special problems of nematode structure. This has been done at Cornell on the study of the cyst wall of Heterodera rostochiensis. Anyone in convenient range of access to the services of an electron microscopist would surely find some worthwhile facts on such important nematode structures as the cuticle and egg shell and, perhaps, nematode relationships with viruses.

X-Ray Microscopy

There is now a new type of microscope which utilizes X-rays instead of a light or electron beam. The greater penetrating power of X-rays reveal structures which may be impossible with the light or electron rays. The presently available X-ray instruments operate in the magnification range of the standard bright field light microscopes and with resolving power said to be as good or better than the best optical microscopes, or on about par with the ultraviolet microscope. The limitations at present are only technical ones, and the X-ray microscope can be expected to improve as it is not at its theoretical limits yet.

In addition to the penetrating properties of X-rays, the design of the instruments permit a very large depth of field. Thus, it is possible to make stereophotomicrographs even at the highest magnification. Such stereographs enable one to see the true shape and distribution of the various structures. The specimen is examined in the atmosphere, that is, it does not have to be placed in a highly evacuated chamber as required in the electron microscope. Nematodes, small roots, and leaves would not be too thick to be observed without sectioning, thus eliminating the need for specimen preparation for microsectioning or in situ staining. Viewing of the image, as with the electron microscope, is done on a fluorescent screen. For greater resolution and for records, photomicrographs are easily made.

The idea of developing an X-ray microscope and an appreciation of its potentialities for new ways in microscopy dates back to soon after Roentgen discovered X-rays. In fact, Roentgen tried to develop such an instrument. His research and that of numbers of others, until quite recent times, failed in finding how to appreciably focus X-rays.

There are now three types of X-ray microscopes evolved in recent years. In the simplest type, the contact X-ray microscope, the specimen is placed in close contact with a fine grain panchromatic film or plate and exposed to X-rays. The resulting image on the film is developed and enlarged optically. This is a simple, easy method quite similar to making a dental X-ray, but presently limited to a resolution of about 1μ and magnification up to 500 diameters. In the second type of X-ray microscope, the reflection X-ray microscope, mirror systems analogous to optical mirrors have been developed. (No refracting lenses analogous to those of the light microscope can be used with X-rays.) Resolutions to about 1μ have been attained with these reflecting systems. The third type of X-ray microscope, the projection X-ray microscope, has so many advantages that production models have been developed in three countries. Resolution of 0.1μ and magnifications of 2000 diameters have been attained, and as mentioned, the theoretical limits have not been met.

General Electric X-ray microscopes are commercially available in this country and represent the projection X-ray system. The problem of focusing the X-rays has been overcome in an ingenious way. First a stream of electrons is focused by means of electrostatic lenses to form a single spot of less than one micron in diameter on to a thin beryllium target window coated with tungsten. The result is an equally tiny spot-source of X-rays. A specimen placed near this X-ray source casts an enlarged image on the fluorescent screen or photographic plate. Thus, one part of the instrument is like an electron microscope, but instead of enlarging the electron beam within the highly evacuated tube, it reduces it to a very small spot image. The other portion of the instrument lies outside the target end of the electron beam tube and is simply a shadow projection microscope utilizing the X-rays that emanate from the tiny spot-source at the end of the sealed and evacuated electron "demagnifying tube."

Whether or not the specimen would be killed by the X-rays depends on the

dosage and the organism, in any case, it will be irradiated. This, of course, suggests the possibility of determining radiation effects on localized areas of the nematodes and study of the resulting hereditary results.

Those of you interested in analytical work will appreciate another advantage of the X-ray microscopes of either the reflection or projection types. X-ray diffraction patterns from selected areas of the specimens can be obtained. Combined with X-ray photomicrographs, quantitative information and specific element detection can be obtained about the composition of small areas on the order of one square micron.

My opinion is that the X-ray microscope probably would have more applications than the electron microscope in phytonematology for those situations where magnifications and resolutions equal to those of the light microscopes would suffice. At the present, we can only guess at the value to microscopy of this new research tool.

Visibility of Transparent Specimen Detail

As previously mentioned, our problem in the microscopic study of nematodes is not one of magnification alone. The study of the nematode is essentially examination for fine details in a transparent material. The well-known solutions to this problem ordinarily involve one or more of the following: Staining of the specimen. This is done only with difficulty with fixed nematode specimens and is rarely satisfactory with *intra vitam* stains. Staining of fixed specimens presents additional problems of distortion, artifacts, and considerable consumption of time. Substage diaphragm adjustments are very helpful in improving the visibility of otherwise nearly transparent specimens. Despite the theoretical disadvantages through reduction of resolution, marked improvement in many structural details of the nematodes result, but there is no denying the loss of definition of very fine structures. Incidentally, stopping down the substage iris results in a sort of phase or interference type of illumination. Darkfield illumination and polarized light have, of course, been tried, but for certain special applications they have not been found of value in improving what one can see of nematode structure.

Before going into some of the newer ways of making the invisible visible, a few words of explanation regarding visibility may prove helpful. The normal colorless or monochromatic microscopic object is seen because it has regions of varying optical density which the eye detects as differences in light intensity. In normal brightfield illumination a completely transparent specimen is very difficult to see in any detail, as all of its parts are equally dense. In darkfield illumination the light illuminates the object from an oblique angle and the completely transparent specimens show up due to their scattering and diffraction of the light. Generally, the surface layers, rather than the internal details, are revealed, and there is an exaggeration of the contrast. In polarized light the completely transparent structure becomes visible if it has light directional

or crystalline properties.

Since World War II, important developments in light microscopy have come about. The theoretical limits to the amount of useful magnification and the degree of resolution of microscope lenses have long been known and refinements to attain the theoretical limits are being incorporated into the new microscopes. Surface coatings of lenses, new optical designs with new kinds of glass, all are helping the microscopist. In addition, there has been a trend in the development of the light microscope to utilize the interference phenomenon of light. This trend has been for the purpose of making the invisible, or near invisible, structures of specimens visible. Such structures are not necessarily difficult to see because of smallness of size, but rather because of their transparency.

This particular kind of problem was first overcome with the development of the phase contrast microscope. Commercially available models became available shortly after World War II. Within the past two years, another new type of microscope became available and has as a main characteristic the ability of making invisible transparent structures visible to the eye. This microscope is called the interference contrast microscope. The principle utilized in both types of microscopes is the phenomenon of interference of light waves. Variations in light intensity come about as two beams of light are combined and their waves reinforce or interfere with each other, depending upon their phase relationships. Variations in light intensity in the specimen render it visible. Despite the implication in having two different names, phase and interference contrast microscopes both are light interference type of instruments. They do have important differences and applications as will now be discussed.

Phase Contrast Microscopy

The optical path in the phase microscope is diagrammed in Figure 1. A single beam of light coming from the illuminating source is focused as a hollow cone of light onto the specimen by the substage condenser. In accordance with the structure and refractive properties of the specimen, the light beam becomes refracted into various individual light rays going in various directions. The phase objective is designed in such a way that the light rays it intercepts from the object structure are divided into two beams of light. These beams are made up of (1) the light rays which enter the microscope objective directly after having passed through the object without being deviated or diffracted by the structure, and (2) the light rays which enter the objective lens after having been diffracted from the original cone of light by the object structure. In order to utilize the interference phenomenon it is necessary to put one of the light beams out of phase with the other. This is done by a phase altering plate which is placed in the objective. The two light beams, consisting of the diffracted and undiffracted rays, are then recombined into focus at the image plane where they are viewed and further magnified by a regular ocular or eyepiece. However, because one of the beams is out of phase with the other, when the two are recombined interference occurs. This results in light intensity differences

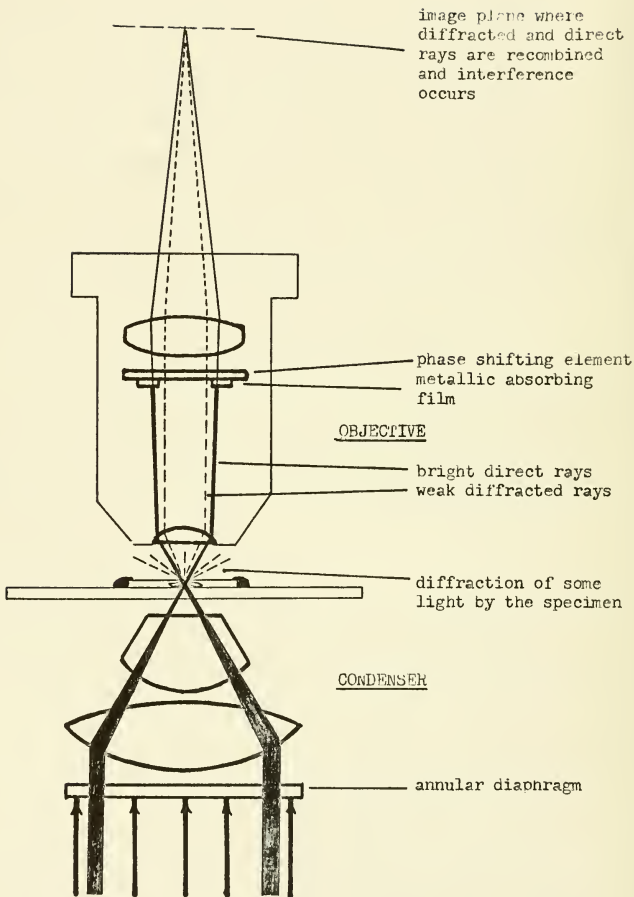


Figure 1. Diagram of phase contrast optics. Eyepiece is not shown in the illustration.

now being visible. These visible intensity differences are related to the ordinarily invisible structural details of the object which do have in themselves slight variations in thickness or refractive index, but which are not sufficiently great to be visible in the bright-field microscope. The phase shifting plate installed in the objective supplies a one-quarterwave shift in phase of the undiffracted light rays; and when these are combined with the diffracted light rays, the resultant interference effects of the two portions of the light which had come from the object form the image of the object.

Various kinds of phase contrast objectives are available and the prospective user may wonder what the differences are and which lenses to choose. Perhaps, some further explanation will help in this matter. Differences in contrast of the specimen image can be obtained depending on whether the undiffracted rays of light, as they pass through the phase shifting plate, are accelerated or retarded one-quarter wave length with respect to the diffracted rays of light. If the undiffracted rays are accelerated, the resulting contrast appearance of the object is called "positive" or "dark contrast." "Dark" contrast reveals the object structures in a manner similar to that seen in a specimen stained with hematoxylin when viewed with a regular bright-field microscope. That is, denser structure appear darker. Thus, in "dark contrast" phase the regions of the object having the greater optical path (thickness X refractive index) appear darker than the surrounding background.

If the phase shift of the undiffracted rays was to retard the rays one-quarter wave length with respect to the diffracted rays, "negative" or "bright contrast" results. Regions in the specimen having greater optical path appear bright against a darker background. The image is similar to that seen by darkfield illumination. The general preference is to use "dark contrast" phase because of the similarity of image appearance to that obtained with stained objects.

The phase altering plate serves the additional function of balancing the intensity of the undiffracted rays of light with respect to the diffracted rays. If one or the other of the two rays is too intense, it "drowns" out the other, obscuring the image contrast. Regulation of this light balance by means of a suitable metallic film deposited on the phase altering plate provides a way of controlling the contrast which can be ranged all the way from no contrast to a pseudodarkfield contrast appearance. The best results for most work lies somewhere in between so that the various degrees of density of the subject are visible as gradations of darkness.

The ability of the phase microscope to make visible structures which are very difficult to see by the bright-field microscope is remarkable and have proved of great value in the study of biological materials. I am sure you know of this. However, there is one inherent aspect to phase contrast microscopy which has limited its usefulness in nematology, at least. This is the necessity of working with very thin subject materials. Even the body diameter of a small nematode renders it too thick for

obtaining good results with phase contrast. Isolated cells or thin structures of the nematodes, such as the cuticle or cyst walls, are thin enough to be seen to considerable advantage with the phase microscope. Obviously, these are rather limited applications. Later, we shall consider in more detail possible applications of the phase microscope to phytoneematology.

Interference Contrast Microscopy

Mainly due to the development of the phase microscope and its many successful applications, interest was revived in developing a satisfactory interference microscope. Interferometry has been used for years, particularly for measuring with great accuracy the refractive indices of many substances. Suggestions for interference microscopes date back many years, but only recently have really good instruments been made and are becoming currently available without being custom made. Thus, for practical purposes, the interference microscope is so new that most biologists know but little of how it operates and what it can do.

It should be pointed out again that the phase contrast microscope operates on the principle of interference of light waves and is essentially a micro-refractometer.

In the interference contrast microscope, as with the phase microscope, two beams of light originating from the same source are necessary. However, in the interference microscope one of the beams passes through the specimen to become altered in phase and the other beam passes to one side of the specimen or is out of focus at the specimen. When the two beams are recombined in the image plane they interfere and produce visible effects. If white light is used for the illumination, contrasts in the subject are apparent as variable colors. If monochromatic light is used, subject variations become visible as differences in light intensity.

Figure 2 diagrammatically illustrates the optical principle of the interference contrast microscope available in this country (A/O-Baker). This instrument is fundamentally a polarizing microscope, but modified to produce the necessary two beams of light which, after passage through the specimen, are recombined again to produce a single image in which the interference effects are visible. Light first is polarized into a single plane and passes through the substage condenser. On top of the condenser is a thin plate of material which has the property of birefringence; that is, it is capable of splitting the polarized beam of light into two separate beams which are plane polarized at right angles to each other. If the two beams are focused by the condenser so that one beam passes through the specimen and the other passes to one side of the specimen, this is called a "shearing system" (Figure 3b). If, however, one beam is focused at the specimen and the other spreads around the specimen and is brought to a focus above it, this is called the "Double Focus system" (Figure 3a). The phase of the light beam

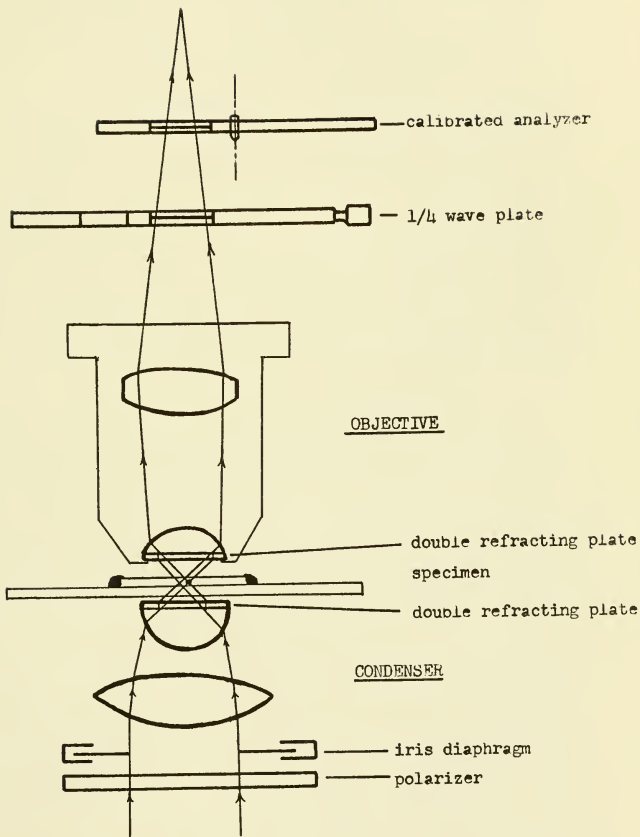


Figure 2. Diagram of interference contrast optics. Eyepiece is not shown in the illustration.

passing through the specimen is changed by local variations in the optical path (thickness X refractive index) in the specimen. Changes in the other beam of light, which is called the reference beam, depend upon the average optical path of the region to one side of the specimen in the shearing system or upon the average optical path of the specimen and the region around it in the double focus system.

After passing through the microscope slide and specimen, the two beams of polarized light are recombined by another thin plate of birefringent material on the front of the objective. The recombined beams are then focused and magnified by the objective lenses, although remaining polarized into beams at ninety degrees to each other. Passage of these beams through the quarter-wave plate changes them into right and left-handed circularly polarized light. In effect, the result of combining the two polarized beams is a single beam of polarized light lying in a polarized plane. The direction of orientation of this plane depends on the phase difference between the two combined circularly polarized beams. The analyser of the instrument is a calibrated plate of polarizing material and is calibrated to measure the direction of the beam. Thus, phase differences in the specimen with respect to the reference beam can be measured.

To obtain results with this type of instrument it is necessary to obtain light entering the system all in one phase. This is done by making adjustments of the substage condenser which produce a series of spectra much as seen as Newton rings between two closely adjacent pieces of glass or much like the rainbow colors seen in an oil slick. By suitable adjustments, a single color (single phase) of the appropriate spectrum is expanded wide enough to fill the field of the objective lens. Thus, the reference beam and the beam which passes through the specimen begin their entry in the identical phase. The other aspects of the instrument make it possible to detect even rather slight changes produced by the details in the specimen.

The interference microscope has been developed and utilized mainly by biophysicists. It has been regarded principally as a measuring tool, and, perhaps, its greatest value lies in this aspect. However, many users will find that otherwise invisible subject contrasts can be seen in different and variable colors as though the structural details were differentially stained. The colors are related to the optical path in the specimen, and with experience one can gain some information from the color differences or changes. So, like the phase contrast microscope, the interference contrast microscope has great potential for the study of living subject matter.

The shearing system of condensers and objectives is preferred for measurement purposes, and I recommend this system for studying nematodes whether or not quantitative work is intended. In the shearing system the reference beam is displaced to one side of the specimen. Therefore, advantage can be taken of the fact that most nematode specimens are relatively narrow in diameter as contrasted with a specimen or structure

which fills the entire field of view of the objective. As the reference beam does not go through the nematode specimen less errors are introduced. With the A/O-Baker instrument the specimen should not be larger in diameter than the central $300\ \mu$ of the field for the 10X objective, $160\ \mu$ for the 40X, and $27\ \mu$ for the 100X water immersion lens.

The interference microscope can measure the optical path differences within reasonably transparent subjects. These difference measurements can be made to an optimum accuracy of $1/300$ wavelength of the light used. The ability to measure optical path difference is in itself not particularly important; the great value is derived from conversion of these measurements into a variety of quantitative information.

Interference microscopy has been available to only a few people for only a few years and much exploratory work is yet to be done. Some of the reported applications indicate that we now have a new measuring tool with a very high degree of accuracy and particularly suited to the study of living, unstained organisms, tissues, and cells. Examples of some of the kinds of work that can be done include the making of wet and dry mass determinations without damage to the living cell. Enzyme action, hydrolysis, partial solution, and changing concentrations can be measured. Refractive indices and protein concentrations of cytological components can be determined.

The question naturally raised is whether or not phase contrast microscopy is now obsolete because of the development of the interference contrast microscope. So a brief comparison of the two may be useful in helping you decide on their possible applications to your own work in nematology. Both phase and interference contrast microscopes make apparent the differences in optical path (thickness X refractive index). The magnitudes of these differences can be measured with interference contrast instruments. Gradients of the optical path within the specimen are shown to greater advantage with phase and there is better contrast for edges, discontinuities, and fine structures. Variable contrast control is possible with both interference contrast and certain new designs of phase contrast instruments. The particular advantages of interference contrast lie in the ability to measure optical path differences, less of the halo effect as seen with phase, colorless structures are made to appear colored (optical staining effect), and it produces images which are more directly related to the structural densities of the specimen than is possible with phase contrast or with darkfield illumination.

The fact that phase contrast is not satisfactory except for rather thin specimen materials has no doubt limited its application in phytonematology. In my own work, which deals with the effects of feeding of the nematodes on their hosts' cells, this is one reason the initial work is being confined to fungi and root hairs which are relatively thin. Similar work being done with the interference microscope has the distinct advantages in that the nematodes are not too thick and that quantitative work may be possible. However, it does have some serious difficulties, too.

The image details are very complex indeed, particularly if white rather than monochromatic light is used. One can learn how to interpret these images only through considerable experience and practice. There is very little in the literature for one to go by, except rather complex physical and biophysical reports, and it will probably be a while yet before more general usages are reported upon. One special problem encountered in these feeding studies is the design of a suitable micro-chamber for sustained observation. Any variations in thickness of any component of the chamber, media, or spaces in the optical path are detected by the interference contrast microscope and can appreciably effect image interpretation. There is no such problem using the phase instrument.

Phase contrast is certainly not obsolete. Mellors said that if the value of a technique can be judged by the information gained by its use and by the number of workers to which the technique is generally available, then phase contrast is probably the most valuable single method available to cytologists for the study of living matter. We in phytoneumatology certainly have not utilized this technique to its fullest capacity yet.

Fluorescence Microscopy

There is another specialized form of microscopy which enables one to detect substances which are not visible with the ordinary techniques. This method involves utilization of the property of fluorescence. Because the technique has been reported in phytoneumatology literature, a brief consideration of it may be of interest.

Some materials react with a beam of light in such a way that light which passes through the material has its wavelength increased. If the light applied is ultra violet light, the reaction with a suitable material results in the production of visible light which is usually colored. I think nearly everyone of you have seen displays of fluorescent minerals and chemicals illuminated with "black light" and showing the beautiful and varied colors emanating from otherwise drab materials.

Plant and animal specimens may contain substances which naturally fluoresce to a certain extent when exposed to a beam of ultraviolet light. Or, there may be substances present whose fluorescence can be increased or in which fluorescence can be induced by special chemical treatments. Also, it is possible to use differential or selective stains which have the property of fluorescence. Examples of some of these stains are coriophosphine, acridine orange, berberine sulphate, rhodamine B, and auramin.

The apparatus needed for fluorescence microscopy need not be expensive or complex unless special high intensity ultraviolet light sources are needed. The ordinary microscope will serve, provided the lenses of the objective and the internal coating of the body tube do not themselves fluoresce under the influence of ultraviolet light.

Fluorescence microscopy has been applied in phytonematology as a technique to distinguish between live and dead nematodes based on whether or not they took up a fluorescent stain such as acridine orange.

Perhaps, the most fertile application of the fluorescence technique would be in intravital microscopy to observe locations and changes of certain materials within plant and animal cells. Some authorities believe that fluorescence microscopy is only just beginning to be developed and has great promise as another valuable research tool.

Reflection Objectives

The final specialized aspect of microscopy, as applied to phytonematology, has to do with the problem of viewing the nematodes at fairly high magnifications (the higher, the better) in action on plant surfaces, in their soil environment, or on special substrates. The problem which arises in using achromatic and apochromatic objectives ranging in magnification 20X to 90 or 100X is due to their having rather limited working distances. Working distance is defined as the distance from the bottom surface of the objective mount to its focus point or, in other words, the amount of clearance that exists between the bottom of the objective and the specimen. The kinds of observations we would like to make of the nematodes require lenses that permit use of simple micro-observation chambers in which ample illumination of the specimen preferably from above, is possible because of opacity of the plant parts under study or of the substrate used in the chamber. As pointed out in the beginning of this talk, the use of lens systems of the optical design, as those found on stereoscopic dissecting microscopes, is not satisfactory. Although these lenses do provide ample working distance, the resolution and the highest magnifications are not sufficient for detailed feeding studies and the like.

There is now commercially available a "new" type of microscope objective referred to as the reflection objective. Such objectives overcome the problems of getting ample working distance so that focusing deep into a micro-observation chamber with adequate incident illumination is possible, even at reasonably high magnifications.

The development of reflecting objectives is really not new. However, it is not generally known among those who now daily use the microscope that in the early days of this instrument both reflecting and refracting objectives were developed and improved and that instruments utilizing both types of lenses were put to practical use. The development of the achromatic objective around 1824 and its subsequent improvements more or less made the reflecting objective obsolete. It is fortunate that interest and progress in the further development of the reflection microscope has been revived, thanks particularly, I think, to the ultra-microanalytical chemists.

Two types of reflecting objectives are available. In one (Figure 4a), the metallic coated reflecting surface or mirror is such that an image of

the specimen is formed at the point of focus of a regular refracting microscope objective. This image is then magnified by the objective and further magnified by a regular ocular or eyepiece. In the other type of reflecting objective (Figure 4b), the mirror system alone forms a magnified image of the specimen; this image is then further magnified by the ocular. The performance of the second type of objective is superior to the first, no doubt due in part to the fewer optical elements.

The regular 43X achromatic objective of the American Optical Company has a working distance of 0.73 millimeter, that of the Bausch & Lomb Company is 0.6 millimeter; apochromatic objectives of comparable magnification have much less clearance, being on the order of 0.18 millimeter. Newton & Company, Ltd. makes a 4 millimeter reflection objective of the first type described above, that is, a reflector plus refractor combination. This device has a working distance of 12.8 millimeters. The well-known British microscope maker, Beck Company, makes reflecting objectives of the second type, which have magnifications and working distances as follows: 15X, 24.0 mm.; 36X, 8.0 mm.; 52X, 3.5 mm.; 74X, 2.5 mm.; and a water immersion objective of 172X (250 mm. body tube length only).

Reflection objectives can be used on regular microscopes, provided there is ample clearance on the nosepiece for the large diameter body of the reflector objectives and provided the body tube can be elevated enough to allow the objective to focus on a subject which may be in an observation chamber having appreciable thickness of its own. These objectives have the standard Royal Microscopical Society thread so they can be interchanged with the regular refracting objectives, if the other conditions for installation permit.

Reflection objectives have other advantages, besides possessing a relatively long working distance, that ought to be mentioned for their value in other applications. Unlike refracting objectives, the reflector units are achromatic throughout the entire spectrum from long wave infra-red, through the visible spectrum, to ultraviolet. Hence, observations and absorptive spectra studies of living cell contents are possible. These objectives are best for microspectroscopy work. There is also less glare because of the reduced number of optical surfaces. Most phytonematologists will, I think, appreciate these objective lenses for the usefulness in viewing nematode activities in micro-observation chambers.

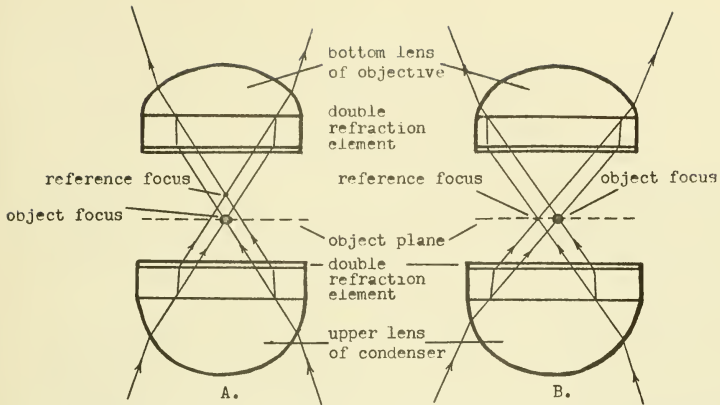


Figure 3. Diagrams of positions of focus of the reference and object beams of light in interference optics.
 A. Double focus system. B. Shearing focus system

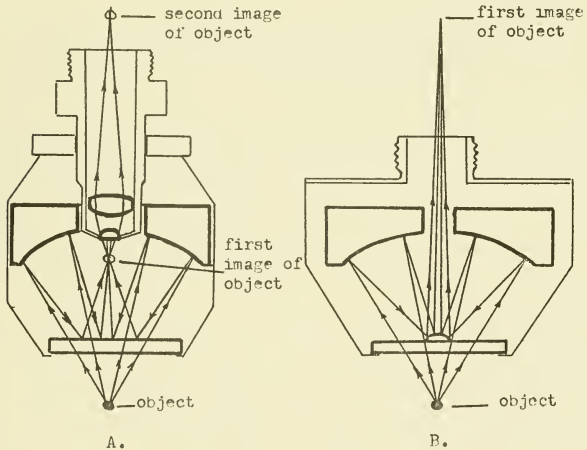


Figure 4. Diagrams of two kinds of reflection objective optics. Eyepieces not shown in illustrations.
 A. Combination of reflection and refraction optical components.
 B. Reflection objective, no refracting lenses used.

Useful References in Advanced Microscopy

1. Cosslett, V. E. 1951. Practical electron microscopy. Butterworths Sci. Publications, London. 299 pp. Electron microscopy.
2. Mellors, R. C. (Ed.) 1955. Analytical cytology. McGraw-Hill, New York. Electron, X-ray, phase contrast, interference contrast, and fluorescence microscopy.
3. Oster, G. and W. W. Pollister (Ed.) 1955. Physical techniques in biological research. Vol. I. Optical techniques. Academic Press Inc., New York. Light, phase contrast, interference contrast, and electron microscopy.

1956. Vol. III. Cells and tissues. Phase contrast, interference contrast, fluorescence, and electron microscopy.
4. Vickers, A. E. J. (Ed.) 1956. Modern methods in microscopy. Butterworths Sci. Publications, London. 114 pp. Electron, X-ray, phase contrast, interference contrast, fluorescence, and reflection microscopy.

All of the above references contain valuable information on other aspects than those cited which only refer to the topics covered in this paper.

DITYLENCHUS
Races, Pathogenicity, and Ecology

J. W. Seinhorst

The attacks of this nematode on various plants have been known since the 17th century. Unfortunately, in the earlier works with the stem nematode new species were made on the basis of new hosts in which the nematodes were found without careful study of the nematodes' morphology. About 1880 Ritzema-Bos studied the morphology of the stem nematode from several of these different hosts and found that morphologically the nematodes were all the same. So he brought them together under the name Tylenchus devastatrix. At the same time, however, he noticed that biologically these nematodes were not the same. He found that he could not always successfully transfer the stem nematodes of one kind of plant to another kind which was also known to be a host of the stem nematode and which was morphologically like the other population. However, because he was thinking along the lines of adaptation theories he never became quite clear about the situation in this species.

Another worker did a good piece of work but unfortunately held to the wrong theory about the biological races. He thought that if you only tried hard enough, eventually you would be able to transfer any stem eelworm from any host plant to any other host plant. In Holland, we have worked with stem nematodes in oats and in red clover and, after a number of years of experimentation, are convinced that the stem nematode of clover will not attack oats. We are also of about the same opinion concerning stem nematodes from hyacinths and from daffodils. That is, one cannot transfer stem nematodes from hyacinths to daffodils or from daffodils to hyacinths. In the clovers there is exactly the same situation. Stem nematodes from red clover do not attack white clover or alfalfa. Those from white clover do not attack red clover, or at least only to a very small extent, and those from alfalfa do not attack white clover and red clover.

This situation in which there are different forms of the nematodes which could not be distinguished, or at least was thought to be indistinguishable on morphological grounds, has existed in other genera, too. For example, this has been the case in the Heterodera and root-knot nematodes. In some of these cases it has been found, after some investigation, that distinctions could be made on morphological grounds. The same has been tried for Ditylenchus dipsaci and so far the only result has been to split off a form called Ditylenchus destructor which had been considered as D. dipsaci. This nematode not only differs morphologically from D. dipsaci, but differs also in the type of symptoms it causes in potato tubers and in other plants which are quite different than those produced by the stem nematode.

The actual stem nematode, D. dipsaci, has not yet been split into different species on morphological grounds, as far as I can determine.

Kirjanova, in Russia, uses the name Ditylenchus allii, but she does not give any good morphological differences. So far, we can only distinguish the different forms of the stem nematode by doing host range tests. Thus, the nematodes are actually host races. Even if we were to find morphological differences, we should not neglect the host range test, because we cannot be sure that there would not be more different forms of the nematodes than we could distinguish morphologically.

At the present time, from my own experience and from the literature, I estimate that there are at least twelve host races of the stem nematode. Thus, there is the question of how these different races are related to one another. As far as I know, all of the races are rather polyphagous. One may find large numbers of different host plants which the different races have in common. There are only a few hosts where one finds differences. These are the plants we utilize for possible tests to separate the different races. For example, the onion race of the stem nematode has been considered for a long time to be the same as the rye race or the race occurring on oats, but it does not attack teasel nor red clover, and it does attack peas. However, in at least some regions of England the stem nematode on onions does attack teasel and onions may be attacked by the teasel race. In Holland, onions are attacked by a race which is different from the rye race and different from the teasel race. So we can go on and by host testing make twelve races of the stem nematode.

Different forms of the stem nematode are distinguishable by different reactions of the plants to the presence of these nematodes. It is not just simply attack resulting in symptoms or no symptoms, multiplication or no multiplication. There are different kinds of symptoms. Feeding and multiplication of the stem nematodes seem always to go together with one particular symptom, and that is dissolution of the middle lamella. When stem nematodes invade the parenchymous tissues, the middle lamellae begin to dissolve and finally the cells are lying loose within the tissue. They cannot, of course, stay alive for a long time like this, so they die soon. If you were to take an attacked rye or onion plant, for example, and place some of the infected tissue in water, you would see that the cells just fall apart from each other. In addition to breakdown of the middle lamellae, in most plants there will also be swelling. Dissolution of the lamellae causes swelling because the intercellular spaces become larger. At the same time, the presence of more air in these spaces make the tissues appear whitish or silvery. Also, other disease symptoms may be found. Especially in growing parts of the plants, there may occur distortions, stunting, swelling, and development of extra parts or extra tillering in onions and in cereals.

* In the case of rye field plantings, attack by the stem nematodes mostly occurs in patches and the affected plants are stunted. In the

* Editor's note: Beginning at this point, Dr. Seinhorst presented a series of lantern slides illustrating symptoms of disease caused by Ditylenchus. Some of his descriptive comments are included here for their value even though the illustrations are not reproduced.

case of onions, the usual condition is stunting of the plants which also have too many short and thickened leaves. Curling of the leaves also occurs, but not so much. Potato plant stems are attacked, and sometimes rather often one finds stem nematodes in the main vein of the leaves with a resulting abnormal bending of the leaves. The foliar parts of carrots when attacked may stop growing and finally rot. Ditylenchus dipsaci, when in potato tubers, spreads through them gradually, making the tissue spongy and yellowish to brown in color. Only when there is a very heavy attack of the whole tuber does a rot caused by fungi or bacteria set in.

This is quite different from the results of attack on potato tubers by D. destructor. The infection sites, instead of being yellow or brown, are whitish and surrounded by tissue where the starch grains have disappeared. The initial invasion is soon followed by secondary rots. Once you have seen potatoes parasitized by D. dipsaci or D. destructor, no further difficulty will be had in distinguishing between the two, as the symptoms are quite different. (The symptoms of stem eelworm on cucumbers are due to the fact that the middle lamellae of tissues between the vascular bundles has been dissolved.)

We are now approaching a discussion of what we call irregular symptoms. Stem nematodes, when entering plants which are unsuitable as food for the nematodes, will cause one of a series of abnormal symptoms. Of course, in some cases no symptoms are produced at all. The most simple, perhaps, of these irregular symptoms is necrosis instead of swelling. For example consider stem nematode resistant clover attacked by the red clover race or any red clover attacked by one of the other stem eelworm races. When the nematodes enter the seedling the tissues show necrosis, the cells dying quite soon after the entrance of the nematodes. There is no development or multiplication of the nematodes in that tissue. Sometimes there appears to be a swelling, but it is due mostly to stunting of the center of the seedling. The cotyledons may bend down, but there is nothing like a real swelling.

Another type of symptom is found in flax. Years ago Ritzema-Bos noted that in flax there may occur symptoms which look like attack by stem nematodes, however, no stem eelworms were found in the plants, at most only a very few. We came across this same thing a few years ago. Again, no Ditylenchus dipsaci nematodes were found in the tissues although the symptoms at first sight certainly looked like the result of stem nematode attack. We performed an inoculation experiment and found that these symptoms did result from the presence of stem nematodes. However, even after inoculation with a few hundred nematodes per seedling, no nematodes or only very few entered the plants. So, one part of the symptom complex may be stunting; stunting that has been caused by these nematodes, perhaps, while trying to enter the plant while it is still in the seedling stage. The other part of the complex, the dissolution of the middle lamella, does not occur; the tissue remains firm, and there is no feeding, development, or multiplication of the nematodes. After some weeks, the plant outgrows the initial effect of the nematodes to the top of the

plant while it was low and near to the ground. We find a similar situation with a weed which grows in sandy soil of Holland. Again, this severe stunting with no swelling and no dissolution of the middle lamellae, and no nematodes in the tissues. Experimentally, it can be shown that the trouble is a result of the presence of the stem nematodes on the young plants. After a few weeks, these plants will grow out rather normally.

Yellow lupine may show symptoms that look anything but being due to stem nematodes, and yet the condition is caused by stem nematode attack. There is stunting, bunching, and irregularity of the small leaflets, and some mottling. It looks almost like a virus disease. This is a condition which can be introduced by inoculating yellow lupine seedlings with stem nematodes of various races. The inoculated plants never recover from this condition. The stem nematode race from red clover attacks yellow clover, but it causes the normal kind of symptoms. The nematodes multiply in the tissues but never is the result a plant with the stunted condition and irregularity of the leaflets.

It seems that cases are never really clear cut in nature, so if I say that in all cases multiplication of the nematodes occur only where there is dissolution of the middle lamellae and normal symptoms; remember that there may be a few exceptions. Thus, it seems that the race of stem nematode from rye multiplies to a certain degree in peas, although it certainly causes abnormal symptoms.*

The abnormal symptoms shown can be considered as different form of resistance of the plants to stem nematodes. Also shown is that these nematodes have different influences on the plant. The salivary secretion of the nematodes may consist of different components. One may dissolve the middle lamella of the plant cells, others may cause distortions, and so forth. It appears that even a very small amount of that saliva can influence the tissue of the plant even at quite a distance from the location of the nematode. The dissolution of the middle lamellae is always around the immediate vicinity of the nematodes in the tissue. However, stunting and other symptoms may occur at some distance away. For instance, strawberry plants attacked by a few stem nematodes from rye or onions may have crinkling of the leaves even though the nematodes are situated about two or three inches from these leaves.

The nature of the symptoms can be used not only for host range studies in differentiating the various nematode races, but also for selection of resistant plants for breeding purposes. This is the present case with red clover in Holland. Selection of resistant red clover is based on selection of plants which show necrosis. It is not the only form of resistance. There is a small percentage of red clover plants which do not show any symptoms at all, or show very slow development of symptoms,

* Editor's note: At this point the talk was no longer based largely on discussion of materials illustrated with lantern slides.

when exposed to the stem nematodes from red clover. However, selection based on necrosis is easier to use because if there is necrosis one can be sure that the result of the inoculation test is positive. With plants not showing symptoms, there is always the possibility that they are merely escapes and are not resistant. Our experiments show that in our inoculation tests plants which do not show symptoms are mostly escapes.

In rye the resistant plants only exhibit very slow development of the nematodes in the tissues and dissolution of the middle lamellae, but to only a low degree. On the other hand, there are resistant rye plants which show necrosis, but as these are not as common in our material as those showing just the very slow development of the nematodes, we do not use them in our breeding program.

Knowledge of the host ranges of different races of the stem nematodes might be of some importance for devising cultural control methods, such as crop rotation. However, it is found that they have hardly any value at all except in such cases where you know that red clover is not attacked by the alfalfa stem nematode, or that rye is not attacked by the red clover race of the nematode. Thus, you know that you can have rye following red clover, or red clover following infested rye, or even red clover in the infested rye without having any difficulty. However, for devising crop rotations, all information of this type is almost useless. That is because the occurrence of stem nematodes in all places largely depends upon the soil type. We can divide the Netherlands into a few regions based on soil types. Heavy soils having stem nematodes are places where serious attacks of some crops occur. On the light, black sandy soil we do not find the stem eelworms. Light clay soils and loamy sand soils are intermediate, crops being attacked under certain conditions. A survey of one of the islands in the southwestern part of the country showed that onions would always have a good chance of being attacked by the stem nematodes when grown on soils containing more than 30% clay. Even if there had been a rotation to a pasture for a long time, the first year back in onions might result in a very heavy attack by the nematodes. However, soils lighter than this, having less than 30% clay, are mostly good onion soils, and there will be nematode trouble if onions are grown more than once in three or four years. Keeping the rotation to onions not more frequently than this assures not having the trouble. The same is more or less the case in growing rye on lighter soils. The farmer can grow rye once in two years and not have much trouble, but if it is grown two times in three years or three years out of four, the whole field may become heavily infested. In slightly heavier soils with less humus, the situation may be that the farmer can grow rye once in four years or so and still have very small patches of plants which are attacked by the stem nematode.

What was the cause or the main reason for the fact that in some places the stem nematodes just do not disappear whatever was done and in other places they were not so harmful as in the heavy soils investigated? We decided to investigate the nematode population fluctuations in a number of fields. We chose the following system for doing this work. It must be realized that, in the case of stem nematodes, which generally are

rather irregularly spread through the field in large or small patches, it is difficult to find a good site for field experiments. Moreover, I think it is pretty clear that there is the influence of soil types. Thus, we had to cover a large area to be sure to include at least a good part of the possibilities; we could not reach any conclusions on just a few fields. So instead of laying out experiments on a few fields with many different crops, we looked for infested spots on some thirty fields and investigated populations in these spots two times a year on very small areas. In the case of rye fields, the infested patches are always more or less elongated with nothing at all in the area around. On the heavier soils, if there was an attack on onions, looking around one finds infested onions spread throughout the field. This is in contrast to the sharper boundaries of infestations in rye plantings. We used sighting reference points to establish the location of the spots in the fields and for refinding these spots on subsequent collecting trips. Measuring to find all the spots would have taken too long.

We were interested in the nematode population fluctuations from autumn to spring and from spring to autumn. To reduce the variation of counts due to irregular distribution, we sampled a small area with a diameter of about ten feet. Of course, what we found on such a small area would not hold for the whole area, but by taking a few areas like this, one gets a pretty good impression of what is happening in the field. Had we taken only a single sample, the variation would have been so large that we could have never reached any conclusions. Now, after having done the sampling for a number of years on different fields and different soils at different times, we compared the differences between the degree of infestation of the stem nematodes in autumn and in spring with the idea that if there is no host crop on the field, the stem nematodes, being obligate parasites, would die. Thus, by measuring the differences in populations between autumn and spring we might get an indication of mortality in different soils when there is no crop present. On the other hand, we could compare the degree of infestation in the spring with that in the autumn and so measure the influence of the different crops.

We found that, generally, the reduction of the nematodes in the autumn, when there were over 100 stem nematodes per 500 grams of soil, was three to one to the spring counts. That is, the number in the spring are about one-third those in the autumn, if the numbers in the autumn were high (above 100); this is on light soils. On sandy soils, the reduction ranged from ten to one to six to one. There is quite a lot of variation because the light soils include different soil types. It was found that when the degree of infestation in heavy soil in the autumn was distinctly below 100 per 500 grams of soil, there was a good chance that there was no further decrease. At least, we can say that in heavy soils the degree of infestation of a spot seems not to go down below ten stem nematodes per 500 grams of soil whatever happens on that soil. Mostly, it remains somewhere between twenty to sixty stem nematodes per 500 grams of soil.

On the light soils the number of the nematodes continues to decrease

until a level of below five nematodes in 500 grams of soil is reached, if only one does not grow onions, rye, or oats. This is a very important difference. We found that when growing onions, rye, celery and even potatoes, if the degree of infestation is over ten stem nematodes per 500 grams of soil, there is a good chance of serious damage to these plants. That explains why onions grown on the heavy soil always have a good chance of being attacked.

Ten nematodes per 500 grams of soil sounds like an unbelievably small number to produce serious damage, but it is a fact. Most likely, the nematodes keep alive on weeds, although most of these onion soils are rather weed free. Another point is that on heavy soils the stem nematode population does not go down below ten in the winter time. Whatever the crop grown in the summer, susceptible or not, it does not matter, the stem nematode population has a general tendency to rise.

We have the impression that the population declines on different soils are not only different between the light and heavy soils but also show differences in the various kinds of light soils. This accounts for the possibility of growing rye once in two years on the very light soils. In these two years, the stem nematode population drops down from about two hundred to below ten, whereas this decline takes a longer time on the slightly heavier soils. Of course, all these studies do not help the farmers very much. They are only very sure now that they cannot grow onions on the heavy soils. Whatever they do, whatever rotation is used, it is impossible to have a good crop of onions in most years on these heavy soils if they are infested with the stem nematode; and it seems that all of our heavy clay soils are infested.

The next step we are trying for horticultural areas which are in this same condition is to completely eliminate the weeds and see if this will improve the situation. However, there are difficulties both in the expense of complete weed control and in the detection with accuracy of population numbers less than ten per 500 grams of soil in reasonable numbers of samples. At any rate, all this illustrates how much different the situations are between Ditylenchus dipsaci and, for example, Heterodera, where crop rotation is one of the answers for getting control. With Heterodera, the situation seems as though it can be simply put like this: If a host crop is grown, there is an increase in the nematodes; eliminate the host crops, and there is a very good chance that within a certain period of years the degree of infestation goes down below temporarily to a level which is dangerous to the plant. Obviously, this is not the case with all kinds of nematodes. It is likely that the situation with Heterodera is more the exception than the rule. The nematode populations in the soil are influenced by crop rotations, of course, but this influence is limited. There are these low infestation levels below which the populations may not drop.



HETERODERA

F. W. G. Jones

LIFE HISTORY

First, a little about life history. Let us begin consideration of the life cycle with the egg enclosed within the cyst lying in the soil. The nematode is usually induced to hatch, although not always, by means of a root diffusate. The second stage larva, there having already been one molt within the egg, emerges from the cyst and migrates through the soil. Finding a suitable host, the larva invades a root leaving a trail of damaged root cells which may be very easily seen by special preparation. Eventually within the plant the larva begins to swell and goes through another molt which brings it to the third larval stage. It is not easy to distinguish at this third stage between the larvae which are to be males and those to become females. (I am using in reference to this the admirable work on Heterodera by Dr. Raski*.) It is possible to see the rudiments of what is to become the testes and in the female the bi-lobed ovary. There is more evident differentiation of the sexes in the next molt. In the male a certain amount of elongation occurs and you can then see by the time of the molting the fourth stage larva coiled within the cuticle of the previous stage. Before the fourth molt, the ovaries have elongated but have not yet been connected to the exterior of the nematode. The fourth molt occurs and we now have the fully mature stages. The male's stylet has developed and the copulatory spicules are visible and the male breaks out of the "cyst", if I may call it a cyst. This "cyst" is usually slightly different from the female "cyst"; it is more flask-shaped. And, I believe I am correct in saying that, in the earlier stages it lacks the little blip at the end of the larval forms such as occurs in the Meloidogyne. In the female, taking as an example one of the lemon-shaped types, the ovaries make contact to the exterior through the vulva and then a small amount of jelly-like material is extruded. At this time the female is ready for copulation. The male goes into this jelly. One can find, if one examines many specimens, the male tightly coiled about the vulva. I think this is, in fact, the act of copulation.

There are species in the Heterodera in which males are not known

* Raski, D. J. 1949. The life history and morphology of the sugar-beet nematode, Heterodera schachtii, Schmidt. Phytopathology 40(2): 135-152.

to occur, for example, H. trifolii. One presumes that reproduction is parthenogenic in such cases. In other species, as for example H. schachtii and H. rostochiensis, evidence is now accumulating, slowly it is true, that the male is required. There is a recent note by Fassuliotis to the effect that he had raised ten isolated individual H. rostochiensis females and these were unable to produce eggs in the absence of males. Ellenby in England has made similar observations, as yet unpublished. Williams in England has made studies using potato slices for cultures. The slices are first surface sterilized and can then be inoculated with suspensions of eggs or with individual nematodes, which can become established quite well. Williams put a hundred single larval nematodes on separate potato slices and obtained only two females which did produce eggs. It is interesting to note that in the other females which did not produce eggs, there was a vestige of an egg sac. After fertilization, ovulation begins and there is a gradual filling of the body with eggs. The larvae develop and molt once to reach the second larval stage. Development is usually arrested at the second larval form.

I don't think we know too much about the browning of the cyst. It is said to be akin to the browning and hardening of insect cuticles. It is my general observation that the browning doesn't actually begin until the death of the female, that is, the really pronounced browning and hardening due to the presence of polyphenol oxidases and so forth. In H. rostochiensis I don't believe that the earlier golden color and condition is the same as the later hardening and browning of the cyst. One also sees an intermediate yellow color in such cysts as H. trifolii and H. galleopsis. This is an intermediate creamy color as compared to the golden color of H. rostochiensis. Species like H. schachtii and H. gottingiana remain white and translucent until death then the browning occurs.

PATHOLOGY

Let us now turn to the subject of feeding. I don't need to say much about this because Dr. Christie has dealt with it fully. The position is almost like that in the Meloidogyne and giant cells are formed but, by and large, there is almost no hypertrophy. In the case of H. schachtii in young roots there may be a slight swelling in the vicinity of the head, but this appears to be due almost exclusively to the presence of the giant cells. There is no real hypertrophy, such as you get with the Meloidogyne. There is a little bit more hypertrophy in some hosts, for example, in tomato attacked by H. rostochiensis. In this case it would be possible to mistake the presence of the cyst nematode for that of the root-knot nematode, although close examination would reveal the difference.

The gross effect of feeding is usually to produce a proliferation of the root system. This is true of all of the species except the pea root eelworm and, as I learned yesterday, the soybean cyst nematode.

There, apparently, the normal proliferation which we get with most of the other species is not apparent. Also, in the leguminous plants nodulation is suppressed.

In looking at a field the first sign that the farmer gets that the cyst nematodes are present is a stunting of the plants. In the case of the sugar beet root, the main root or tap root is attacked and there is a proliferation of lateral roots which in turn become studded with the swollen and white female forms. In peas the main root may be somewhat thick and fleshy and one may be misled by not seeing the nematodes on the surface. However, upon dissecting the root large numbers of the males and females which are imbedded in the roots may be easily found. When the lateral roots which are smaller in diameter are inspected, the female nematodes may be found showing at the roots' surface. One may get heavy infestations of this type without root proliferation and loss of the nodules.

TRENDS: Egg sac development

Within this nematode group there are certain fairly obvious trends, for example, consider the egg sac. Let us start with the Meloidogyne. In this genus there is a large egg sac and, if I am not mistaken, virtually all of the eggs are extruded into it. When we come to the Heterodera we find some that are not quite reminiscent of that, for example, H. cruciferae and H. carotae, in which the egg sac is quite large and absolutely full of eggs. There may be fully half of the eggs produced laid in these sacs. If one is lucky, he may see these eggs maturing and the larvae hatching. This having eggs in an external sac is of some importance in making larval counts, that is, encysted egg counts. The next stage is found in H. gottingiana which has an intermediate size egg sac containing a fair number of eggs. Then we go on to types like H. schachtii and H. cactii where only when the cyst is fairly mature and well-filled with eggs are eggs laid in the sac. Next we come to forms like H. humuli and H. avenae which have a perfectly good egg sac, but without eggs being pushed into it. It may be, although I am not sure, that this is due to the fact that the vulva is too small for the eggs to be pushed through. Now, at the end of the series we have H. rostochiensis in which no egg sac is formed, although there may be a slight trace in earlier female stage. Certainly, in this case the vulva is not large enough for the eggs to go through as is also true for H. major. I, personally, believe that the egg sac is a constant feature of all of the species, but that it is usually overlooked as it is sticky and may adhere to the soil particles and so be lost.

TRENDS: Response to root diffusate

Another trend that one may distinguish is the response to root diffusates. I suppose that the response to root diffusates is a sort of ultimate refinement to parasitism. The idea being the nematodes wait in

the soil until a root comes close by and stimulates hatching. It is not known for certain that root exudates are necessary in the soil to stimulate hatching. Personally, I think there is a lot of spontaneous hatching going on in the soil. We must not get the idea that diffusates are absolutely essential. In this matter of response to diffusates we can divide the nematodes into a number of categories. There is first of all those types which just do not respond to the diffusates at all. In H. major at certain times of the year you will get no response at all, but if you collect cysts from the soil in the spring and keep them moist, this is quite important with this nematode, and incubate them at the temperature of 55°C. then you can get perfectly good hatch. Using grass root diffusates makes no difference. It is apparently only the water which is operative. There is another group, although I don't know you can call these groups yet because in some there is only a single species, in which there is another type of response. This is exemplified by the pea root eelworm, H. gottingiana, in which you can get no hatch whatsoever. This is quite a puzzle, for if you take exudates from pea roots and expose H. trifolii to them you do get a stimulation of hatching. Let me remind you that this is with a diffusate from a plant which is not a host for the H. trifolii, and so we have another puzzle. The point is, the pea roots do produce a substance which is capable of inducing hatching but the pea root eelworm does not respond to it. In the field there are some leguminous plants which can reduce cyst nematode populations. These plants are non-hosts, but they do stimulate hatching. We can confirm this in the laboratory.

Another group based on hatching response is what I call the H. schachtii group and I think H. trifolii falls into this group. In it one finds quite a marked water hatch; quite a lot of larvae come out. As a sideline, H. schachtii is therefore a very useful laboratory animal because you can do work in hatching studies without having to rely on root diffusate which would complicate the experimental work. In this group there is a much higher hatch with diffusates from host plants and from some non-host plants within the same family. That is, we have a high water hatch with a much higher hatch from host diffusates.

Then we come to the last group which we may regard as more highly adapted parasites. The water hatch is very low and the host hatch is very high. In that group will come H. rostochiensis, H. carotae, H. cruciferae, and H. humuli. So you see we have in all these groups mentioned a range of physiological responses to root diffusates.

One can look at this thing in two ways. That is, we can propose two hypotheses. One is that these diffusates are a single class of substance and that the minor differences in specificity are due to modifications of the same molecule; we have one kind of basic material with some differences in structure. Alternatively, one can consider the hypothesis that we are not dealing with a single kind of material,

but rather are concerned with a complex of substances. Some support is lent to this by the fact that in some species there may be stimulation due to such materials as inorganic salts. Most of this kind of work has been done with H. schachtii. If you use such a substance as common salt or even such an unlikely substance as mercuric chloride, you will find that you can get at certain concentrations quite a noticeable increase in hatch. Of course, as concentrations get too strong the hatch drops off to zero. You can get this result with a variety of things such as urea, amino acids, and some sugars. So you see that there are a number of materials which one might find in the rhizosphere, mercuric chloride excepted, and which one might expect to contribute to the hatching. I would like to mention here we have tried a substance found by two American workers which they have called galactinol, which they have isolated from sugar beets. In their paper they have said this is one, if not the, hatching factor. In England we have tried this chemical with our sugar beet nematode and have found no evidence of it having any hatching value at all. Its effect is quite indistinguishable from that of water. Now it may be that we are wrong and they are right, or that we are both right. I suppose a little more work is indicated. Whenever you get differences in work of this type, it does not mean that the other chap is wrong, rather it means more investigation is necessary.

TRENDS: Dessication

Another trend within the genus is the ability of withstanding dessication. Some species, like H. rostochiensis, can put up with quite a lot of drying. These cysts when extracted from the soil can be stored almost indefinitely and hatching will occur if they are given a sufficiently long soaking in water, which may be as long as fifteen days or so. Almost anything you do to H. schachtii seems to reduce its hatching. For example, lowering the oxygen content in the water and dessication. H. schachtii, particularly our H. schachtii, doesn't seem able to stand up under dessication as does H. rostochiensis. Our H. schachtii may be different from yours. Drying appears to kill H. major. You must therefore be very careful in doing work with hatching tests. You must not use glaring lights which may heat them and do not do any other thing which may effect their hatching. These considerations are quite important.

HOST RANGES

Now I would like to turn to host ranges. The systematist is obviously a very important man; he tells us which nematodes we have. But from the farmers point of view, the man who is more important is the one who can tell us what the host range is. Economically, the host range is, I think, of paramount importance. Among the Heterodera I think we can divide them roughly into two types. There are those like

H. schachtii, H. trifolii, and H. galeopsidis where we have species that are fairly polyphagous and attack plants in several families. They may be a bit choosy in some plant families, missing out on some genera and attacking others, but they do cover quite a range of families. As far as the beet nematode is concerned, which is fairly polyphagous, the two chief families are the Chenopodiaceae and the Cruciferae. Other minor host families are ones like the Caryophyllaceae and Polygonaceae. It is interesting to know that host range studies done by Dr. Kaski in the U.S.A., by Oostenbrink in Holland, and by myself and Winslow in England, do agree very well, although there is quite a difference between the source of our materials. The only difference that I have detected is that in America one would include the Labiatae. We so far have not found this. When we turn to the other species, species like H. major, H. cruciferae, H. rostochiensis, H. tobacum (which is fairly similar), H. gottlingiana, H. humuli, and H. carotae, they are a good deal less polyphagous and tend to attack plants in one family and sometimes restrict themselves to only one or two genera. I suppose of all of these, based on our present knowledge of host ranges, which I must say is far from complete, the most specialized is H. carotae which attacks only one or two species of the genus Daucus. I don't think we can divide up the groups on the basis of host ranges beyond this; that is, divide them up soundly on the basis of scientific evidence. It is of great interest that some of these host ranges overlap. For example, H. schachtii and H. cruciferae may have their host ranges overlap among the Cruciferous plants. This would, I think, make a very interesting bio-chemical study to see what it is that these two eelworms find in their common host plants and yet do not find in the parts of their host ranges which do not overlap. In other words, what are these substances which go into making up the differences in host ranges? The same thing is seen, perhaps more acutely, with H. rostochiensis and H. tobacum where we have two very similar eelworms with a certain amount of overlapping of their host ranges within the same genera, Solanum and Nicotiana. This would be a very interesting study indeed for a bio-chemist.

Well now, it is all very well to study a host range and find which plants are attacked, but there are varying degrees of host efficiencies in raising the nematode population. There are some hosts that are more efficient than others and that is a matter of some importance to the farmer. There are, in fact, two attributes of host plants. One is this business of supporting invading nematodes and the production of new cysts and the other is susceptibility to invasion by the nematodes. I submit that these two things are not necessarily related. For example, in H. schachtii you have a host like the sugar beet which is an efficient plant, but which is highly susceptible to injury by the larvae. On the other hand, you have in the Cruciferae plants which are perhaps even more efficient in raising the nematode population, but which don't suffer to anywhere near the same extent due to larval invasion. If you were to put sugar beets and these crucifers in the same soil, the sugar beet may collapse whereas the crucifers may continue to grow showing only a

partial diminution in growth and not collapsing because of nematode injury. So you see there are these two attributes and they are both very important in connection with breeding. Most of our breeding programs so far have gone on the basis of host efficiency, but I think we ought to turn our attention to resistance to troubles due to invasion, remembering that these two are not necessarily the same thing.

ECOLOGY

Now to turn to something different. We can break down the Heterodera life cycle into a number of phases. The first is the hatch from the egg and the second is emergence from the cyst. I point out that there may be conditions which can effect emergence even after hatching. It is conceivable that there may be larva which hatch within the cyst but are unable to get out, so emergence can be a different phase from hatching. The third phase is movement in the soil towards host roots. Fourth in the series is the actual act of invasion. When infection is completed the organism is out of the soil, but up to that point it had been entirely dependent upon the soil as its environment.

It is true that the plant itself may be modified by certain conditions such as length of day and by the soil as a medium for plant growth, but after invasion occurs it is the plant primarily which provides the environment for the nematode. Stage five is its remaining development and then mating. There are times, of course, when the male goes back into the soil environment and its actions and movements are there governed by the same conditions which regulate the earlier stages. The process of producing eggs by the female is, I am sure, very dependent upon the nutrition obtained from the plant. That then marks the end of dependence on the plant for, I think, that once the eggs are fertilized and have their egg shell around them they become to a large extent independent of both the plant and the soil. It is probable that the embryo can go on developing up to the second stage, provided there is enough moisture.

These thoughts can be summarized as follows:

	<u>Environment</u>	<u>Life Cycle</u>
I	Soil	1. Hatch 2. Emergence 3. Movement in soil 4. Invasion of plant
II	Plant	5. Remaining development 6. Mating (male may go back to soil) 7. Ovulation
III	More or less independent of soil and plant. Needs moisture.	8. Embryonation

I have working with me at Rothamsted a man who followed me from Cambridge named Wallace. He is concentrating particularly on studies of the various factors in hatching, emergence, and movement in the soil, but hasn't yet gone on to effects of invasion. Personally, I think he is doing some really fine pioneering work using techniques borrowed from soil physics. Perhaps, there may be opportunity to say more about that later; I regret I do not have the time right now.

POPULATION STUDIES: Pathogenicity

I would like to pass on to the effects of host populations and initial Heterodera populations on yield. This is being studied at two or three different places using pots and microplots. In studies with H. cruciferae and H. schachtii, and in the U.S.A. with H. tobaccoe, the consensus of opinion is that low inoculum levels can increase plant yields in pot experiments using sterilized soil. In one experiment which I did, the difference was significant. One therefore supposes there can be a stimulating effect from low population levels. As the nematode population levels rise, yield begins to decline in a manner that can be plotted. Figure 1 shows this on a logarithmic basis and on an arithmetic scale.

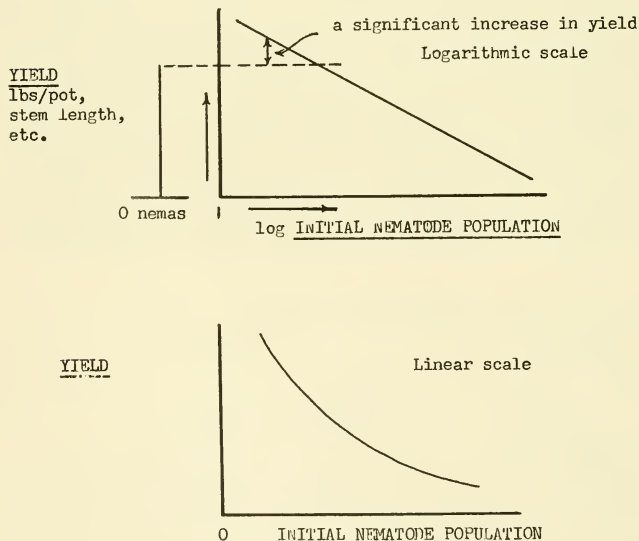


Figure 1. Yield in relation to initial nematode population.

The interpretation is briefly this. As the nematode population gets higher it takes relatively more nematodes to create the same amount of injury as it did initially. I think the reason for that is perfectly straightforward in that, when there are a lot of nematodes in the root they compete with each other and modify to some extent their own effect. Thus, it requires relatively more nematodes to cause a big diminution in yield than it does to cause a small one. The first few nematodes produce relatively more effect.

In the case of sugar beets one is not only interested in yield but also in sugar percentage. In some of the old literature you will find that the sugar beet eelworm is said to decrease the sugar percentage. Plotting the effect of the initial nematode population against the percentage sugar (Figure 2) does show an apparent increase in the sugar.

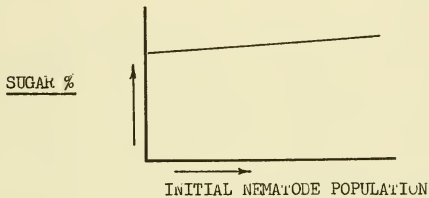


Figure 2. Sugar content of beets in relation to initial population.

The explanation is that this is the normal thing in that, the smallest roots have the highest sugar percentage. Sugar beets exposed to increasing levels of nematode population produce smaller roots, whereas the more normal large roots which develop from being exposed to lower inoculum levels have a lower sugar count. It appears that the eelworm has a very limited effect, if any at all, on the sugar physiology of the sugar beet root. This would suggest that the toxins which are produced by the nematodes remain localized and haven't spread far into the main tap root. That, of course is only a hypothesis, and I wouldn't want to press this too far, because many other things go on in the sugar beet root besides sugar production.

POPULATION STUDIES: Generations

Next, I would like to deal with the number of generations of the nematode which may develop in a year. In order to get the maximum number of generations, the soil must be in the right sort of physical condition and above all have sufficient moisture. During the summer months in the eastern part of England and probably, for all I know, in the United States and elsewhere, one finds that there is in well-drained soils a period during July and early August that soil moisture is limited. When the amount of moisture in the soil is not adequate to permit suf-

ficient hatch, larval movement, and proper invasion of the roots, there is created a kind of hiatus in the population development. There are also regions in England certainly, and probably elsewhere, on muck soils where there is a high water table and that hiatus does not occur and the nematode generations go straight through.

Suppose we are working with soils in favorable conditions and we start with the beet nematode. It so happens that if we take the size of the nematode, starting with the larval of about 500μ up to a fully grown female which is about 1000μ , certain stages in development occur at fairly fixed points in size so we can set a number of relatively well defined stages. You can pull up the roots and taking always the largest cyst, which represent the products of earliest invasion, you can chart the stage of development reached. Sow the host plants at different times and you can produce in that way what I call developmental contours.

Let us consider as an example, work done with H. carotae on carrots. Carrots were grown at intervals. One finds that as soon as decent sized roots are produced they are invaded almost at once. Then you next find the swollen females, or early cystic forms as I call them. The cortex is ruptured, the eggs are forming and becoming fully embryonated. There is another stage beyond that, which is the liberation of larvae, which in a sense completes the life cycle. This latter is a very difficult thing to observe in the soil. Now in this particular case with the carrot, there were 2, or perhaps, $2\frac{1}{2}$ generations. If you plant as early as February you get slow development first, because of the low soil temperatures. At the later higher temperatures you get more rapid development and then in the autumn, as the temperatures begin to cool down, we observe a rapid earlier development and later dying off.

With the pea eelworm I have actually taken it right through the winter on beans as a host. Development is merely arrested and the nematode by no means killed under our conditions so development again commences as soon as soil temperatures rise. It looks as though under our conditions, provided the crop grows long enough, for most species except H. major, which is rather different, you can get the maximum of about $2\frac{1}{2}$ generations per year. On sugar beet which grows for us from about March right through to November we could get $2\frac{1}{2}$ generations. The last generation is often in the form of very many small cysts which you can see on the roots in October and which don't develop any further. Potato root eelworm with us probably doesn't go through more than say $1\frac{1}{2}$ or, at the very most, 2 generations, more likely 1 generation; H. major only 1 generation. Beet eelworm development depends on the vegetative period of the crop and whether there is an adverse drying condition in the soil during the summer. That seems to be the situation with us. Whether it would be the same under your conditions I don't know. In some of the soils, for example in Mr. Thorne's area in Utah where you have irrigation and high soil temperatures, I wouldn't be a bit surprised but that the sugar beet nematode goes through more than 3 generations. It would seem to me to

be quite possible. If one went north, say into Scandinavia or northern Europe, it may very well be that the maximum number of generations reached is 3.

POPULATION STUDIES: Host effect

We shall next turn to the effect of the host on the soil nematode population. This work, which I am going to describe to you in outline, has been done in two ways. The early work was done in fields using gridded plots to try to reduce effects of variability. Later, I turned to microplots which were 2 feet long by 3 feet wide and 2 feet deep. The soil before being put in the microplots was mixed to destroy uneven fertility effects and to even up the cyst nematode population. This reduces to a considerable extent the within-field variability. Most of this work has been done with sugar beet eelworm and one can get a fairly good measure of the effect of the host on the soil population. In the first experiments I grew the host plants as crops, that is, the plants were spaced in the plots as they normally would be in the field. Thus there would be more plants of one kind per microplot than there would be of others. For example, there were only four sugar beet plants in a plot, whereas there were many rape plants per plot. The nematode population levels can be set up as low, medium, or high, as desired. Nematode population levels in the fallow plots, in a sense, represent the check during the course of the experiment. The levels in these plots taken at the end of the season represent the maintenance level for the nematodes without plants being present as an influence. After the plots are used for a year, the eelworm populations may be considered to be the one factor which has varied the most, whereas there is still a fair degree of homogeneity in fertility and soil structure, although these will be shifted a little. In the next year, having created different cyst nematode population levels, you can do further work with them.

Using these microplots it has been possible to study the effects of numerous kinds of plants on the soil population.*

The findings can be summarized into a graph in Figure 3.

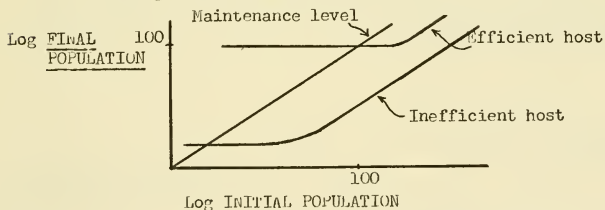


Figure 3. Effects on final nematode populations on hosts of different suitability or efficiency.

*Editor's note. At this point Mr. Jones presented and discussed considerable data in tabular form on lantern slides, but these are not reproduced here.

The graph plots the initial cyst nematode population levels of the soil against the final population levels. The diagonal straight line represents the maintenance level, where the final populations equal the initial populations. If the data are plotted in terms of logarithmic values for convenience, the effects of the plant on the populations are shown by the remaining two curves. If the host is an efficient host and the initial nematode population is not too great, there is a resulting increase in the population, as shown by that portion of the curve to the left of the maintenance level line. If the population is too high, then there may be a decrease in actual level, as shown by that portion extending to the right of the maintenance level line. The curve is more or less straight, though I am not suggesting it always is so. It seems there is some sort of a ceiling effect on the population level, so that regardless of the initial population, the levels build up to this value. I don't know that we have an explanation for this.

The remaining curve illustrates what happens to the soil population if the host is an inefficient one. At low initial population levels there may be a slight increase, but at higher population levels there is a decrease in nematodes to below maintenance levels. How much the decrease varies with the kind of plant.

ROOT DIFFUSATES

I would like to deal with one other matter which I think of very great importance. I shall talk of some of the work of Dr. Fenwick, who is really doing pioneer work on the standardization of root diffusates. Eliminating a lot of the details, which I really out to give you, the essence of his work is this. Carrying out of hatching tests is done by using batches of cysts of not less than 100 in five-fold replications, making sure the cysts are drawn from randomized plots and are thoroughly mixed to assure a uniform standard cyst product to begin with. Also needed is a standard batch of root diffusate of high potency, which is kept in the refrigerator except when in use. A series of replicate cyst batches are set up in the usual manner in watch glasses, diffusate is added, and these cysts are incubated usually at 25°C. For example, set this up as a dilution test using the diffusate at full strength, 1/10, 1/100, and with water as a check. The results of this hatching test can then be plotted (Figure 4.)

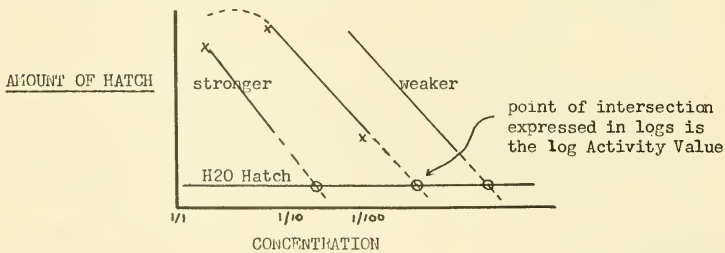


Figure 4. Determination of log activity values for root diffusates.

The water hatch, of course, is the same throughout and may be represented as a straight line. The diffusate can be too strong giving an inhibiting effect. The important thing is that there is a region on the curve through which a straight line can be drawn. That straight line cuts through the line of the water hatch and this intersection is used as a reference point. The dilution value at this point can be conveniently expressed in logarithms, for example, a dilution of 1/100 has a log of 2. We would then express the strength of the diffusate as having a log activity value of 2. Use of this procedure does give us a means of standardizing the diffusate.

Suppose the biochemists are working on the active factor in a root diffusate. They may extract it and produce what we call unknowns; there may be several of them and we want to test their activity. The man who is responsible for the assay has to ask the chemist about the level of extraction, or how much it has been concentrated. That is, in making up these tests the fractions have to be of such a concentration or dilution that the straight line portion of the curve will be found. For example, if the unknowns are too concentrated they may give an inhibiting effect and one couldn't get any measure of hatch. So you try to set up about five dilutions, at least three of which you hope will fall on the straight line portion of the curve. Then you set up the batches in five-fold replications with not less than 100 cysts per replica, as mentioned before. Often the batches of cysts are weighed out, not counted, which is just about as accurate and much quicker. You also set up a check with the standard root diffusate of known log activity. From the results you can estimate the activity of the fractions produced by the biochemist. And to conclude, I want to add that there is not much use in taking the hatching results as of only a week or two. The tests must be permitted to run to completion. I have heard Dr. Fenwick tell chemists who have come with their series of chemicals and ask for the results the next day, "You take a hen and put some eggs under her. You just cannot get chickens in a day. You simply have to wait for them to hatch out. It is the same with nematodes, you cannot get hatching in a day; you have to wait until the test has gone to completion."

HETERODERA TAXONOMY

A. L. Taylor

The genus Heterodera was placed in the family Heteroderidae of the Tylenchoidea by Thorne (1949) with the genus Meloidogyne (root-knot nematodes). Prior to 1949, species of both these genera were placed in the genus Heterodera. Prior to 1940, there was a strong tendency to refer all of the cyst-forming nematodes to a single species, H. schachtii Schmidt, 1871, though some effort was made to differentiate races, strains, or varieties according to the host plants attached. However, little was done toward study of morphology, and identification was difficult unless the host plants were known. Some progress has been made in recent years, though the situation is yet far from satisfactory.

Morphology

The adult females and cysts of Heterodera are the forms most commonly encountered. Adult females or cysts will be found on roots of various plants if these are carefully removed from the soil and washed. The nematodes are attached to the roots by the neck only, with most of the body outside the root. The females are white or yellowish in life, and the cysts are light to dark brown. Average size is about 0.5mm by 0.75mm. Some species are lemon-shaped, others are pear-shaped. Cysts are very highly resistant to decay and may be found in soil in which infected plants have grown, even many years afterward.

The males are slender worms shaped very much like Meloidogyne males. That is, they are about 1.25 to 1.75mm long, slender ($a = 35-40$), taper slightly anteriorly, and have a short rounded tail (Goodey, 1951, Fig. 70). Males will be found in abundance at certain times of the year, but may be very scarce at other times. The larvae have an average length of about 0.5mm. They differ from root-knot nematode larvae in that the stylet is 20 to 30 microns long (Meloidogyne, 10-11 microns) and in the shape of the anterior end.

Excellent drawings of the larvae and other stages of H. schachtii will be found in "The Life History and Morphology of the Sugar-Beet Nematode, Heterodera schachtii Schmidt," by D. J. Maki (Phytopath. 40(2): 135-152, 1950). Larvae are seldom found free in soil, but can easily be obtained from the cysts.

The cysts are important contaminants of imported plant material and also are searched for in soil in connection with quarantine and rotation programs in various countries. Consequently, they have been intensively studied, and most of the present information on identification of species is based on characters of the mature cysts and their contents.

A key to aid in identification of cysts is presented. Key characters will be found on the mature cysts.

Life History

Larvae in the soil enter the roots of plants near the root tips and begin to feed on the developing tissues. Here they undergo three moults, breaking through the outer root tissue at the last one. Females remain attached to the root by the neck. Males leave the larval cuticle and go in search of females. Apparently all of the females of the H. schachtii and H. gøstingiana groups deposit some eggs in a jelly-like substance, forming an egg mass or "egg sac." However, these species also retain eggs in the body so that by the end of the life of the female, the body is tightly packed with eggs. Females of the H. rostochiensis and H. cacti groups do not deposit any eggs, retaining all in the body. The female finally dies and her cuticle becomes transformed into a cyst filled with eggs. Eggs in the cyst develop to the first larval stage, then moult once, becoming second stage larvae. Apparently hatching may then take place immediately, or the larvae can remain in the eggs in the cyst indefinitely.

It has been shown that root excretions of various plants can stimulate hatching. Most work on this subject has been done with H. rostochiensis. When cysts of this species are placed in leachings from a growing potato plant the rate of hatching of their eggs is enormously increased. However, it is seldom that all the eggs in a cyst hatch even under the most favorable conditions. In the absence of a host plant only a few eggs hatch each year. Hatching after 17 years has been reported in the literature. Probably the maximum time under most conditions is less than half of that. Once hatched, the larvae make their way to a host plant completing the life cycle.

Because of the limited host range of most of the Heterodera species, it is usually easy to devise crop rotation methods for control. On the other hand, delayed hatching of the eggs means that the rotations must be very long; in heavily infested sugar beet fields, as long as 5 or 6 years must be allowed between beet crops. Since it has been shown that larvae die within 12 to 18 months after hatching if they do not reach a host plant, efforts are being made to analyze the "hatching factor" in root leachings in the hope that it can be synthesized and used in control. Some progress has apparently been made.

The literature on Heterodera is voluminous, European workers having studied the sugar beet nematodes and other species of this genus since around 1860. A summary to about 1938 will be found in "A Manual of Agricultural Helminthology," by I. N. Filipjev and J. H. Schuurmans Stekhoven (1941). A later and shorter summary, "The Cyst-forming species of Heterodera," by Mary T. Franklin, was published by the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, in 1951. "The Golden Nematode of Potatoes," by B. G. Chitwood, (USDA circular No. 875) summarizes information to about 1951 on H. rostochiensis in this country. There are several host lists, but because of the confusion as to identity of species, most are apt to be misleading. Probably the best information on hosts is found in reports of experiments by F. G. W. Jones, "Observations on the beet eelworm and other cyst-forming species of Heterodera." (Annals of Applied Biology 37(3):

407-440), of 1950.

Available names of the genus Heterodera are listed in Table 1. Identification of some of these by characters of the cysts and contained eggs and larvae is doubtful, and it is possible that some of the species are not valid. The final answer to this question must await careful study and description of the males, females, and larval stages. It should also be mentioned that while the writer is certain there are a considerable number of undescribed species, very few cysts have been seen which could not be placed in one of the species groups described below.

Species formerly referred to this genus are as follows:

Heterodera raditicola (Greef, 1872) Miller, 1884, was shown by Goodey (1932) to be a species of another genus and is now known as Ditylenchus raditicola (Greef, 1872) Filipjev, 1936.

Heterodera vitis Phillipi, 1884, was shown by Giard (1894) to be an insect Margarodes vitium (Phillipi, 1884) Giard, 1894.

Heterodera javanica Treub, 1885, is a root-knot nematode, Meloidogyne javanica (Treub, 1885) Chitwood, 1949.

Heterodera exigua (Goldi, 1887) Loos and Foaden, 1902, is also a root-knot nematode, Meloidogyne exigua Goldi, 1887. (The species name was misspelled exiqua by Loos and Foaden.)

Heterodera marioni (Cornu, 1879) Goodey, 1932, is a root-knot nematode, Meloidogyne marioni (Cornu, 1879) Chitwood, 1952.

Heterodera lupuli Filipjev and Schuurmans-Stekhoven, 1941, is an obvious error, H. humuli having been intended.

Heterodera viale (Lavergne, 1901) Chitwood, 1949, is also an obvious error of transcription for Anguillula viale.

Special attention should be called to the fact that several additional names to be found in Cooper's paper published in 1955 have no nomenclatorial standing, having been specifically designated a provisional name by the author.

Table 1

The Species of Heterodera with Type Hosts and Localities

The Species:	Type Host:	Type Locality
<u>H. schachtii</u> Schmidt, 1871	<u>Beta vulgaris</u>	Halle, Germany
<u>H. gottlingiana</u> Liebscher, 1892	<u>Pisum sativum</u>	Göttingen, Germany
<u>H. rostochiensis</u> Wollenweber, 1923	<u>Solanum tuberosum</u>	Rostock, Germany

Species	Type Host	Type Locality
<u>H. punctata</u> Thorne, 1928	<u>Triticum vulgare</u>	Saskatchewan, Canada
<u>H. major</u> (Schmidt, 1930) Franklin, 1940*	<u>Avena sativa</u>	Halle, Germany
<u>H. trifolii</u> (Goffart, 1932) Oostenbrink, 1949	<u>Trifolium pratense</u>	Schleswig-Holstein, Germany
<u>H. humuli</u> Filipjev, 1934	<u>Humulus lupulus</u>	Kent, England
<u>H. galeopsidis</u> (Goffart, 1936) Filipjev & Schuurmans- Stekhoven, 1941	<u>Galeopsis tetrahit</u>	Lauscha, Germany
<u>H. cacti</u> Filipjev & Schuurmans- Stekhoven, 1941	<u>Epiphyllum</u> <u>ackermannii</u>	Haartensdyk, Holland
<u>H. cruciferae</u> Franklin, 1945	<u>Brassica oleracea</u>	England
<u>H. weissii</u> Steiner, 1949	<u>Polygonum pennsylvanicum</u>	Beltsville, Maryland, U.S.A.
<u>H. carotae</u> Jones, 1950	<u>Daucus carotae</u>	Isle of Ely, England
<u>H. glycines</u> Ichinohe, 1952	<u>Glycine max</u>	Tokachi Province Hokkaido, Japan
<u>H. leptonepia</u> Cobb and Taylor, 1953	Unknown	Probably Peru
<u>H. tabacum</u> Lownsbery and Lownsbery, 1952	<u>Nicotiana tabacum</u>	Hazardsville, Conn. U.S.A.
<u>H. fici</u> - Kirianova, 1954	<u>Ficus</u> sp.	U.S.S.R

*/There is some confusion as to the proper name for this species. H. avenae (Mortensen, Kistrup, and Kolpin Ravn, 1908) Filipjev, 1934, has been used in some recent literature. However, as pointed out by Franklin (1957), this name was never accompanied by an adequate "indication" as required by the Rules of Zoological nomenclature, and is therefore invalid.

The Cysts

Cysts of Heterodera are of two general types which are easily distinguished by examination of the lower end. 1/This part of the cysts of H. rostochiensis and H. punctata has a smooth rounded contour (figs. 1 and 2). The cysts of all other known species is shaped somewhat like the end of a lemon, that is, the vulva is located on a definite protuberance. This is shown in figures 3 to 6, the variety of forms illustrated being representative not only of the species shown, but of the other known species as well. The first type of cyst is conveniently referred to as "round" and the second type as "lemon-shaped", or it might be said that the vulva does not or does protrude. At the upper end of the cyst is a distinct neck

1/For convenience here and in the following parts of this paper, the cysts will be described as viewed laterally with the vulva at the lowest point. The "lower end" of the cyst would then refer to the region around the vulva, the vertical axis would extend from the vulva along the center line of the cysts, horizontal lines would be at right angles to the center line, etc.

which varies in length, shape, and position with reference to the vertical axis of the cyst. Shape, size, and proportions of the cysts are highly variable; and while it can be shown statistically that averages of these dimensions or of the relations between them differ between species, such averages are of little value for identification of small lots of cysts.

As has been illustrated by Chitwood (1951) for *H. rostochiensis*, the cysts of all species are made up of several distinct layers. When the cysts of some species are fresh, they may be covered or partly covered by the "subcrystalline layer." This is a waxy, translucent substance which apparently persists for only a short time in the soil.

The color of mature cysts is always some shade of brown. The cuticle of living females may appear white, colorless, or yellow.

The outermost layer of cysts is marked by grooves and ridges which form distinctive "patterns." These vary in detail with individuals, but are sufficiently constant within groups of species to be of value in identification. The pattern is visible on immature females and can nearly always be seen on part or all of cysts even when these are very old. In certain species, the basic element of the pattern at the middle of the cyst is a short zig-zag line which may appear as light on a dark background or dark on a light background, according to the focus of the microscope (figs. 7, 8, and 26). The segments of the line are straight, and the angles between them are well defined. Usually these lines near the middle of the cyst show no trace of regular arrangement. Near the base of the neck and around the vulva there may be parallel lines (fig. 9) or wavy lines (fig. 10). The size of the elements of the pattern may vary greatly, being small as shown in figure 8, relatively large as shown in figure 26, or intermediate between these two. A variation which seems rare is the network pattern shown in figure 12. An occasional cyst with partly zig-zag and partly network pattern has been seen. So far as is known at present, there is no constant difference in pattern between species in the *H. schachtii* group, though it is possible that fine, coarse, or network patterns will be found more frequently in some species than in others.

A second type of pattern is found in the group of species which includes *H. weissii*, *H. cacti*, and probably a number of undescribed species. This pattern has as its basic element parallel lines running around the cyst at right angles to the vertical axis (fig. 13). These may be interrupted at intervals by short vertical or oblique lines (figs. 14 and 15). Sometimes this pattern may appear somewhat like the zig-zag pattern but differs in that some trace of the parallel lines always remains.

The cysts of *H. rostochiensis* and *H. punctata* have a third type of pattern. Around the vulva it is made up of wavy lines (fig. 19). On the lower portion of the cyst, there are short, crooked lines, sometimes in horizontal rows (fig. 22). On the upper part of the cyst, the lines tend toward the vertical, sometimes appearing as nearly vertical striae (fig. 23).

Some of the species with zig-zag patterns have in the lower end a striated object shaped somewhat like a sheaf of grain, apparently the cuticular lining of the vagina (fig. 11). This is nearly always accompanied by a number of dark bodies of irregular, though never angular shape. These may be few or numerous. No constant number or arrangement has been observed. These are absent in cysts of other species having zig-zag patterns and can be used for separation of these cysts into two groups.

Punctuation is found on a layer of the cyst below that which carries the pattern. According to Franklin (1939) punctuation is minute pits in one of the layers of the cyst. Under high magnification these appear as round dots of uniform size, either light or dark, according to the focus of the microscope.

Punctuation is usually very prominent in H. rostochiensis and H. punctata, with the dots often being arrayed in distinct parallel horizontal rows (figs. 20 and 24). In cysts of the H. schachtii group, punctuation is of several types. One of these is a prominent feature of most H. avenae cysts, but also occurs on other species. The dots are about one-half micron in diameter, and there is little or no trace of regular arrangement (figs. 25 and 26). This is called "coarse irregular" punctuation.

Cysts of H. trifolii have dots of about the same size as those found on cysts of H. avenae, but these are often arranged in parallel lines on part of the cyst at least. This is shown in figure 27 with the lines running diagonally from lower left to upper right across the photograph, but the rows are seldom as long as those shown.

In other species of the H. schachtii group, fine irregular punctuation occurs. The dots of fine irregular punctuation are much smaller than those of the coarse type, being difficult to see even with the oil immersion objective of the microscope. Unfortunately, punctuation is a somewhat variable character, being easy to see on some cysts and difficult or impossible to find on others. Its presence is therefore a useful character, but its absence cannot be taken to indicate that a given specimen does not belong to a species for which punctuation is described.

Punctuation has not been seen on H. weissii or H. cacti, though it may occur on some of the undescribed species of this group. But cysts of these species often have a grainy appearance (figs. 17 and 18) due to the presence of dots of somewhat irregular size and shape on the outer layer of the cyst.

The anus of H. cacti is shown in figure 17. All lemon-shaped cysts have the anus located in about this same relationship to the vulva. The anus of H. rostochiensis is shown near the upper edge of figures 19 and 20. The pattern runs around the vulva, but the anus is marked only by a slight irregularity. The anus of H. punctata is located at a thin spot on the cyst, which is about the same size as the vulvar opening. Figure No. 21 shows this clearly, though the cyst wall was split in the process

of preparing the slide for photographing. This difference, together with the round shape of the cysts, permits the identification of H. rostochiensis and H. punctata from examination of the cysts alone.

The Larvae

Larval characters used in the key are average length, relation of body length to breadth, shape of stylet knobs, location of the dorsal gland orifice, and relation of the tail terminal to the stylet length.

Identification by average length of the larvae has distinct limitations due to the fact that variation in length within a species might be as much as 20% of the total length. Relation of larval length to width is useful for separation of only one species, H. leptonepia.

Stylet knobs are of two general types, concave anteriorly and convex anteriorly. With most species, there is no doubt as to the type of knobs, since the concavity or convexity is distinct, but some forms have stylet knobs intermediate in type and difficult to distinguish.

Location of the dorsal gland orifice is used mostly to distinguish between H. trifolii and H. glycines. As was pointed out by Hirschmann (1956), the dorsal gland orifice in larvae of H. glycines is located 3.0 to 5.2 microns posterior to the stylet knobs; in H. trifolii, the location is 5.6 microns posterior to the stylet knobs.

The tail terminal is defined as the hyaline portion of the tail posterior to the body cavity. This portion of the tail is usually clearly defined, since the contents of the body cavity are more or less granular. In poorly preserved specimens, the body contents may be shrunken, making the tail terminal appear longer than it is in reality.

Key to The Mature Cysts of Species of Heterodera

Note: This key is designed to facilitate identification of the species of Heterodera, using only characters of the mature cysts and their contents, that is, eggs with second stage larvae. Certain characters used in the key may not be visible on other than fully mature cysts.

Measurements of larvae are from Fenwick and Franklin (1951) for most species, from Jones (1951) for H. carotae, from Ichinohe (1952) for H. glycines, and from Kirianova for H. fici.

1. Body of cyst ovoid to globular, that is with posterior portion rounded and vulva not located on a distinct protuberance (figs. 1 and 2)-----Heterodera rostochiensis group 4.

Body of cyst lemon-shaped, that is, with vulva located on a distinct protuberance (figs. 3 and 6)-----2

2. Basic element of pattern of outer layer of cyst wall at middle portion of cyst short zig-zag lines with little or no trace of regular transverse arrangement (figs. 7 and 8) sometimes modified to appear as network (fig. 12)-----3.
- Basic element of pattern at outer layer of cyst wall at middle portion of cyst straight or wavy lines (figs. 14 and 16) lines at right angles to axis of cyst; sometimes broken by short oblique or vertical lines; outer layer of cyst may have grainy appearance (fig. 18)-----Heterodera cacti group-----7.
3. Mature cysts with dark bodies (brown knobs) and often sheaf-shaped object (lining of vagina) at posterior end (fig. 11). On immature cysts, these seldom visible, and then do not appear dark-----Heterodera schachtii group-----8.
- Mature cysts without brown knobs or sheaf-shaped object at posterior end-----Heterodera göttingiana group-----11.
4. H. rostochiensis group. Cyst often ovoid, anus located at a transparent spot on cyst so that anal and vulvar openings appear to be about the same size when seen by transmitted light (fig. 21). Hyaline portion of larval tail much longer than stylet-----Heterodera punctata
- Cyst ovoid to globular; anal opening appears much smaller than vulva opening (figs. 19 and 20). Hyaline portion of larval tail about the same length as stylet-----5.
5. Larvae very slender; length about 39 times greatest width; orifice of dorsal oesophageal gland about two-thirds stylet length posterior to stylet knobs--Heterodera leptonepia
- Length of larvae about 22 times greatest width; orifice of dorsal oesophageal gland about one-fourth stylet length posterior to stylet-----6
6. Distance between vulva and anus about one and one-half times diameter of vulva-----Heterodera rostochiensis
- Distance between anus and vulva about two and one-half times diameter of vulva-----Heterodera tabacum
7. H. cacti group. Hyaline portion of larval tail about as long as stylet; stylet knobs concave anteriorly-----Heterodera weissii
- Hyaline portion of larval tail usually shorter than stylet; stylet knobs convex anteriorly--Heterodera cacti
8. H. schachtii group. Cyst always with distinct punctuation consisting of dots of uniform size but not in rows (fig. 25);

brown knobs closely clustered around vulva. Hyaline portion of larval tail at least one and one-half times longer than stylet-----Heterodera major

Cyst with or without punctation, mostly in rows if present; brown knobs not closely clustered around vulva. Hyaline portion of larval tail about as long as stylet-----9.

9. Average length of larvae 480 μ or more-----10.

Average length of larvae about 460 μ ----Heterodera schachtii

10. Average length of larvae 484 μ -----Heterodera glycinis

Average length of larvae 502 μ -----Heterodera trifolii

Average length of larvae 518 μ -----Heterodera schachtii
galeopsidis

11. H. göttingiana group.

Average length of larvae 414 μ -----Heterodera cruciferae

Average length of larvae 454 μ -----Heterodera carotae

Average length of larvae 474 μ -----Heterodera göttingiana

Average length of larvae 405 μ -----Heterodera humuli

Average length of larvae 406 μ -----Heterodera fici

Host Plants

Many lists of host plants of Heterodera species have been published, but it seems probable that many of these are inaccurate in that they include plants which are not hosts of the species discussed. This is especially true of the older lists and of those based on information compiled from the literature rather than from host tests. In order to avoid this particular error, the following list of hosts includes only the type host of each species and an indication of the other plants which it attacks so far as there is general agreement or information on host tests available. That is, the list is not intended to be complete, but is believed to be accurate so far as what is included is concerned.

The species of Heterodera and their principal hosts are:

H. schachtii. Type host, sugar beet (Beta vulgaris L.). Also other Chenopodiaceae, many species of Cruciferae (Oostenbrink, 1950 and Jones, 1951) and various species of other plant families (Thorne 1932). It seems possible that H. schachtii attacks a wider variety of plants than any

other known species of Heterodera.

H. göttingiana. Type host, garden peas (Pisum sativum L.). Also other Leguminosae, but according to Oostenbrink (1951), this species does not attack beans (Phaseolus vulgaris L.), clover (Trifolium spp.), alfalfa (Medicago sativa L.), or soybeans (Soya max Piper).

H. trifolii. Type host, red clover (Trifolium pratense L.). Also other Leguminosae, including beans (Phaseolus vulgaris L.), but not peas (Pisum sativum L.), alfalfa, or soybeans. (Oostenbrink, 1951).

H. glycines. Type host, soybean (Glycine max L.). Also snap bean (Phaseolus vulgaris L.), Adzuki Bean (P. angularis), vetch (Vicia sp.), Annual lespedeza (Lepedeza stipulacea Maxim.), Henbit (Lamium sp.).

H. major. Type host, oats (Avena sativa L.). Also other Gramineae (Oostenbrink, 1950).

H. cruciferae. Type host, cabbage (Brassica oleracea L.). Also other Cruciferae.

H. carotae. Type host, carrot (Daucus carotae L.). Wild carrot (Daucus carotae) is the only other known host (Jones, 1950b).

H. humuli. Type host, hops (Humulus lupulus L.). Also other Urticaceae.

H. galeopsidis. Type host, hemp nettle (Galeopsis tetrahit L.). Also other Labiatae and some species of Chenopodiaceae and Carophyllaceae (Jones, 1950b).

H. fici. Type host, rubber plant (Ficus sp.)

H. weissii. Type host, knotweed (Polygonum pensylvanicum L.). No other hosts known.

H. cacti. Type host, Phyllocactus (Epiphyllum ackermanni). Also other Cactaceae.

H. rostochiensis. Type host, potato (Solanum tuberosum L.). Also tomato (Lycopersicon esculentum Mill.) and a few other species of Solanaceae (Oostenbrink, 1950), but not tobacco (Nicotiana tabacum L.) (Taylor, 1952).

H. tabacum. Type host, tobacco (Nicotiana tabacum L.) and tomato.

H. punctata. Type host, wheat (Triticum vulgare Vill.). Also other Gramineae.

Species of other plant families than those mentioned above have been reported as infected by nematodes of the genus Heterodera. Some of these may be attacked by known species, but it is highly probable that others are attacked by species as yet undescribed. Among these should be mentioned the species found attacking sea marram grass (Ammophila arenaria

(L.) Link) by Triffitt (1929), one found by Thorne on Shadscale (Atriplex confertifolia (Torr. and Frem.) S. Wats.); one found by Chitwood (1949) in soil from North Dakota, and several found by Oostenbrink (1950) attacking plants of various species.

Captions for Illustrations

- Plate I. Shapes of cysts of Heterodera species. Fig. 1. H. rostochiensis. Fig. 2. H. punctata. Fig. 3. H. schachtii. Fig. 4. H. avenae. Fig. 5. H. weissi,
- Plate II. Cyst patterns of Heterodera schachtii and related species. Figs. 7 and 8. Zig-zag line pattern near middle of cysts of H. trifolii and H. schachtii respectively. Fig. 9. Pattern at junction of neck and body of cysts of H. schachtii. Fig. 10. Pattern near vulva of cyst of H. gottingiana. Fig. 11. Sheaf-shaped object and dark bodies at lower end of cyst of H. schachtii. Fig. 12. Network pattern, a variation of that shown in Figs. 7 and 8. Magnification of figure 11 is about 200X, all other about 410X.
- Plate III. Cyst markings of Heterodera weissi and H. cacti. Fig. 13. Lower part of cyst of H. cacti. Fig. 14. Pattern near middle of cyst of H. weissi. Fig. 15. Pattern at junction of body and neck of H. weissi. Fig. 16. Pattern near middle of cysts of H. cacti. Fig. 17. Lower end of cysts of H. cacti showing anus. Fig. 18. Grainy appearance of cyst of H. cacti. All about 410X.
- Plate IV. Cyst patterns and punctation of Heterodera rostochiensis and H. punctata. Fig. 19. Pattern at vulva and anus of H. rostochiensis. Fig. 20. Same as Fig. 19, but with deeper focus to show punctation. Fig. 21. Anal and vulvar openings of cyst of H. punctata. (Cyst split in process of preparation). Fig. 22. Pattern at about middle of cyst of H. rostochiensis. Fig. 23. Pattern of upper part of cyst of H. punctata. Fig. 24. Punctation of H. rostochiensis. All about 410X.
- Plate V. Cysts and eggs of Heterodera species. Fig. 25. Punctation of cyst of H. avenae. Fig. 26. Punctation and pattern of cyst of H. humuli. Fig. 27. Punctation of cyst of H. trifolii. Fig. 28. Punctation of egg shell of H. cacti.

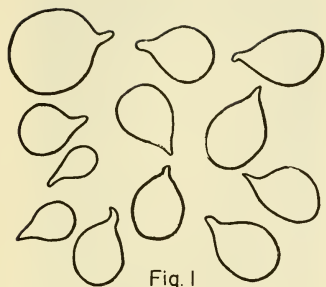


Fig. 1

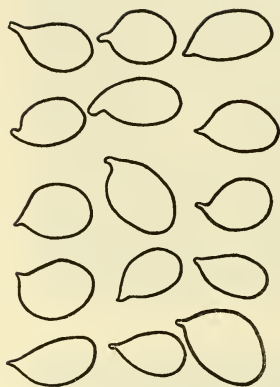


Fig. 2

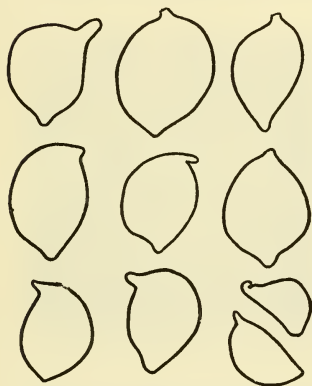


Fig. 3

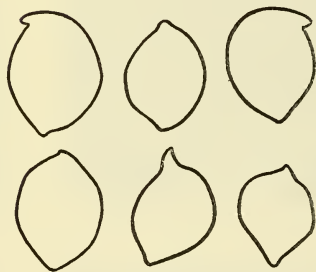


Fig. 4

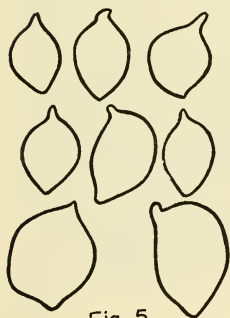


Fig. 5

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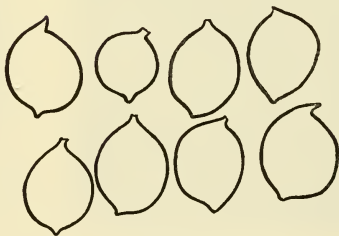
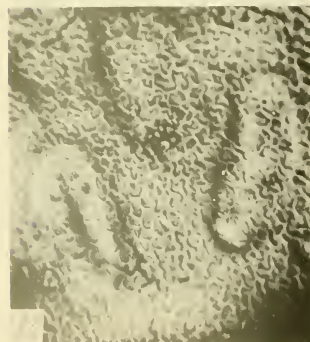
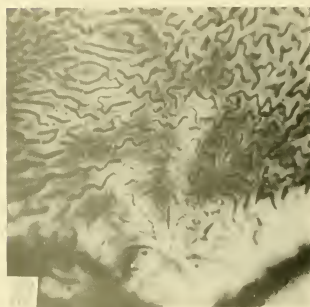
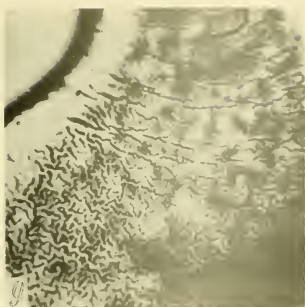
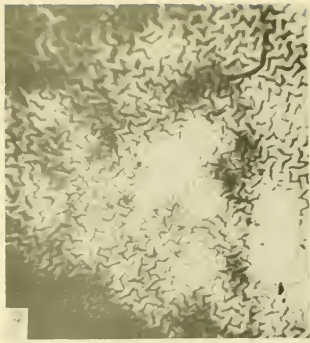
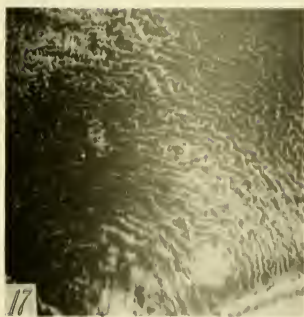
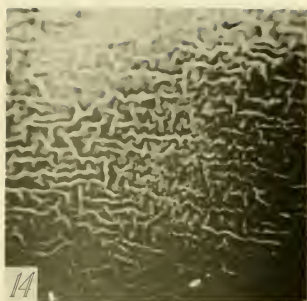
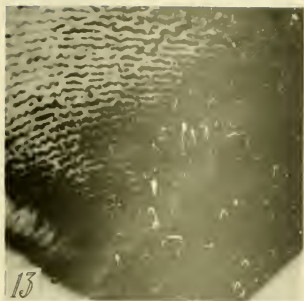
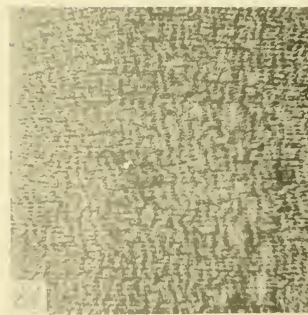
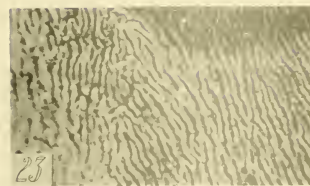
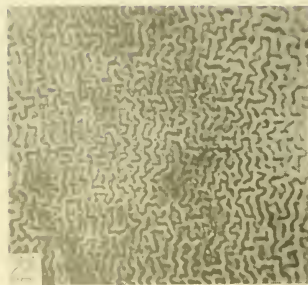
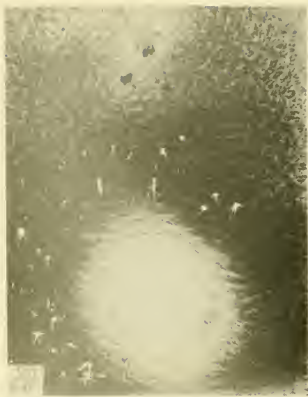


Fig. 6

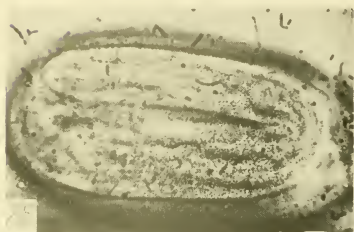
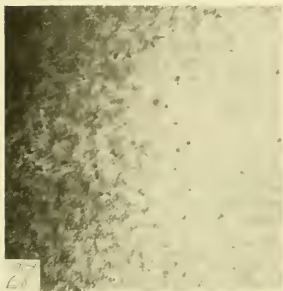
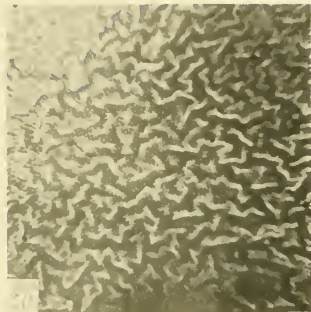
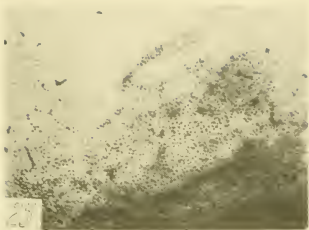














HETERODERA GLYCINES, THE PRESENT SITUATION

J. N. Sasser

The soybean cyst nematode, Heterodera glycines, we know is present in Japan, China, and Manchuria. Up until August of 1954, it was known only to occur in the orient. There it causes a disease known as "yellow dwarf." The term is very characteristic of the symptoms in the field. Considerable work has been done by the Japanese workers, particularly Ichinohe and his co-workers. We have had difficulty, however, in getting all of those publications from abroad and even more difficulty in getting them translated. Most of the work that I will report on today, by necessity, will be the work that we have done in North Carolina, as there was no other source from which to make a review.

The pest was found, as is usually the case, by a grower noticing poor spots in his field over a period of several years. He finally called in one of the plant pathologists at the station within a few miles of this area. Upon examination of the root in the laboratory, numerous nematode cysts were found and were later identified to be H. glycines.

Immediately after its finding, we were concerned about knowing how widely distributed it was. Our own research staff made a rather hurried survey. It was found that the nematode was rather widely spread within that one community. That is, it was not limited to one field, but there was a rather uniform infestation through several hundred acres. To find out if the nematode was spread into our major soybean producing region and how extensively or how widespread it was in the particular region where it was found, the Plant Pest Control group from Washington came to make a survey.

The present extent of the known infested acreage in this one area in the state is now something over 1800 acres. Although the disease at that time was rather minor with us, causing only a very small percentage of the total nematode damage in the state, we did realize its implications, the possibility of spread and the potential threat it might be to the soybean industry. Therefore, we tried to do as much work as we could with the facilities and manpower that we had.

One of the first tasks that we realized as necessary was to be able to accurately distinguish the species. We have known for sometime that H. glycines and H. trifolii very closely resemble each other morphologically, and we knew that we had H. trifolii throughout the state on clover.

The first test was to set about finding definite morphological differences between those two because of the possibility of quarantine action. We knew that if cysts were found on a given property, we would need to

have some means of being relatively sure that the nematode was the soybean cyst nematode and not H. trifolii. Dr. H. Hirschmann of our staff was given the assignment. This work has been completed and published.

Definite differences have been found and recorded between these two species. The differences are evident in the second stage larvae. There were no statistical differences in the cyst characters. Fortunately, in the second stage larvae, differences, which never overlap, were found for several characters. These are as follows: the overall length of the nematode, the length of the stylet, the distance from the stylet knobs back to where the dorsal gland empties into the lumen of the esophagus, and the tail length.

The next phase of the work was to learn something about the nematode's biology. For the most part, Dr. G. B. Skotland, who is a plant pathologist with U.S.D.A., did this work. He made a study of the life history mainly by staining roots in the various developmental stages, using the osmic acid technique. This work has also been published. He found that there were mature males in 14 days after infection and that the complete life cycle, under the conditions he performed experiments, was completed in 21 days.

Another line of work immediately started was, of course, a host range study. This was initiated to enable us to advise the growers, in the particular region of infestation, of crops that they could grow which would not build up the soybean cyst nematode. First tested were all of the crops that are commonly grown in the area, plus others. By no means is the host range study complete. It is being continued at North Carolina, as well as at some other institutions, now that the soybean cyst nematode has been found elsewhere.

By and large, the host range of this pest is rather restricted. Apparently it is not as limited as for H. rostochiensis and some of the other cyst forms, but the positives are relatively few. The nematode reproduces by far the best and most rapidly on soybeans. Soybeans undoubtedly are a very suitable host. Snapbeans also are infected, and the nematode reproduces, but not to the same extent as on soybeans. Lespedeza and vetch are also hosts, but to a still lesser degree than snapbeans or soybeans. At least, that is the way we feel about it now. We have not run extensive tests comparing the relative host suitabilities of these crops, but I think we can be reasonably safe in saying that soybeans are by far the most suitable host among crops grown in this country at least.

Some of this work on host range had been done earlier by Ichinohe in Japan. Dr. Skotland has more or less confirmed some of the hosts, and he has added new ones to the list.

We are also interested in how or what the effects of desiccation might be on the nematode. We know that cysts' forms are commonly spread in

debris of various sorts in baggage, burlap bags, and things of that nature. In this particular area of North Carolina, soybeans are grown rather incidentally during the summer months as a soil-builder and also to provide some shading for gladiolus bulbs. We were interested in whether or not a period of storage after digging the bulbs has any material effect on killing the nematodes, or whether they could remain viable in a dry condition, like H. rostochiensis and some of the others, for an extended period of time.

While this work is by no means complete, Dr. Skotland did get convincing evidence that desiccation has a very pronounced effect. Drying the cysts at room temperature for a period of two, three, or four weeks reduced the population about 99%. He never did get all the nematodes in the cysts completely killed by this method, but it is quite evident that desiccation is a very important factor in the survival of this nematode.

Hot-water treatments were worked out for the growers who wished to dig bulbs and to sell them. While I do not remember the details, this likewise has been published in Phytopathology, if you desire specific information. The combination of hot-water-formalin treatment for a given period of time was found very effective in eradicating the nematode on bulbs. Also, a chemical material called Dowcide D, at given concentrations and at varying periods of time, proved effective. Thus, we did have a means of treatment whereby the growers could sell or move their bulbs. I would like to stress that the bulbs are not a host for the nematode. They could be a source of spread--the nematodes as contaminants adhering to the bulbs.

Another line of research that was started was a five-year rotation program. This was set up last year. We are in the second year with that now, and, while Dr. Skotland initiated the work Dr. Ross is now in charge of it, as Dr. Skotland left for another assignment.

The rotation consists of cowpeas, which are not a host, and soybeans. The experiment is set up over a five-year period, in which soybeans in some plots will be planted continuously. Other plots will be planted in one year rotations of soybeans, followed with cowpeas; two years of cowpeas, followed with soybeans; three years of cowpeas, followed with soybeans; and so on.

I visited the plots last week and observed that there are some very striking differences already evident in plots that were of soybeans last year and are planted with soybeans again this year, as compared to rotated crop plots. I believe that I will be safe in saying that not a single bean will be produced on those plots. The plants were about four inches high and had a yellow chlorosis. I doubt that they will live throughout the season. On the other hand, in the plots that were planted in cowpeas last year but have been planted in soybeans this year, in other words, just one year of a non-susceptible crop, the soybeans are about knee-high and look as if they will make a good yield. I would like to mention that this field in which this experi-

ment is carried out, in 1955 was grown solid with soybeans, and soil assay indicated a fairly uniform infestation throughout the field.

Another phase of the work that I personally have followed, perhaps most closely, has been chemical control. We have leased the field just mentioned for a period of five years. Plots twenty feet square, with four-foot wide alleyways between, were set up for experimental work. We established three replications and are using methyl bromide at three rates: 1, 2, and 3 pounds per hundred square feet; D-D at three rates: 20, 40, and 60 gallons per acre; ethylene dibromide at three rates: $4\frac{1}{2}$, 9 and 13 gallons per acre; telon at 20 and 40 gallons per acre; nemagon at 3 and 5 gallons per acre; and the necessary control plots.

In gauging the effects of the chemicals, we took what we call a "white cyst index." It was a means for rather quickly obtaining data on the effectiveness of the chemicals. We found that if one removes the bean plants from the ground approximately 30 days after planting, the white cysts can be seen and judged by an arbitrary rating scale. The roots can either be washed by dipping in a bucket of water or, if the soil is readily shaken off, the roots can be examined without washing. The white cysts are very easily seen. We used a rating scale of from 0 to 5. "0" represented no cysts being found in the examination of from 10 to 20 plants from a plot. "5" represents the heaviest degree of infection. Use of this method serves to give a good idea of the level of infestation at the time the beans were planted, as, of course, the white cysts had come from invasion by active, viable larvae in the soil.

Now, as to the results of the fumigation experiment. The plants in the untreated check plots had index readings of about 5. While we do not claim to have eradicated the nematode from the methyl bromide treated plots, we were unable to find white cysts on the roots when they were pulled as soon as one month after planting, so we gave these a rating of 0 for the time being. Telon, at 20 gallons per acre, was quite ineffective, but gave very good control at 40 gallons per acre. Nemagon was very good at 3 and 5 gallons per acre, particularly at the 5 gallon rate. D-D was only fair at the 20 gallon rate and very good at 40 and 60 gallons per acre. Ethylene dibromide was somewhat erratic, in that it seems the more we put on, the less control we had. However, we are repeating this same test again to make sure we are not being unfair in our appraisal of this material.

We knew that methyl bromide would not be a practical field method of control. However, we did want to learn if it could control the nematode, as it would, perhaps, be useful for treating small spots or infested objects. Methyl bromide did give the best looking plots. The 1 and 2 pound rates were about equal in effect, but at the 3 pound rate there was some toxicity to the soybean plants. One difficulty was had in the check plots. Where there was poor growth of the soybean plants, hence little shading out of the weeds, control of the weeds was quite a problem.

About three months after planting, the plants in the Nema-gon plot treated at 5 gallons per acre were about shoulder high. They were very green in color and gave one of the highest yields.

We made a larval emergence study throughout the year, taking samples every month. We were particularly interested in the build-up of the nematode during the growing season. At the end of the growing season, we took samples from all of the plots, recovered the cysts and let the larvae hatch out in Baermann funnels. The counts represented the number of larvae per half-pint of soil. The check plots yielded almost the lowest larval counts; not the lowest, but very low. One would expect this, because of the very poor plant growth during the season. The nematodes presumably did not have enough root system available to them. We did find relatively few larvae in the methyl bromide plots. We do not know if these represent forms missed in the white cyst checks made earlier at one month, or whether they were due to contamination. It was very difficult to take care of the plots in such a way as to avoid the possibility of contamination. There was some build-up of the population in the Telon treated plots, larva counts being very high in the 20 gallon rate plot. D-D and, again, the ethylene dibromide gave somewhat erratic results and, in general, fairly poor control of the nematode build-up.

The final aspect of the experiment was the yield of beans. The plots were, as mentioned, 20 feet square with 8 rows of plants. Yield data were taken on the center 4 rows. The untreated checks yielded something like 7 bushels of beans per acre. Some of the better treatment plots had yields of about 22 to 23 bushels per acre. All of the yields were better than those of the checks, although not substantially so in all cases. Most treatments gave fairly good yields. Poor yields were found in the Telon plots and in some of the D-D treated plots.

Another phase of the overall work has been a study of resistance. The first year, 1956, two or three thousand lines of soybeans were tested in these infested fields in the search for resistance. This work has been carried on jointly, with Dr. Herb Johnson of the U.S.D.A. working with Dr. Skotland and Dr. Ross, at Raleigh, North Carolina. Last year as far as I know, none of the tested lines showed any resistance; but this year, out of some 29 hundred lines screened, they have run across about seven or eight promising lines. When I talked to Dr. Ross last, he said there were between two and five lines that look extremely good.

I would like to say a little about this screening work. The field used was checked before planting and found to be highly infested with the soybean cyst nematode. The plant lines to be tested were planted in short rows five feet long. In order to make sure the plants were exposed to infection and were not just "escapes," along side each plant being tested there was planted a known susceptible plant. These indicator plants were of a glabrous type, so as not to be mixed with the plants under test. Thus, if the indicator plant had roots well covered with cysts, and the adjacent test plant did not, there could be some

degree of confidence in the possibility of resistance occurring in the test plant. If both plants were free of cysts, the results on that test plant were disregarded. Lines which showed signs of resistance are, of course, being tested again this season.

Dr. Ross is currently checking the roots of some of the resistant lines to determine if the larvae have entered. He is finding that the larvae do enter, but fail to develop. This is about as far as the work has gone, to date, along the line of resistance as a means of control. It does appear that in the soybean germ plasm there certainly is some resistant genetical material for the plant breeders to work with.

Now, regarding the distribution of the soybean cyst nematode. It apparently is confined to a small area within North Carolina, but, as you know, it has been reported now from at least five other states. Perhaps one reason it showed up as it did in North Carolina was because of the intensive soybean cultivation in the particular area for about 15 to 20 years. Every summer the farmers grew soybeans as a cover crop. It seems that the history is similar in the infested areas in Tennessee, that is, soybeans were grown continuously over a long period of years. I do not know that we can say the pest was introduced from the Orient, although it could have been. Certainly we have no proof. I believe it is going to show up in this country wherever, as Mr. Al Taylor has pointed out, we have grown soybeans or some other susceptible crop for a good many years.

In concluding, I would like to give you the little bit of information we do have on the longevity of the nematode in the soil. This past January I collected soil from the field where the nematode was originally found in North Carolina. Cysts screened from this soil, which had not been replanted to soybeans since 1954, yielded viable larvae. There had been two complete years with non-host crops in the field. I cannot, at the present, give you the degree to which the population has been reduced.

I would like to call on Dr. Andes of Tennessee for information of the soybean cyst situation and work begun in his state.

J. O. Andes

In September of 1956 we were processing soil from Lake County, which is in the northwest corner of Tennessee, and we found cysts in some of the samples. We sent them to Dr. Sasser for checking and later to Mr. Taylor at the Section of Nematology. They were identified as being the soybean cyst nematode. Following this discovery of the pest in our state, the Plant Pest Control workers came to begin a survey. The distribution of the nematode extended into various areas in several of the counties in the western end of the state. A meeting was called in Memphis to have a hearing on the situation. One result has been the establishment of a quarantine which went into force yesterday. I have

not yet seen the statement , so I do not know of its details or just what is covered. Following the meeting at Memphis, an agreement was worked out with the U. S. Department of Agriculture with the result that a field station and laboratory are to be established in Tennessee. The laboratory will be at Jackson, and the field work will probably be conducted in Lake County, or wherever we find suitable infested areas available for our use. The laboratory building is about completed and will soon be in operation.

Since the discovery of the soybean cyst nematode in Tennessee, we have been doing some work in a fumigation experiment. We have a total of six chemicals under test. They are being applied at four rates each and in both row and broadcast applications. The rates are the normal recommended rates, one-half normal, one and one-half normal, and double the normal rates. These are applied in plots of five rows, thirty feet long. The plots have only been planted for about one month, so we have no results to report as yet.

We have also been checking to see if there are other hosts for the nematode in our area. We have planted many cultivated crop plants and as many wild or weed plants as we could obtain seed. We have found that white lupin is readily attacked, producing many cysts when grown in pots containing soil from infested areas. We have found cysts being produced on a common weed which is not a member of the legume family. As this may be a first report of a host outside of this plant family, you may be sure we are having this checked very carefully. A compilation of the host range testing work will soon be printed in the Plant Disease Reporter.

Discussion

- Q. Do you know what the quarantine regulations are that went into effect yesterday?
- A. No, not exactly, as I only have a copy of what is in the register. I have not as yet seen the quarantine statement.
- Q. Is the quarantine a state by state one?
- A. No, at the present time the quarantine covers North Carolina, Tennessee, and Missouri. Hearings are to be held July 24 on the question of including Arkansas and Texas. I think the regulations will be uniform from state to state.
- Q. How effective did you find Telon to be relative to D-D?
- A. Telon was not as effective as D-D, but you know things can go wrong in experimental work of this sort. The applicator device can become stopped up, or some other incident of that kind can happen. We are going to repeat the test again this year to know for sure that

we are fair in our appraisal of the materials, as I have said before.

Dr. Nusbaum of the North Carolina staff has done work on control of nematodes in tobacco; he finds Telon to be as good as D-D, or even a little better, at comparable doses.

MELOIDOGYNELIFE HISTORY, BIOLOGY, RACES, PATHOGENICITY, AND
OTHER TOPICS DEALING WITH ROOT-KNOT NEMATODE RESEARCH

J. R. Christie

I am going to begin without preliminaries but with a few generalities.

The root-knot nematode has been long in that group of plant infecting species that are sometimes referred to as sedentary parasites. There have been named at least ten genera, the species of which belong in this category.

In every one of them the adult female, or the female once it has become established in the tissues of the host, remains thereafter in a fixed position, feeding on the tissues within reach of its head. In every case, the female loses its original nematoid shape and becomes more or less sac-like. The final form varies from what might be called sausage-shape to almost spherical.

In every one of these genera, the adult male retains its slender, nematoid form.

In the genera Meloidogyne and Heterodera, larvae of both sexes enter the plant. (Usually they enter the roots.) That is to say, larvae destined to become males, as well as those destined to become females, enter the plants. In early development, the male undergoes the same changes as the female—the same widening of the body; but as it reaches maturity, it undergoes a series of molts and a metamorphosis, from which it emerges as a slender, nematoid form. This type of development for the male is unique for these two genera, being found nowhere else in the phylum Nematoda, so far as I am aware.

The larvae of these two genera do not necessarily pass any stage of their development in the soil. To be sure, they are in the soil, but the sojourn in soil is strictly incidental. Larvae are ready to penetrate the roots at the time of hatching. They are, in both genera, technically second stage larvae, because they have already molted in the egg.

In the genera Tylenchulus and Rotylenchulus, the larvae pass their entire development in the soil. It is a hurried up development. They pass through a series of quick molts, but they end up technically as adults. Only the female enters the root; and the female, at the time of penetration, is technically an adult, having completed its molts. The male does not become parasitic. Both sexes pass through these early stages with no appreciable change in size and apparently without feeding.

This type of development, in which the male at least passes through its

larval development quickly, reaches the adult stage apparently without feeding and without any appreciable change in size, occurs in other groups of the Tylenchoidea, Tylenchidea, Tylenchina, or whatever you wish to call it, for it is the typical Allantonematoid life cycle.

We do not yet know much about the life cycle of the new genera, Meloidodera, Trophotylenchulus, and Trophonema. It looks as though their life cycle might be similar to that found in Tylenchulus. I think the life cycle in Nacobbus is different.

With those generalities over, I am going to go on from here and recount some of my own observations and experiences in working with this group and make a few suggestions as to what may perhaps be the reasons for this behavior. In so doing, I am making them merely as suggestions, with no insistence that they are correct. I expect a good place to start is with the egg.

The egg of the root-knot nematodes does not have delayed hatching in the sense that is found in the genus Heterodera. Eggs of the root-knot nematode are said to hatch at maturity, providing conditions are favorable. The question then arises, "What are favorable conditions?" or perhaps one should say, "What are the unfavorable conditions that might retard hatching?" One condition that may retard hatching is drought—dry conditions, lack of moisture.

I remember when Oliveira was spending a summer in Washington. She demonstrated to me the method they were using in Hawaii for obtaining root-knot larvae for experimental work. She went out, or sent someone out, into the fields in the area of Washington and got her material, infested plants. They were field grown. She brought them into the laboratory, put them in a suitable container with a small amount of water, sloshed them around, and soaked them awhile. Then she poured off that water into a filter paper and set up her apparatus. It was a modified Baermann funnel.

It was Friday afternoon when she was demonstrating this to me, and she brought it to me the next Monday morning. She had what looked like a half thimble full of root-knot nematode larvae, absolutely free from debris and practically free from other kinds of nematodes. I was very much impressed. I had been hatching larvae and doing all right, but with a good deal more work. Therefore, I forthwith did as she had. There were about twenty populations of root-knot nematodes growing in the greenhouse at that time. I got my material and set it up as she had.

The next morning I had nothing. At first I thought perhaps Miss Oliveira had been so in contact with the mystic Orient that she had developed some magic touch in her fair hands, but I thought that was no way for a scientist to think. The explanation was, or what I feel confident it was, is that she had got field material in which eggs had accumulated. The larvae had matured but had failed to hatch, so that in her material

there was a quantity of larvae already to hatch but in which the larvae had not really emerged. As soon as she put it in water, they began to hatch. My material was greenhouse grown in moist soil subject to daily watering, and there was no accumulation of larvae to hatch. I think one will get accumulation of larvae in dry weather and a fresh attack of the roots sometimes following rain.

Of course, moisture is not the only factor that influences hatching. There are also oxygen and temperature. I have been wondering who knows of any information on the effect of temperature on hatching, because I cannot find any. Therefore, I am going to pass over temperature. I cannot say much about oxygen, except that I am interested in a little more information about it.

I do know that when hatching larvae for experimental work, one picks the egg masses off the roots, puts them in a Syracuse dish or Petri dish in a very thin film of water, and keeps them in a moist chamber. They practically all hatch in a week. If they are not picked off the roots, they do not hatch. One can cut off the roots with the egg masses intact, put them in the same Petri dishes, and in a week's time may get practically no hatch. I have assumed that that was an oxygen relationship, in that when the eggs are dislodged from the roots, they are exposed from the backside, and the resulting hatching would be due to the dissolved oxygen in the water. We are considerably interested in this in Florida, because we are interested in flooding as a possible means of controlling root-knot. We know that a summer flood is effective, and it has occurred to us that these oxygen relationships might have some importance in understanding the factors that influence the efficacy of such a procedure.

In the older literature, one can read that after the larvae hatch, there are two things that can happen. One is that the larvae may migrate or move slightly to one side in the root tissues in which they were produced. That is, they may remain in the same root and there become established, or they may escape into the soil and seek new roots. After working for a year or so at Beltsville using the tomato almost exclusively as an experimental host plant, I came to the conclusion that there was no reinfection of the same plant. The larvae escaped into the soil. I sectioned and studied the stained sections of many roots of various ages, and I never saw any evidence that larvae ever re-established themselves in the same root. I began to doubt that it ever happened.

Not long ago some *Caladium* tubers came to our laboratory in Florida in connection with some experimental work which is not pertinent to this discussion. We examined them, and we found that they were infected quite heavily with root-knot. We found numerous females with the accompanying eggs distributed throughout the tissues of the tubers. Some were quite deeply embedded, some were fairly near the surface. There was no particular pattern of distribution, so far as we could see. We took about a dozen of those tubers and potted them in nematode-free, treated soil and grew them for about ten weeks or three months. Then we brought

them back to the laboratory for examination.

In the meantime, they had made quite a growth and had established quite large root systems. On examination we could find no galling on the roots whatever. When we went back to the tubers, there they were, as heavily infested as ever, perhaps more so. In cutting those tubers, we saw some small circular areas, not brown, having a kind of water-soaked appearance. In those tubers we found adults, old adult females, eggs, larvae, and all stages of development up to young egg laying adult females. Obviously those parasites were going through generation after generation in the tissues of that plant. They were not escaping into the soil to reinfect the roots. So, as is often the case, these early investigators were right—both can occur and does occur, but whether the one or the other occurs is not fortuitous. It depends entirely on the character of the tissues in which the females are embedded.

In the old days (I think of the old days as the '20's, about the time when I first began work in the Department of Agriculture.) it was the general impression among everyone who was working on root-knot that the reason these so-called resistant plants did not become infected was because the larvae did not go into the roots. I know that Mr. Arzberger, who was with the Division of nematology back in the days when I first joined it, was at that time quite busily running sections of root tips to see if there was any structural peculiarity in these root tips which might act as a barrier to the entrance of the root-knot nematode larvae. Nothing ever came of that work; nothing was ever published. I presume that he did not find any.

It was not until about 1940 when Barrons published his paper which showed that in his work just as many root-knot larvae went into the roots of *Crotalaria* as went into the roots of a tomato plant, when the two had an equal opportunity to become infected. Starting from there I have always regarded Barron's work as quite an important one—one of the landmarks in root-knot nematode investigation, because it changed our thinking a good deal. However, I was not at the time quite convinced that that was true of all plants.

I am sure you could not question his results, because he had them well documented. I knew the situation was true for marigolds, that is the horticultural form of the species Tagetes erecta, but I had a suspicion it was not true of all plants. I had at that time in the greenhouse a population of root-knot from alfalfa from the West. I took an alfalfa root tip and a Lantana root tip, put them side-by-side, and surrounded them with large numbers of root-knot larvae of the population that originally came from alfalfa. After about twenty-four hours, the alfalfa root-tip looked like a porcupine's tail, there were so many larvae trying to get in and partly sticking out. None had gone in the Lantana root. With repeated trials, the most I ever got into the root tip of the Lantana was two. Judging by this example, at least, the larvae of some of the root-knot nematodes do not go into the roots of some plants.

The next item is factors influencing the development. Just a word or two about temperature. Many years ago, Jocelyn Tyler, working with root-knot in California, found that the optimum temperature for development of the form with which she worked centered around about 85° F. or about 29° C. But if she raised the soil temperature up about 92.3° F. or thereabouts, or 33° C., the development ceased at that point; if she dropped it to 60° F., it also ceased. Recently Ferris has published data to the effect that the optimum temperature for the development of Rostochiensis centers around about 65° F. or 18.3° C., and if the soil temperature is raised to 85° F., development ceased. There, in fact, is quite a difference in temperature relationships compared to the root-knot nematodes with which Miss Tyler worked, at least. We do not know what the root-knot species was, but it apparently was more nearly a hot weather organism than one of its counterparts in the other genus.

Let us now consider that we have the larvae hatched and we have them more or less in the root. Perhaps, the next logical matter to comment on would be some of the relationships with regard to factors that influence infection. One of the factors, of course, is the suitability of the plant. Quite a long while ago, Godfrey and Oliveira pointed out that it took considerably longer for the root-knot nematode with which they were working to develop in the roots of pineapple than it did in the roots of cowpeas. Further work, later on, has shown that there is a big difference, other factors being equal, in the rate of development, depending on the plant in which the parasite is developed and whether it is what I choose to call a suitable host or an unsuitable host.

*These illustrations are all of tomato roots. They are all photomicrographs of galls of known ages, known to within 24 hours. A gallery may be left in the tissues by the migration of a larva through the tip. It is said that the larvae tend to pass between the cells, pushing them apart. This can be seen clearly in sectioned material. Twenty-four hours after infection it can also be seen that cells of the epidermis are somewhat enlarged. Although the nematode's path may be between the cells, many of the cells are crushed and destroyed.

One of the effects of most plant-parasitic nematodes, and certainly that of root-knot larvae, is the effect on the cell walls. One of the first things you will see in many instances is a change in the staining quality of the cell walls. In the case of root-knot, the cell wall staining property changes, and then the wall begins to dissolve. Or, at least, many of the cell walls do. They either dissolve or they are

*Editor's note: At this point, Dr. Christie's talk was based on discussion of lantern slides which were shown to the group. As much as possible of this portion of the talk which could be used without having the pictures at hand is presented. It has been necessary to alter the wording somewhat to fit this situation, but the content has not been changed.

digested away; at any rate, they disappear. There is a flowing together of adjacent cells to form what is known in the literature as "giant cells." It is also characteristic of the cells in the region of invasion to show nuclei with the nucleoli beginning to swell. The nuclei always stain densely when brought under the influence of the nematode's secretions. Another thing that happens in the tissues of a developing root around the nematode is that the development pattern of these cells is retarded or stopped. Thus, they may never develop into the structures they would have normally.

There is a tendency for new roots to form at the region of invasion, particularly in the case of Meloidogyne hapla. It is not so pronounced with the other root-knot nematode species. In fact, I do not know if in some of these cases many more roots than normal are formed.

Cross-sections of the giant cells, on which the parasites feed and from which they get their food primarily, show that the nuclei are large. The nuclei disintegrate and disappear as the giant cells get older. Giant cells formed by the fusion of a few cells have fewer nuclei than those formed by fusion of numerous cells.*

The parasites enter the root tips—most of them do this, but not all by any means. It is the favored place of entry. They take up their position in undifferentiated tissue; and as the root matures, the tissue surrounding the parasites differentiates into the vascular elements and so forth, while the tissue immediately surrounding them is entirely undifferentiated. It is on this tissue, of course, that the parasite feeds.

In the first place, the reason that the larvae, in the tomato root with which I was working, did not infest the roots is that they could not if they had wanted to. They would have nothing on which to feed. The cells had already matured. The roots were beyond the stage at which the nematodes could either retard their differentiation and keep them in a condition whereby they would supply the parasite with food, or would contain enough food to maintain them, even if they were able to feed on it. In other words, in these cases, it is necessary for the parasite to influence the normal maturation of the root tissues. Thus, in the case of those roots where the larvae fail to develop, I suggest that the reason they fail is because they are unable to influence the normal maturation of the root tissues. The root tissues go on and develop in an almost normal manner, and the parasites die of starvation.

I had thought once that the reason the parasites died was because they killed all the tissue around their heads in the region of invasion.

*Editor's note: This was the end of that portion of the talk based on discussing features to be seen in the lantern slides.

The term "super-sensitivity" has been used for such relationships. I flirted with that idea quite awhile. It was an attractive theory, and looking at some plants like peaches, where there is so much necrosis around the nematodes, it looked as though there might be something to the idea. Certainly, the matter required investigation.

I repeated my work with tomatoes on marigolds to a considerable extent. I used marigolds as a plant in which the parasites did not develop. I very quickly found that there was no necrosis around the head of the parasite. It was just the opposite, nothing happened around the head of the parasite, or, at least, very little happened up until about eight or ten days after invasion. At the end of about a time like that, I do not remember the exact figure, there developed a condition that compared favorably with the condition in a tomato root tip about two days after larvae entered. That is as far as it went, because by that time the parasites were dead.

I think in those cases, and in the case of marigolds and probably *Crotalaria*, that the reason the parasites fail to develop is because they do not, or are not able to, modify the roots in such a manner that they can feed on them. I offer that as a suggestion.

What about the *Caladium* tubers I spoke of before? I suggest that in some tissues like *Caladium*, for example, the parasite can feed on the tissue without any changes whatever, just as it is. Therefore, it does not make any difference whether they can modify or not modify it; it is already in such condition that they can feed on it.

Quite a long while ago when Mr. Machmer was in Beltsville, he was working with rose geraniums, one of the Pelargoniums. He was growing them in root-knot nematode infested soil. Some little hard spots were produced on the roots which looked somewhat like galls. However, no root-knot nematodes could be found in this tissue. All the plants were grown from cuttings. One day he noticed at the base of one of the cuttings where the roots originated quite a little gob of tissue, a soft, succulent tissue. I expect, perhaps, you would classify it as callus tissue. It was found to be just loaded with females and lots of egg masses. I suggest that the reason the parasites developed in that tissue is because it was of such a nature the nematodes did not need to do anything to it at all. The reason nematodes did not develop in the roots is that the roots just went on in normal development and starved the parasite out.

Not long ago I had an occasion to look at some soybeans that one of the experimental people in Gainesville was growing. He had several varieties of soybeans that he considered to be fairly root-knot resistant. We went out into the plots and began pulling up plants. There was one variety that certainly did look good. We could not see any galls on the roots; they seemed to be free from galls. I took some plants back into the laboratory to look at them a little more carefully. I found plenty of female root-knot nematodes with egg masses in the bacterial nodules.

I am suggesting that the reason the females developed in those nitrogen nodules is that the tissue of those structures was of such a nature that the nematodes could feed on it, while, perhaps, they could not feed on the other parts of the root system.

There has been great speculation on why it is that an occasional root-knot nematode will develop on a resistant plant. Every now and then some are found that will, for one reason or another, come through and lay a few eggs. It has been suggested that if one could just gather those eggs, rear them, then put them on the same plant again, and maybe get a few more that layed a few eggs, that in a little while one could probably breed a strain of root-knot nematodes that would not be susceptible to this particular plant's selective elimination proposition.

I suggest that the reason those individuals happened to grow up and lay a few eggs was due entirely to their position on the plant. They were lucky enough to get somewhere on the roots where there was a little plant tissue that retained enough protoplasm in the cells that they were able to feed on and get enough food to reach maturity. It was only those lucky ones that happened to get in such positions that were able to mature.

Just one more comment or observation before closing. Quite a number of years ago, when I was working with Dr. Arndt at Clemson, we were interested in the possible role of nematodes in certain cotton seedling diseases. We were interested in their possible connection with the diseases of "sore shin" and "big shank." The latter was a condition in some of the fields in South Carolina in which the hypocotyls were enlarged. The two disease symptoms did not look alike. In the case of big shank there were no lesions.

I took quite a number of plants from fields showing big shank back to the laboratory at Clemson, and I teased them out with needles. I could find nothing. There were no nematodes in them that I could recognize, although I looked with considerable care. At that time the problem rested there.

Then some years later I had occasion to grow cotton seedlings in root-knot infested soil at Beltsville. Everyone of them developed big shank. That put me to thinking, so I really went after it that time. We cut and studied sections and easily found the remnants of females that had gone in and lived long enough to cause the swelling but had finally died. They were no longer living, I am sure, when we teased them out, but they were still there. They are not easy to find, but one can find them by sectioning.

That reminded me of some carrots that came to the laboratory at Beltsville. They had beautiful root-knot on them. Of course, everybody knows that carrots are very susceptible to root-knot, and everybody knew exactly what was on them when they were questioned about it. Somebody just suggested in sort of an off-handed way that one of the girls

dissect out some of the parasites. She could not find any. Of course, no one would believe her, but everybody had a hand in it, and nobody else could find any root-knot. There were root-knot galls but no root-knot nematodes.

I think that there are cases where these parasites do go into plants, and they do produce galling, sometimes considerable, and then die, leaving no remnants that are easy to find; they can be found, but not easily.

Well, those are my thoughts.



RESISTANCE AS A FUNCTION OF TOLERANCE LEVELS

W. B. Mountain

The breeding of resistant varieties has been of tremendous help in our efforts to control plant disease. A great deal of progress has been made in breeding for resistance to nematode-induced diseases. However, in some crops, at least, breeders are being forced to use a mechanism of tolerance, rather than one of true resistance. A mechanism of tolerance is being used in Canada in the tobacco breeding program for resistance to Pratylenchus. As this undoubtedly occurs in other crops, and breeders may have to use it quite frequently, I thought we might look at some of the problems which arise when a nematologist is asked to establish the degree or level of tolerance a plant possesses in relation to a particular nematode.

In Canada, several lines of tobacco have been developed which possess apparent resistance to the root lesion nematode. These lines were derived from selections based on plant size and without any idea of what nematode was involved. It was found later that, in certain areas following certain crop rotations, the resistance was not nearly so marked. A few years ago, we found that a susceptible tobacco variety is one which is severely stunted when a sufficiently high population of Pratylenchus invades the roots. In contrast, a so-called resistant variety is one in which no measurable amount of stunting occurs, although the roots contain as high a population of Pratylenchus as did the roots of the susceptible variety. So, obviously, the mechanism which the breeders have been using is tolerance. Our interest became aroused when we found that this tolerance was quite relative and could be upset rather easily by increasing the number of nematodes within the roots or, as might be expected, by substituting another species of Pratylenchus. It was then apparent to us that the reaction which our breeders were using in their search for resistance would be very difficult to define, since it was relative to the inoculum level (i.e. the number of nematodes within the roots) and to the species of Pratylenchus involved. Therefore, it will be very difficult for the breeder to base his selections on differences in plant size unless the nematologist can tell him whether or not these actually represent true differences in the tolerance level. This will not be as easy as it may appear.

At present, I am trying to find a technique whereby I might indicate to the plant breeder the precise tolerance level of his material by indicating the number of Pratylenchus per gram of root of a certain species required to reduce the growth of that plant by some arbitrary amount. This figure would be accompanied by a standard deviation and would give him a precise comparable measurement of the tolerance of his breeding material.

As a basis for this technique, we are making use of two relationships we found during the earlier work with brown root rot of tobacco. First,

With a susceptible variety there is a regression of growth to root population, and the coefficient of regression is highly significant. Secondly, the population build-up of Pratylenchus in the roots of the tobacco plant can be altered by soil temperature. Because there appeared to be a linear relationship between growth of the tobacco plant and the number of Pratylenchus within the roots, and since the number of Pratylenchus which entered the roots could be controlled by soil temperature, it appears feasible that by using these two characteristics we might be able to construct a growth curve which would be characteristic for each tobacco variety in relation to several species of Pratylenchus. Theoretically, at least, by following along these growth curves, it should be possible to express the tolerance in terms of the root population of a certain Pratylenchus species required to reduce growth to some arbitrary level; and we have chosen a reduction of 50%. This technique is not new, of course. It is the same technique as one uses to evaluate the tolerance of a fungus spore to a fungicide, or an insect to an insecticide.

This technique could only be carried out under rigidly controlled environmental conditions. The various temperatures of the soil must be held constant, and conditions for the growth of the plant must also be constant, so that the growth response will not vary from one test to another.

The actual procedure we are following, at present, is this: The various species of Pratylenchus are maintained the year around in the greenhouse in the roots of selected host crops. The species include Pratylenchus penetrans, P. minyus, and two other single-striated populations which do not fit our present keys. At the time the test is to be carried out, the roots of the host crop are chopped and mixed into the soil, which is then placed in steamed five-inch pots. These pots are then placed in six special Wisconsin soil temperature tanks, each maintained at a separate temperature within a range of 1° F. In each tank, a control is set up consisting of the identical soil treated with D-D at a rate corresponding to 40 Imp. gallons per acre. Seedlings of the variety to be tested are planted in the soil, one plant to each pot. The plants are grown under constant light of 1,000 fc for a 17 hour day during a period of exactly 50 days. At the end of the test, the plants are weighed to the nearest 1/100 of a gram, and the weight is then expressed as a per cent of the potential weight at each temperature. The potential weight, of course, is that in the absence of the nematode, i.e. in the D-D treated controls. The roots are incubated, using a modification of Young's technique, whereby the roots are placed in pint jars and stored in the dark at 65° F. for 2 months. At weekly intervals, a solution of ethoxyethyl mercury chloride and streptomycin sulfate is sprayed over the roots in each jar by a compressed air sprayer. This treatment inhibits bacteria and fungi. The numbers of Pratylenchus which emerge are counted, and the populations are standardized on the basis of the numbers per gram dry weight of root. Growth-population curves are then plotted on log-normal paper, and probit analyses are carried out. We then establish the population required to reduce growth 50%, and

the standard deviation of this figure is calculated. Theoretically, at least, we have reached the stage where we can make a critical comparison of the tolerance of this variety with that of some other.

I would like to emphasize, at this time, that there is still considerable doubt as to whether this technique will work at all. We have been able to get the standard deviations under control, although we are getting a very good fit of the points to the line. We hope we can reduce the standard deviations by some further refinements in our techniques, which we will try this fall. However, there is a possibility that the soil temperature is having an effect upon the dosage response curve and that we are not getting true linearity. In any event, we have only worked with this technique for a year, and that has not been sufficient to eliminate all of the difficulties. Thus far, we have tested three varieties of burley tobacco and five varieties of flue tobacco against four populations of Pratylenchus.

This work may appear to be quite academic, and we all realize it can only be carried out where rather elaborate facilities are available. I am the first to admit that the method may never have any practical value. On the other hand, if one ever can define the relationship between growth and the nematode with mathematical precision, one has uncovered a very valuable research tool. We will actually be measuring the interaction between the two organisms, and, therefore, one could study the reaction of either organism by holding the other constant. For instance, by testing different strains of the host against one Pratylenchus species, one can determine variations in the tolerance of these plant strains which, basically, is the reason for this work. This, of course, should be of great benefit to the plant breeder. On the other hand, if one were to test different populations of the nematode against one variety of the plant, one should detect variations in the pathogenicity of the nematode which, of course, reflect differences in the physiology of the two populations. A knowledge that such physiological differences occur should be of benefit to the taxonomist in his search for morphological differences between the populations.

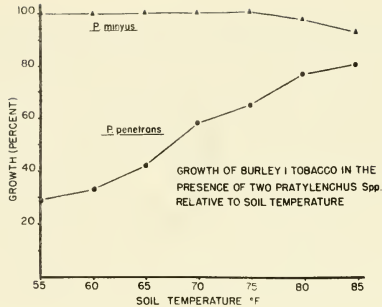


Figure 1. This variety is tolerant to P. minyus and stunting occurs only at very high soil temperatures. In contrast, the variety is very susceptible to P. penetrans.

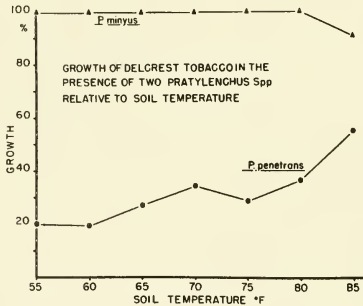


Figure 2. This variety behaves exactly as that shown in figure 1.

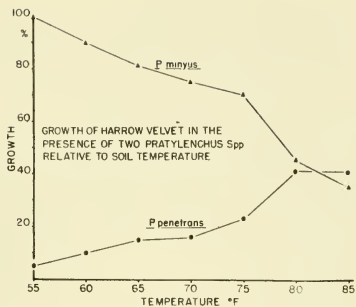


Figure 3. This variety of tobacco (Harrow Velvet) in contrast to the varieties illustrated in figures 1 and 2 is susceptible to both species of Pratylenchus.

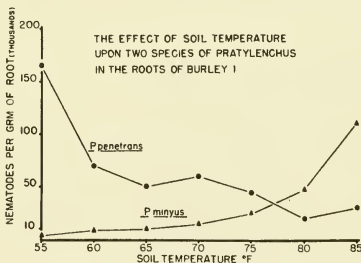


Figure 4. The optimum soil temperature for Pratylenchus minyus and P. penetrans is shown to be quite different. This observation was used to maintain different levels of nematode inoculum in establishing the "dosage-response" curves.

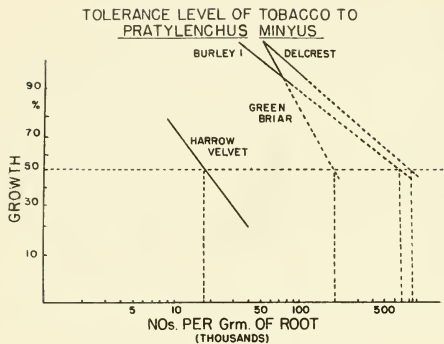


Figure 5. The dosage-response curves for *P. minyus*. Notice that the 50% growth reduction value is much lower for Harrow Velvet than for any of the other varieties. We can now measure quantitatively the tolerance to the different varieties.

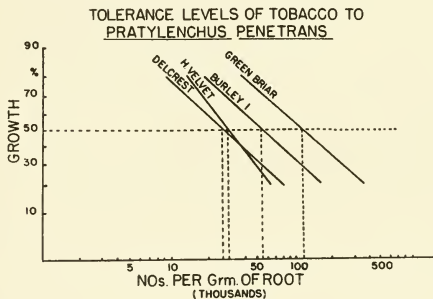


Figure 6. The dosage-response curves for *P. penetrans*. Notice that the 50% growth reduction value is not very much different in any variety, i.e., there is not the tolerance there was for *P. minyus*.

BREEDING FOR RESISTANCE TO DITYLENCHUS

Dr. J. W. Seinhorst



Breeding for resistance against the stem nematode, Ditylenchus, has been tried now in three agricultural crops: rye, red clover, and alfalfa. The breeding work for resistance in rye is the oldest of the three. In Europe there are a few varieties of rye which appear to be more or less resistant against attack by stem eelworms. This disease of rye occurs in Germany, Holland, Belgium and France. We do not know much about the stem eelworm of rye in France.

In Holland there appear to be two local varieties of rye which are more or less resistant. There is one variety in Germany and one or two in Belgium. The German variety had been reported resistant in Holland somewhere around 1903. Some breeding had been done just after the first World War on the so-called Ottersum variety. Ottersum is a small locality in the southern part of our country. Actually Ottersum rye was derived from the German local variety of rye.

The breeding in red clover has been done in the field by making crosses, sowing the seed in the infested land, and doing the selection in the field. Starting from material developed by field selection, it was possible to improve the variety and to get reasonable commercial quality and resistance against stem nematodes.

The alfalfa breeding work for resistance against the stem eelworm has been done in the United States. Attacks by stem nematodes in alfalfa seem not to be very bad in Europe. In the United States, South Africa, and Australia the disease occurs more or less regularly. Breeding for resistance in this crop was certainly done in the first part by field selection.

In all these crops, field selection has only limited possibilities. Take, for instance, the difficulties with rye breeding. If one sows rye in infested fields, one is by no means sure that all the plants will be infected. To have a chance of obtaining a reasonable degree of infection of rye in our country, we have to sow the rye in October or November. If we chose another time of year when the breeding of rye sometimes is possible, we would have little chance of attack of the plant occurring.

Although the soil is infested when sowing rye in Spring, the chance of infection occurring is very small. Moreover, even in an infested field and sowing the rye at the right time of the year, the degree of infection is extremely variable. So, when we decided in Holland to take up the breeding of resistant rye again, we decided that a good result could be obtained only by devising laboratory methods for the inoculation of the rye.

The same approach to the problem had been done already in Sweden by Binge-fors. He found from his work that inoculation of seedlings in the laboratory was the only possibility of making fast progress in the breeding program. The first to develop a good method for laboratory inoculation of plants was Binge-fors. His technique is to place seeds or seedlings along the edge of a long strip of filter paper. Over this strip is laid a second sheet of filter paper. Both sheets of filter paper are moistened; the seeds then will stick to the paper. The sheets are rolled up together and the roll stood on end in a beaker with some water in the bottom. The edge of the roll with the seeds is uppermost.

In two or three days the seeds will germinate. As soon as they start germinating they are inoculated either by putting a nematode suspension on top of the whole roll of paper or by putting a drop with a certain number of stem nematodes on top of each germinating seedling. After three or four days the first symptoms will appear, or a little longer time can be allowed.

In about four days the paper is taken out of the beaker and unrolled. One of the papers is folded back and the plants are examined. The infected ones are discarded, keeping only those free of disease. The paper is folded back, rolled again, and replaced in the beaker for a week or so and then rechecked.

It is really a very handy method. In about two weeks one can select the resistant plants, take them from the paper, and plant them in soil. In this way one can inoculate and investigate thousands of seedlings in a very limited space, and, moreover, one can keep the seedlings at the same temperature winter and summer. This is about 10° to 15° C. There is usually no objection, as far as the observation of the infestation of the plant goes, to using a higher temperature, but generally more molds and bacteria develop on the paper. So it is better to keep the temperature rather low.

In Holland, we were rather interested in having a similar kind of laboratory test for rye plants. However, the rye seed is too thick to be rolled between thin sheets of filter paper. We finally developed a method that solved the problem. We used a rectangular shaped filter paper pad that is 1 mm thick. We cut slits in the filter paper, using a sharp chisel, and the seeds were inserted in the slits. The paper was then moistened a little, but not too much as it becomes too soft. Rye seed has a sharp point at the end where the embryo is situated. The seeds are pushed into the paper with the sharp point forward, using a pair of forceps with grooves in the tips. The filter paper pads are supported in an aluminum frame, and these can be stood one next to another in a box.

To make the inoculation of the red clover, when used in this technique or in some other way, we simply take a drop of nematode suspension containing about ten stem nematodes and place that drop on the plant. Then

at this low dosage rate we produce a very good percentage of infected plants, whether resistant or not. In the work with rye, however, such a simple method of inoculating does not work at all. It is necessary to get the nematodes between the unfolding leaves.

To inoculate the rye seedlings we use a hypodermic syringe. It is not possible to use a plain water suspension of the nematodes for inoculum, as the nematodes soon clump together and will not come through the needle. To avoid difficulties like that, we prepare the nematode suspension with methylcellulose which keeps the nematodes from settling out. Precautions must be taken to eliminate air from the syringe. For routine work we use a large syringe with an accurate metering device which permits giving any uniform doses of the nematode suspension. The suspension can be prepared to contain any desired number of nematodes per fraction of a milliliter of the suspension used for each inoculation.

The rye seedlings are inoculated by putting the needle into the plant just about at the point where the roots come out of the seedling. About 500 nematodes are applied per plant. This is a very high number as compared with the low numbers of stem nematodes which can cause damage in the field. We do not know what is the reason for it, but to be sure of getting attack and symptoms on about 98 percent of the plants, we have to use that high number of nematodes. In the case of red clover, about 10 nematodes per plant is sufficient to produce symptoms.

In the testing of rye seedlings the use of a low temperature is necessary to obtain recognizable symptoms, as well as for retarding growth of the molds and bacteria. If rye seedlings are kept at higher temperatures, they grow so fast no symptoms develop. We, therefore, use a 5° C night temperature and 18° C during the day, or a mean temperature below 10° C, in order to get recognizable symptoms.

The two methods described can be used for various monocotyledonous and many dicotyledonous plants, too. We have used the methods for oats. In this case, it is necessary to take the hulls off the seed to get easier access to the coleoptile. Stem eelworm of oats is a problem in England and Scotland.

We rate the plants from these tests as follows: no symptoms, doubtful (question of whether it is nematode attack or damage by inoculation), light attack, and heavy attack.

The next part of the work is the evaluation of the resistance. In previous talks we have already indicated what types of resistance there are in plants against attack by stem nematodes. In the case of red clover, we mostly use necrosis as an indication of resistance, although necrosis is not the only symptom. In rye, however, we must use another method, as the resistant rye plants do not show necrosis.

In the resistant red clover, it is possible to see the necrosis which is indication that the plants have been attacked and that nematodes are in the tissues. However, no normal disease symptoms develop, and there is

no multiplication of the nematodes in these resistant plants. Because the nematodes do cause this necrosis in the resistant plants, it is necessary to be careful with the dosage of stem nematode. If the number of nematodes is too high, the resistant plants may be killed. Plants which do not show necrosis nor show normal stem nematode disease symptoms are only escapes. If they are kept for a few weeks, they may later develop swellings if infection occurs later.

It is not accepted that if a plant is more or less resistant, all of its tissues have the same degree of resistance. Some tissues may prove to be more or less susceptible even in resistant plants. Beets, for instance, when infected with the rye stem nematode show two types of symptoms. In beet seedlings the normal symptom is damage to young tissues causing crinkling. There are hardly any nematodes in these irregular tissues. By the time the beet has developed its swollen root, there may be a heavy nematode attack in the top of the root. In Holland I have never seen any attack by nematodes in the beet leaves even when there have been enough chances for the leaves to contract the nematode infection. I think this must mean that the leaves are not susceptible, but the top of the root, or beet itself, is susceptible. In England the leaves of the sugar beets are infected. This may mean that the English races of the stem nematode are different from ours.

Fortunately, red clover plants which show the necrosis type of resistance occur in all the red clover varieties. It is not a high percentage, but it is enough to start a breeding program. Thus it is not necessary for us to cross local varieties with the highly resistant varieties from other countries. To have to do that would cause difficulty in Europe, as we would have to go to the Swedish varieties. These are late varieties and this character of lateness in the Swedish varieties is a very persistent character not easily rid of in breeding work. Now, we just take the local varieties and select the resistant plants.

In the work with rye plants, we use the absence of stem eelworm disease symptoms as the characteristic for resistance. Unfortunately, in rye there are all sorts of degrees of resistance. There is a full series, ranging from highly susceptible to highly resistant, and one has to draw the line somewhere. That was one of the difficulties that people doing breeding of resistance of rye in the field ran into. They could only find and eliminate the heavily infected plants and go on with the remaining material which contained a rather high percentage of susceptible plants. The result of their work at the end of about five years was that they had not obtained more than 25 percent of really resistant plants; 25 percent were just as susceptible as the usual susceptible varieties; and the remaining 50 percent were susceptible, but not very susceptible. In the heavier inoculation tests on the filter paper, we could throw out so many of these half susceptible plants that in three years of breeding we had material which showed hardly any susceptibility, and we had over 50 percent of highly resistant plants. This took only three years, beginning with material which consisted only at most of 5% or, perhaps, only 2 or 3 percent of resistant plants. Thus with this crop, work in the laboratory

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They should not be kept at too high a temperature because secondary rots develop.

Q. Would you explain how the nematodes are dried and kept in the refrigerator?

A. We extract the nematodes from the infected tubers or plants and sieve them to get a clear suspension of the nematodes. We then filter out the nematodes on filter paper. We hang the paper up to dry after which the nematodes can be stored in the refrigerator for about two years and sometimes more. Just put pieces of the filter paper in water to revive the nematodes.

Q. Do you think it is possible that the reason these nematodes fail to grow in the resistant plants is that the enzymes produced by the nematodes fail to dissolve the middle lamellae?

A. Yes, I think that is the reason in all those cases where there is absolute resistance and no development of the nematodes. This is true, not only for red clover, but also rye. There is a very slight degree of dissolution of the middle lamellae in resistant rye. The nematodes may develop to the fourth larval stage in rye, but cannot go on any further.



BREEDING FOR RESISTANCE TO POTATO ROOT BEETLE,
HETERODERA MOSTOCHIENSIS WOLL.

F. G. W. Jones

An expedition went from Great Britain in 1938 to collect tuber-forming species of Solanum from mountainous areas in south and central America. Many of the plants brought back were screened by Ellenby (1954), who was able to demonstrate resistance in S. vernei (=balsii) and in five tetraploid lines of S. tuberosum var. andigena. Work in Holland, Great Britain, and Germany (Toxopeus & Huijsman 1953, Jones 1954, Huijsman 1955, 1956, Howard 1955, Williams 1956) has confirmed Ellenby's results, and the resistant material has been used as a basis for the breeding of new varieties. Resistance has also been reported in S. sucrense (Mai & Petersen 1952) and in S. capsicibaccatum, S. aff-famatinae, S. microdontum, and S. suaveolens (Goffart & Ross 1954). S. vernei and S. catarthrum are reported to be used as resistant parents in Russia (Hawkes 1956). Table 1 summarises the material used for breeding in Great Britain.

Breeding from the tetraploid S. andigena is easy, since the cultivated S. tuberosum varieties are all tetraploids. S. vernei and the other resistant species are diploid and must first be treated with colchicine to obtain plants with 4n chromosomes. Crossing in S. vernei is then satisfactory. Certain complications arise from tetraploidy (Haldane 1930). Assuming that resistance is due to a single dominant gene (H), then five types of plants are possible, as shown in column (1) of Table 2. The usual symbols and the nomenclature adopted for these types are shown in columns (2) and (3), while the expected gametes and the results of crossing with the recessive (susceptible = 0) or selfing, are set out in columns (4), (5), and (6) respectively. Small deviations from these expected ratios would be expected on a random chromatid rather than random chromosome hypothesis. Deviations are small for crosses with simplex and duplex plants, but are chiefly of interest, because they explain the appearance of susceptible plants in triplex crosses where only resistors are expected.

Breeding from resistant lines of S. andigena is now well advanced. Resistance is apparently due to a single dominant gene (H), (Table 3), which, when simplex, gives approximately 50% of resistant plants in crosses with susceptible plants and approximately 80% of resistant plants when duplex, column (5) Table 2 and Table 3. Toxopeus (1956) has indicated that the gene is known from a limited area in the Andes (S. Peru, Bolivia, and N. Argentine; absent from Central and N. Peru, Ecuador, and Colombia).

Types of resistance have been reviewed by Ellenby (1945), Jones (1955), and Williams (1956).

Plants may be placed in three arbitrary categories, namely:

- (1) Absolutely resistant. Not invaded.
- (2) Partially resistant. Invaded.
 - (a) larvae fail to develop,
 - (b) larvae develop but fail to mature, and
 - (c) larvae mature, but the females are reduced in numbers and are not very prolific.
- (3) Susceptible. Large numbers of highly prolific females are produced.

Other considerations entering into the problem are whether or not the roots produce the hatching factor, whether or not the roots produce an additional and quite separate substance responsible for attracting larvae to them, and whether or not the roots respond to invasion by the production of giant cells.

Screening tests are based on pot work which assesses best efficiency, not tolerance of invasion. Table 4 shows the results of investigations by Williams into the larval invasion of resistant plants. All the plants examined were invaded, formed giant cells, and produced subsequent stages in the proportions shown in columns (3) to (7). Generally, the unmodified larva, as found in the soil (arbitrary stage one), made up the greatest percentage of forms observed. A small percentage of males which appeared normal were also noted, together with the virtual absence of forms distinguishable as females; although in the root samples under test, one small immature female was observed on the roots of each of two plants. The occasional appearance of a few cysts on resistant plants is sometimes a source of embarrassment in the determination of the ratios of resistant to susceptible plants. More must be said of this later. Evidently, resistance is only partial (category (2) c.), and this holds equally for the four lines of S. andigena (lines H1 - H4), for the crosses of S. vernei with S. stenotomum (the cultivated diploid potato), and for S. vernei itself.

The final column in Table 4 gives the rate of invasion/gm. of root. Fibers and stolons are less resistant than roots. The falling off in the rate of invasion from the susceptible types at the head of the column to the S. stenotomum x S. vernei crosses at the foot is apparent, rather than real, and may be connected with the month in which the observations were made. The very low figures for S. vernei, obtained in mid-August, may possibly indicate somewhat greater resistance to invasion, but requires confirmation.

In experiments conducted upon the production of the hatching factor, 27 plants of S. andigena, drawn from the four lines (H1, H2, H3, and H4), all produced a root diffusate active to a greater or lesser degree, although the average was a little below the activity of S. tuberosum varieties, Langworthy, Red Gladstone, and Golden Wonder. S. stenotomum x S. vernei crosses and S. vernei itself produced root diffusates of low activity, but there was some evidence for segregation of this factor in the former, one plant in twelve producing diffusate fully as

active as that of *S. tuberosum*. In breeding for resistance, it would obviously be an advantage to have two types of resistant plant, one producing little or no hatching factor, which would tend to 'guard' or enhance resistance; and another type, producing much of the hatching factor, which would make the ideal trap crop, after suitable breeding, to give tubers of economic value, if only for feeding stock. The preliminary experiments outlined above suggest that the first type is more likely to be found by crossing with *S. vernei* than with *S. andigena*. Unfortunately, however, active diffusates are produced quite widely amongst Solanaceae, so that response is not highly specific to hosts.

The effect of resistant plants on soil populations has been investigated in pot experiments and in the field. The results of a pot experiment are shown in Figures 1 and 2. In Figure 1, the effect of five individual resistant plants from each of the lines H1, H3, and H4 are compared with fallow soil (O), susceptible *S. andigena*, and *S. tuberosum* seedlings (H13 and H14 respectively), and with plants grown from Gladstone tubers.

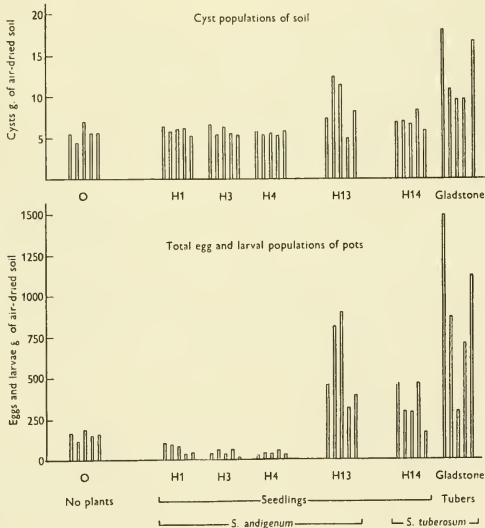


Figure 1

Results of a pot experiment.
Resistant plants have reduced the soil population.

At the start of the experiment, the pots of 10" diameter were filled from a mixed bulk of infested soil and were sunk into the ground to prevent drying out. At the end of the experiment, the cyst counts (not 'viable' cysts) from the pots containing resistant plants showed no increase over the unplanted controls (0), whereas the susceptible plants caused increase in varying degrees. The cyst contents were investigated by a squash technique, and there was evidence of a decrease in egg and larval counts for the pots containing resistant plants, and large, but variable, increases for the pots containing susceptible plants.

Further batches of cysts were submitted to the action of a standard root diffusate from *S. tuberosum* and produced the hatching curves shown in Figure 2.

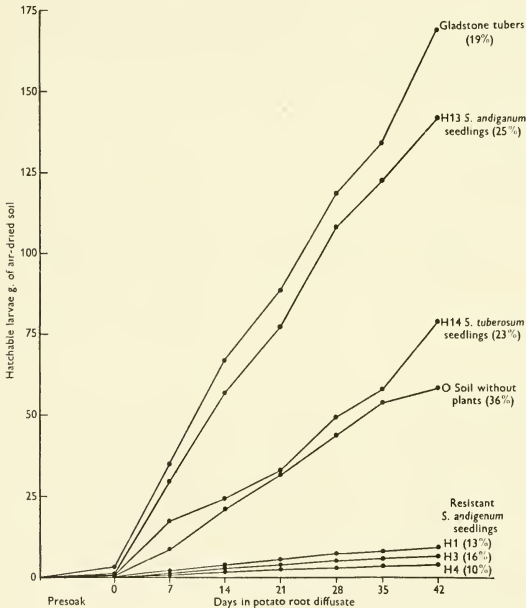


Figure 2

Hatching tests on cysts after growing resistant plants. Susceptible plants have produced cysts from which many larvae hatch. Susceptible plants have apparently removed most hatchable larvae from cysts.

Although the hatch was not taken to completion, marked differences in the 'hatchable' contents of the different batches of cysts are apparent. It seems clear that the resistant plants produced few or no new cysts and that their active root diffusates caused partial emptying of the cysts already present.

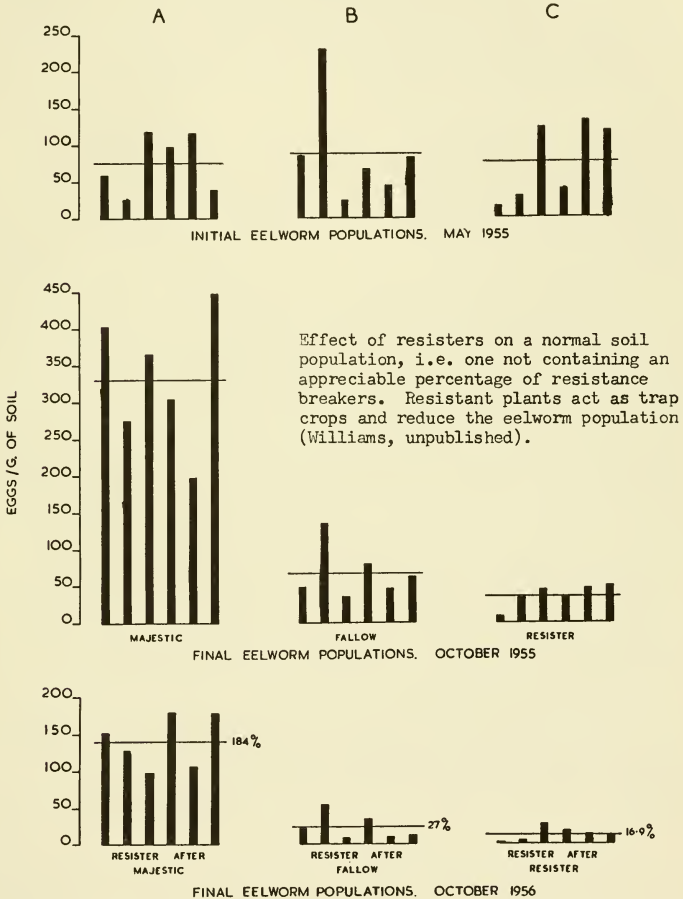


Figure 3

Field tests have given results comparable with pots. Figure 3 gives the results of two years' plot work (Williams, unpublished). In the first year the cultivated variety Majestic gave a large population increase, while a resister gave a slight reduction compared with fallow. In the second year, the whole area was sown with a resister. This reduced the population after Majestic, after fallow, and after the resister. Two years' cultivation of the resister resulted in a very low potato eelworm population, indeed, and, with this particular population, did not suggest any breakdown of resistance.

Reference to the presence of occasional cysts on resistant plants has been made. The different lines of *S. andigena* vary, C.P.C. 1673 especially producing fewer than others. The appearance of these cysts was puzzling. They might be explained by variations in the susceptibility of plant roots, variations in the conditions of tests, or by the existence of resistance-breaking biotypes. Evidence is now accumulating in favour of the last explanation. Van der Laan (1957) obtained cysts from Peru and found that these broke resistance, and this was subsequently confirmed by tests of tubers sent from Holland to Peru (Quevedo, Simon & Toxopeus 1956). Meanwhile, evidence of a similar kind had been found by Dunnet (1957) in Scotland. Here, one garden population was found to be very aggressive. Confirmatory tests done by Jones (1957 and unpublished work) have shown that British populations vary considerably in their content of resistance-breaking biotypes (Tables 5, 6, 7, and 8).

In one series of tests, the proportions, crudely measured, ranged from less than 1% to 75%. Subsequent tests with these populations have indicated that there is only a very slow swing, when resisters are grown, towards increase in the percentage of resistance-breakers at 1% level, and that increase is more rapid at higher percentages. This is shown in Table 9 and also in Table 10. In the latter, a population raised on susceptible and resistant plants was grown on resistant plants in single cyst culture (i.e. 1 cyst/pot). On the susceptible plant, the population remained static at 4% of resistant-breakers, while on the resistant plant, the proportion of resistance breakers increased 3-4 times. These results are not inconsistent with the hypothesis that the resistance-breaking character in nematodes is recessive, but the results, so far, are slender and require confirmation of various kinds.

Dunnet (1957) could find no evidence that resistance in *Solanum vernei* was broken by his aggressive population. It would be valuable if resistance here were of a different type, although breeding from *S. vernei* is likely to be more difficult than from *S. andigena*.

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I should like to thank T. D. Williams, H. W. Howard, and P. A. van der Laan for allowing me to mention some of their work.

REFERENCES

- Dunnett, J. B. (1957). Variations in pathogenicity of potato root eelworm (Heterodera rostochiensis Woll.) and its significance in potato breeding. Euphytica, 6, 77-89.
- Ellenby, C. (1945). Susceptibility of South American tuber-forming species of Solanum to the potato root eelworm (Heterodera rostochiensis Wollenweber). Emp. J. Exp. Agric., 13 (51), 158-168.
- Ellenby, C. (1954). Tuber-forming species and varieties of the genus Solanum tested for resistance to potato root eelworm, Heterodera rostochiensis Wollenweber. Euphytica, 3, 195-202.
- Goffart, H. & Ross, H. (1954). Untersuchungen zur Frage der Resistenz von Wildarten der Kartoffel gegen den Kartoffelnematoden (Heterodera rostochiensis Wr.). Züchter, 24, 193-201.
- Haldane, J. B. S. (1930). Theoretical genetics of autopolyploids. J. Genet., 22, 359.
- Hawkes, J. G. (1957). Potato breeding in Russia. Euphytica, 6, 38-44.
- Howard, H. W. (1955). Breeding potatoes for resistance to root eelworm (Heterodera rostochiensis). Heredity, 9, 150.
- Huijsman, C. A. (1955). Breeding for resistance to potato root eelworm II. Euphytica, 4, 133-140.
- Huijsman, C. A. (1956). Breeding for resistance to potato root eelworm in the Netherlands. Nematologica, 1, 94-99.
- Jones, F. G. W. (1954). First steps in breeding for resistance to potato root eelworm. Ann. Appl. Biol., 41, 348-353.
- Jones, F. G. W. (1957). Resistance-breaking biotypes of the potato root eelworm (Heterodera rostochiensis Woll.). Nematologica, 2 (in the press).
- Laan, P. A. van der (1957). Tindschr. PlZiekt. (in the press).
- Mai, W. F. & Petersen, L. C. (1952). Resistance of Solanum Ballsii and Solanum sucrense to the golden nematode, Heterodera rostochiensis Wollenweber. Science, 116 (3009), 224-225.
- Quevedo, A., Simon, J. E. & Toxopeus, H. J. (1956). Estudios de resistencia a la anguillula dorada de la papa. Inf. Estac. agric. La Molina, 347, 10-15.
- Toxopeus, H. J. (1956). Collecting cultivated potatoes in South America for potato breeding. Euphytica, 5, 97-200.
- Toxopeus, H. J. & Huijsman, C. A. (1953). Genotypical background of resistance to Heterodera rostochiensis in Solanum tuberosum var. andigenum. Nature, Lond., 170, 1016.
- Williams, T. D. (1956). The resistance of potatoes to root eelworm. Nematologica, 1, 88-93.

TABLE 1

Some breeding material used in Holland and Britain
and derived from resistant material
found by Ellenby.

Lines of *S. andigenum* containing resistors
(Holland and Britain)

H1	=	C.P.C. 1692 selfed.	Cochabamba,	Cereado	Bolivia
H2	=	C.P.C. 1690 selfed.	Puno,	Puno,	Peru
H3	=	C.P.C. 1685 selfed.	Juli,	Puno,	Peru
H4	=	C.P.C. 1673 selfed.	La Paz,		Bolivia
		C.P.C. 1595 selfed.	Uroro,		Bolivia

Susceptible *S. andigenum*
(Britain)

H13	=	C.P.C. 1787
		C.P.C. 1470

Lines of *S. vernii* (= *S. ballsii*)
(Britain)

		C.P.C. = 2487)
		C.P.C. = 2488)
		} <u><i>S. vernii</i></u> selfed
D3	=	<u><i>S. stenotomum</i></u> x <u><i>S. vernii</i></u> F ₂
		C.P.C. = Commonwealth Potato Collection

TABLE 2

Ratios expected in tetraploid crosses when
resistance is due to a single dominant factor H.

(1)	(2)	(3)	(4)	(5)	(6)
Type	Symbols	Nomenclature	Gametes	Back-crossed to susceptible (0) Resistant : Susceptible	Selfed. resistant : Susceptible
FFFF	FFFF	Quadruplex	All FH	All resistant (100%)	All resistant (100%)
FFHh	FFHh	Triplox	LHh : lHh	All resistant (100%)	All resistant (100%)
FFhh	FFhh	Duplex	LHh : lHh : lhH : lhh	5 : 1 (83.3%)	35 : 1 (97.2%)
Fhhh	Fhhh	Simplex	lHh : lhh	1 : 1 (50%)	3 : 1 (75%)
hhhh	hhhh	Multiplex	All hh	All susceptible (0%)	All susceptible (0%)

Other crosses: Simplex x Duplex 11 : 1; Simplex x Simplex 3 : 1.

TABLE 3

Ratios of resistant to susceptible plants in crossesof resistant *S. andigenum*s

Parentage	Number of Seedlings		Type of Cross	Expected Ratio	Number of Seedlings Expected	
	Resistant	Susceptible			Resistant	Susceptible

A. <u>From Toxopeus and Huijsman 1953</u>						
H4 x H4	(70	4	HH x HH	34:1	72.0	2.0
	(291	25	HH x H	*(15:1)	(69.4)	(4.6)
	(119	51	H x H	11:1	289.3	26.3
<u>H4 x</u> <u><i>S. tuberosum</i></u> <u>varieties</u>	(565	193	HH x O	5:1	631.5	126.3
	(215	263	H x O	*(3:1)	(568.5)	(189.5)
	(1:1	239.0	239.0
				*(5:7)	(199.2)	(278.8)
<u>H3 x</u> <u><i>S. tuberosum</i></u> <u>varieties</u>	94	311	KL x O	1:3	101.2	303.7
Susceptible H4 x <i>S. tuberosum</i>	0	129	O x O	0:00	All susceptible	
B. <u>Tests made in Cambridge</u>						
<u>H3 x</u> <u><i>S. tuberosum</i></u> <u>varieties</u>	83	99	H x O	1:1	91	91
			KKL x O	5:7	75.8	106.2
<u>H1 x</u> <u><i>S. tuberosum</i></u>	17	13				
Susceptible H3 x Susceptible C.P.C. 1470	0	50	O x O	0:0	All susceptible	

* = Possible alternative ratios (in brackets). It is now clear that only a single gene (H) is responsible for resistance, and the alternative KL system does not obtain.

TABLE 4

Degree of invasion of the roots of resistant and
susceptible *Solanums*

Type	Number of Plants Tested	*Stage one %	*Stages two to four %	Males %	Small females %	Total all stages/ gm root	Date counted
<u>Susceptible</u>							
Majestic	3	5.5	53.9	30.2	10.4	903	June
Gladstone	5	26.1	33.8	16.4	23.7	517	July
<u>Resistant</u>							
H1	9	56.6	34.6	5.8	0	413	July
H2	4	82.3	17.1	0.6	0	610	July
H3	13	64.5	28.4	6.5	0**	349	July-Aug
H4	29	90.5	7.3	2.2	0	167	July-Aug
<u>Stenotomum x vernii</u> F ₂	12	71.0	22.8	6.2	0	167	Mid-Aug
<u>Vernii</u>	13	94.3	0	5.7	0	53	Late Aug
Total tested	88						

* = These are arbitrary stages and do not relate to true stages between moults.

** = One small immature female observed on the roots of two plants in the samples under test.

TABLE 5

Tests of various resistant crosses against five local races of
potato root eelworm. Cumulative results of three
visual assessments of cysts on the peripheral root system.

Source of Cysts	Duddingston Midloth.	Bog Hall Midloth.	Feltwell Norfolk	Frampton Lincs.	Rothamsted (1) Herts.
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Resistant plants:C.P.C. 1673 line

2201(174)	S. <u>andigena</u>	+++	-	+	+	-
2296.2(64)	Fl cross	+++	-	-*	-*	-*
Y2/26	Fl cross	+++	-	-	++	++
2371b(1)	Bl cross	+++	-	+	++	++

C.P.C. 1685 line

NY27/3	Fl cross	+++	+	-	+++	+
Z3/7	Fl cross	+++	-	+	++	++

C.P.C. 1690 line

H2/2	S. <u>andigena</u>	+++	-	-	-	+
Y15/4	Bl cross	+++	-	-	++++	+

Control plants:

Potato (Arran Banner)	+++	++++	++++	++++	++++
Tomato (Klondyke Red)	+	+	+	+	+

Key: - No cysts visible on external mat of roots.
+ Up to 5 cysts visible.
++ 5-15 cysts visible.
+++ 15-50 cysts visible.
++++ Over 50 cysts visible.

* = Grown from cuttings and therefore retarded in development compared with other plants.

TABLE 6

Tests of various resistant *S. andigena* - *S. tuberosum* crosses
against five local races of potato root eelworm

<u>Source of cysts</u>	Duddingston	Bog Hall	Feltwell	Frampton	Rothamsted
No. of cysts added per pot..	52	42	50	56	54
Estimated egg content.....	7,540	7,602	7,672	7,452	7,500
Hatchable larvae in 21 days at 25° C.....	4,750	3,150	5,500	3,900	4,450

New cysts per pot at the end of the test

Resistant plants:

C.P.C. 1673 line

2201	<i>S. andigena</i>	1,047	5	0	79	0
2296.2(64)	Fl cross	2,089	0	0	57	12
Y2/26	Fl cross	677	5	3	117	1
2371 b(1)	Bl cross	1,453	12	8	480	17

C.P.C. 1685 line

NY27/3	Fl cross	2,253	70	52	293	150
Z3/7	Fl cross	1,673	19	23	318	117

C.P.C. 1690 line

H2/2	<i>S. andigena</i>	355	19	14	44	18
Y15/4	Bl cross	1,466	40	0	1,081	7

Means for resisters		<u>1,377</u>	<u>21</u>	<u>13</u>	<u>309</u>	<u>40</u>
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Control Plants:

Potato (Arran Banner)	310	1,615	2,272	593	2,790	Means <u>1,516</u>
Tomato (Klondyke Red)	48	220	146	83	152	<u>130</u>

TABLE 7

Tests of the resistant *S. andigena* - *S. tuberosum* cross, 23/7,
against various local races of potato root eelworm:
cumulative results from three visual assessments
of cysts on the peripheral root system.

Source of Cysts	Tomato (Klondyke Red)	Potato (Arran Banner)	1	23/7 2	3
<u>1st Batch</u>					
Duddingston	+	++++	+++	+++	+++
Bog Hall	+	++++	+	+	-
Feltwell	+	++++	-	-	-
Frampton	+	++++	++	++	++
Rothamsted (1)	+	++++	-	+	+
Shelford	+	++++	++	+	+
<u>2nd Batch</u>					
Oxlode	++	++	-	-	-
Cadishead	+	+	+++	++	++
Albrighton	+	++++	-	+	+
Colyton	++	+++	+++	+++	+++
St. Leonards	+	+++	+++	+++	+++
Thornbury	+	+	+	-	-
Newcastle	+	++++	+	-	-
Jersey	-	++++	+++	++	++
<u>3rd Batch</u>					
Ramsey	-	++++	-	-	-
Rothamsted (2)	+	++++	-	-	-
Fosdyke	++	++	+	-	-
Deeping	+	++	-	-	-
Scilly	+	+++	-	-	+
Gulval	-	++	++	-	-
Controls (1)	-	-	-	-	-
(2)	-	-	-	-	-
(3)	-	-	-	-	-

Key: See foot of Table 5.

TABLE 8

Tests of the resistant *S. andigena* - *S. tuberosum* cross, Z3/7,
against various local races of potato root eelworm.

Source of Cysts	<u>New cysts per pot at the end of the test</u>					Means	% mean for potato
	Tomato (Klondyke Red)	Potato (Arran Banner)	1	Z3/7 2	3		
<u>Batch I</u>							
Duddingston	30	1,412	1,570	1,035	687	<u>1,097</u>	75
Bog Hall	70	1,636	0	12	0	<u>9</u>	<1
Feltwell	222	1,341	6	0	0	<u>2</u>	<1
Frampton	85	1,246	292	122	334	<u>249</u>	17
Rothamsted (1)	169	1,727	11	122	21	<u>51</u>	4
Shelford	223	1,418	72	37	75	<u>61</u>	4
Means	<u>133</u>	<u>1,463</u>					
<u>Batch II</u>							
Oxlobe	199	930	0	0	0	<u>0</u>	0
Cadishead	120	935	573	805	483	<u>620</u>	73
Albrighton	253	1,466	10	0	0	<u>3</u>	<1
Colyton	146	824	364	742	476	<u>527</u>	62
St. Leonards	234	700	340	440	489	<u>423</u>	50
Thornbury	141	299	76	-	0	<u>38</u>	5
Newcastle	157	777	29	28	5	<u>20</u>	2
Means	<u>179</u>	<u>847</u>					
Jersey	-	723	235	127	34	<u>132</u>	18
<u>Batch III</u>							
Hamsey	52	716	0	6	0	<u>2</u>	<1
Rothamsted (2)	36	373	0	0	10	<u>3</u>	<1
Fosdyke	45	190	38	22	4	<u>21</u>	6
Deeping	145	533	12	5	2	<u>6</u>	2
Scilly	73	269	84	93	69	<u>82</u>	22
Gulval	12	207	120	67	76	<u>88</u>	23
Means	<u>60</u>	<u>381</u>					
Controls (1	1	0	1	0		
(3	0	0	2	0		
(1	0	3	1	2		

TABLE 9

<u>Less than 1% resistance breakers</u>				<u>10-25% resistance breakers</u>				
Source	Scores 1956 Arran Banner Resister	Scores 1957 Arran Banner Resister	Source	Scores 1956 Arran Banner Resister	Scores 1957 Arran Banner Resister	Source	Scores 1956 Arran Banner Resister	
Fog Hall	++++ (+ (- (-	+++ + -	Frampton	++++ (++ (-	+++ - ++			
Feltwell	++++ (- (- (-	++ ++++ +	Jersey	++++	+++			
OxLode	++	+	Silly	+++	++ *			
Albrighton	++++	+++	Gulval	++	++			
Ramsey	++++	++++	Mean Score	3.3	1.9		3.0	
Eothensted (2)	++++	++++	Relative Score		0.58		0.97	
Mean Score	3.7	2.4	<u>50-75% resistance breakers</u>					
Relative Score	0.2	0.8	Duddingston	+++ (+++ (+++	+++ +++ +++			
<u>1-10% resistance breakers</u>								
Relative Score	0.05	0.33	Mean Score	3.0	3.1		3.9	
Eothensted (1)	++++ (++ (++ (+	++++ ++++ ++	Relative Score		1.03		1.00	
Shelford	++++	+++	Cadishhead	+++ *	+++			
Thorbury	++ *	+++	Colyton	+++	+++			
Newcastle	++++	+++	St. Leonards	+++	+++			
Fosdyke	++	++++	Mean Score	3.0	3.1		3.9	
Deeping	++	++++	Relative Score		1.03		1.00	
Mean Score	3.0	3.5						
Relative Score	0.50	0.71						

* = based on cysts

TABLE 10

Single cyst tests with Rothamsted (1) cysts raised
on susceptible and resistant plants: initial
population with about 4% of resistance breakers.

Test Plants	Total Pots	Pots +	Pots -	Total Cysts	Cysts/ pot.	% Pots with cysts
<u>Cysts bred on Arran Banner (Susceptible)*</u>						
Z3/7	139	6	133	6	0.04	4.3%
Arran Banner	10	10	0	219	21.9	100%
Controls	10	0	10	-	-	0%
<u>Cysts bred on NY27/3 (resistant)**</u>						
Z3/7	105	14	91	14	0.13	13.3%***
Arran Banner	10	8	2	64	6.4	80%
Controls	10	0	10	-	-	0%

* = Average content 374 eggs/cyst.

** = Average content unknown, but probably about 100 eggs/cyst.

*** = Becomes 16.7%, assuming 80% take on Z3/7 as in Arran Banner.

The original population contained approximately 4% of resistance breakers which agrees well with 4.3% of cysts giving positive pots in the new generation on Arran Banner, i.e. no change. The expected figure for the population bred on a resister would be <20%. The value 13.3% to 16.7% obtained for the same line raised on NY27/3 is also in fair agreement.



BREEDING FOR RESISTANCE TO MELIODIOPYNE SPP.

Albert L. Smith

Historical

The breeding for resistance to root-knot is historically significant. Webber and Orton, in 1902, reported a root-knot resistant cowpea, the Iron variety. This variety was also resistant to Fusarium, in which they were interested, so that, accidentally, root-knot resistance was discovered. This stimulated the search for root-knot resistance in other crops.

Importance

Root-knot resistance breeding is of primary importance to the southeastern area for several reasons as follows:

1. Primary plant parasitic nematode in the south, particularly on tobacco and cotton, the major cash crops, as well as a host of lesser crops, particularly vegetables.
2. It is important in the initiation of the wilt disease of cotton and has more recently been found important in initiating or intensifying several other diseases.
3. Improvement in root-knot resistance may improve the resistance of crops to other nematodes. Clayton has made this observation in tobacco, and the writer believes the same situation exists in the cotton crop.

The several species of Meliodiopyne, because of the wide host range and the adaptability of this parasite to infest a wide range of soil textures, are considered the most important plant parasites in the south. This wide range of adaptation to soil textures is illustrated by their attack on the cotton crop. The general statement is made that the root-knot nematode is a problem in all the lighter soils from Virginia to California. Going to the extreme, there are a few soils which are too sandy for the survival of the root-knot nematode. These soils are primarily near the coast in South Carolina and Georgia. Holdeman and Graham have found areas in South Carolina where the sting nematode and other ectoparasites are the primary parasites and where apparently the soil is too sandy for root-knot. A small area of the Agronomy farm at Auburn, which mechanical analysis shows is 80 per cent sand, is also too coarse for root-knot survival and it is replaced by Trichodorus, Pratylenchus, and other ectoparasitic species. Although root-knot can become serious in rather heavy soils, workers report an area on the Experiment Station Farm in Louisiana where the kidney-shaped nematode,

Rotylenchulus reinformis, is the predominant type, and this soil is apparently too heavy for root-knot. The sting nematode, Belonolaimus gracilis, the kidney-shaped nematode, and the two root-knot species, M. incognita and M. acrita, are all important in providing openings for the Fusarium wilt pathogen of cotton. Root-knot is the predominant species and covers the widest range of soil textures, but the sting nematode and kidney-shaped nematode infest lighter and heavier soils, respectively.

The Problem of Root-Knot Species

Considerable confusion was created among root-knot resistance breeders by the publication of Chitwood's revision of the genus in 1949. Perhaps this tended to delay the work somewhat temporarily in some cases. However, this remarkable piece of work has placed breeding on a firm foundation, and greater progress can now be anticipated for the future.

While it is necessary when initiating a breeding program to consider the five species of Meloidogyne as independent species, it has often been found that one gene or group of genes provides resistance to two or more species. Hare, working with peppers, and Crittendon, working with soybeans, found that certain varieties were resistant to M. incognita, M. incognita acrita, M. javanica, and M. arenaria. In neither crop none of the varieties were resistant to M. hapla. Thus, M. hapla, the northern root-knot species, appears somewhat removed from the resistance standpoint from the other four species. Once the range of resistance is determined for a set of genes in a given host then testing in the breeding program may be limited to one species. To illustrate this point, in cotton M. incognita acrita gives the best differentiation for resistance in segregating populations. With tomatoes M. incognita apparently gives the better differentiation and is preferred for testing.

Procedure for Breeding for Root-knot Resistance

While much of the breeding for root-knot resistance in the past has been done by small increments and in dispersed particles, it is now apparent that a well defined formula can be outlined for breeding for resistance. This formula consists of six distinct and well defined operations as follows:

1. Determination of the species of Meloidogyne involved by testing with pure cultures of the nema; survey area of the host range for prevalence and species.
2. Develop techniques for infecting all plants in progeny populations.
 - a) Satisfactory greenhouse techniques have been developed for several crops which may be adaptable to additional crops.

- b) Develop field techniques for testing crops which are not well adapted to greenhouse production.
3. Determine the type of resistance involved and method of evaluation of resistance.
 - a) Type of resistance:
 - (1) Limited entrance.
 - (2) Limited development and reproduction after entrance.
 - (3) Tolerance due to rapid root extension downward or laterly.
 - b) Evaluation of resistance by:
 - (1) Disease index, yield, green weight, other.
 4. Search for resistant parental material in:
 - a) Established commercial varieties or closely related material.
 - b) Wild material and related species.
 5. Determine the inheritance of resistance as a guide for breeding.
 6. Cross and backcross until the desired types are established.

Inheritance of Resistance, Chromosome Numbers and
Polyploidy in Crops Bred for Root-Knot Resistance

The table at the end of the paper summarizes the available information on number of factors involved, dominance or recessiveness, basic number of chromosomes and polyploidy in breeding for resistance to Meloidogyne. From this table it can be seen that good information on inheritance of resistance is rather sparse. The problem of transferring resistance is not difficult in most cases where one or two dominant factors are involved. However, with crops such as tobacco and sweet potato where tetraploids and hexaploids are involved the problem is more complex. In cotton, an amphidiploid, where several recessive factors are involved, considerable difficulty in transfer of resistance is anticipated. With tomatoes where a single dominant gene is involved the transfer of resistance from the wild peruvianum has been difficult because of the linkage of resistance with undesirable commercial traits. This work is now perhaps in the 25th generation, with numerous backcrosses, and no commercial varieties have yet been released.

Perhaps the work of Hanson, Robinson, and Wells on the heritability of resistance to root-knot in Lespedeza represents a more practical approach to the genetics of resistance where inheritance is complex.

This paper also raises another question concerning the necessity for absolute or extremely high root-knot resistance. In field crops where small increments of tolerance may be economically valuable it may be wiser to utilize the best resistance available in readily accessible material rather than delay improvement until high resistant or immune sources of resistance are located which may be difficult to transfer.

Slides were shown giving results of studies on root-resistance in commercial cotton varieties and the relation of this resistance to Fusarium wilt and yield. Root-knot resistance is positively correlated with Fusarium resistance and yield on heavily infested soils. By the use of soil fumigants in row applications the difference in yield on treated and untreated soils gave an indication of total nematode resistance. It was shown that Auburn 56, a cotton variety developed at the Alabama Experiment Station by Mr. H. B. Tisdale, was outstanding in root-knot and wilt resistance in comparison with other varieties.

Discussion

In a discussion of root-knot resistance breeding with tomatoes, Dr. Al Harrison from Yoakum, Texas, stated that very high resistance to Meloidogyne species had been obtained in combination with resistance to Fusarium and some other diseases. North Carolina workers praised Dr. Harrison for developing this material and stated that in the North Carolina test his material appeared almost commercial in type.

Dr. Holdeman raised the question of the possibility of the occurrence of races within the several species of root-knot created by Dr. Chitwood. He pointed out that most workers have tested their breeding material using the original isolates made by Dr. Sasser as representative of Chitwood's species. Dr. Holdeman reported that Dr. Hartwig was supplying the seed of 25 soybean varieties to a number of people across the Southeast to get a regional measure of root-knot resistance in these varieties and to determine whether different strains could be found in the root-knot species involved.

The finding of a good source of root-knot resistance in tobacco was reported. Resistance was found determined by a single dominant gene which was not deleterious to yield, and it was anticipated that rapid progress would be made in developing resistant varieties. (Other discussion not recorded)

GENETIC INFORMATION ON SEVERAL CROPS BRED FOR
RESISTANCE TO MELOIDOGYNE SPP.

Genera and Crop	: Number of : Factors : Determining : Resistance	: Dominant : Inter- : mediate : Recessive	: Number of : Basic : Number : in Genus	: Chromosomes : Number : in Crop : Studied
1. <u>Phaseolus</u>				
String Beans	2	R	11	22
Lima Beans			11	22
2. <u>Lycopersicum</u>				
Tomato	1	D	12	24
3. <u>Capsicum</u>				
Pepper	1	D	12	24
4. <u>Vigna</u>				
Cowpeas	?	D	11	22
5. <u>Prunus</u>				
Peach	?	D	8	16
6. <u>Vitis</u> (grape)				
Several Species	?	D	19	38
7. <u>Gossypium</u>				
Wild Barbadosense	M	R	13	26
8. <u>Nicotiana</u>				
Commercial Tobacco	1	D	12	48
9. <u>Ipomoea</u>				
Sweet Potato	M	I	15	90
10. <u>Lespedeza</u>				
Korean	M?	70 to 90% heritable	10	20



ORGANISMS OF THE SOIL WHICH ATTACK NEMATODES

C. L. Duddington

The suggested title of my paper, "Organisms which attack eelworms in the soil," is for a very wide topic. I could not possibly cover so broad a subject here. Actually, I am proposing to deal totally with one group of nematode attacking organisms, the predacious fungi, which are the ones I have been particularly interested in over the last twenty years.

Predacious fungi are to be found, I think I am right in saying, in every group of fungi. The particular ones that I am interested in are found in two rather widely separated groups. One is the Saprolegniales, which is a group of Phycomycetes fairly closely allied with Mucorales. The other predacious fungi that I especially want to discuss are found in the Fungi Imperfecti, those fungi which have no sexual reproductive process and which, therefore, cannot be properly classified.

These predacious fungi divide into two groups: First, those which capture eelworms alive and consume them, the truly predacious types, such as Arthrobotrys and Dactylella; and second, those which are internally parasitic, attacking the eelworms usually by means of spores which stick to the cuticle of the eelworm, penetrate it with a germ tube, and so form a mycelium inside the worm. (That type, I suppose, should be described as parasitic, rather than predacious.) Common, too, are the eelworm trapping fungi, which are more important. So, I am going to start by describing some of the ways in which they capture their prey.

There are two kinds of eelworm traps, the sticky trap and the contractile trap. The simplest form of sticky trap is found in the zoopagales, where such genera as Stylopage have mycelium that are sticky all over. If the eelworm come into contact with such a mycelium, at any point, it becomes stuck like a fly on fly paper. That is the least specialized type of eelworm trap; but in the predacious Fungi Imperfecti, much more specialized trapping organs are found.

The most common and best known type of sticky trap is a sticky network. A hypha on the fungus produces a branch which curls around and joins again. Other hyphal branches form in that way, producing a system of networks with the loops usually standing at right angles to one another, much like the semi-circular canals in the inner ear. Only the networks are sticky, and they appear to be sticky just for eelworms, at least while they are in their normally moist condition. The networks will not stick to such things as insect cuticle, cuticle of earthworms, and

the like, unless they are partly dried. Any eelworm that comes in contact with such a network is held fast. One can easily see under the microscope that there is a secretion of quite a large quantity of sticky fluid. It is extremely efficient; if anyone could manage to isolate it, to synthesize it, and to market quantities of it as a glue, he would make a fortune. I have never known of an eelworm getting away once it is fairly held.

After the worm has been captured in the network, there is then a small outgrowth from the fungus which penetrates the eelworm. The outgrowth develops into a large bulbous structure; and from this infection bulb, hyphae grow inside the body of the eelworm, absorbing its contents. Within about 24 hours there is nothing left but the empty cuticle of the eelworm filled with the hyphae which absorb the body contents of the nematode. Incidentally, the worm apparently dies in from one to two hours after capture. Death takes place before the intrusion of the bulbous structure. What exactly kills the eelworm, we do not yet know. The only thing I can think of is that it dies of fright.

A rather simpler form of sticky trap is the adhesive process, or adhesive branch, type of nematode trapping fungi. The very common Dactylella cionopaga, for example, has branches of one, two, or three cells which go out from its hyphae. These natural branches are sticky and function in the same way as the networks.

From this sticky process type of structure, it is not a very long distance to the adhesive knob type of traps. These have hyphae with short erectile branches on which there are small knobs. One of these knobs usually measures rather less than 10 microns in diameter. Again, these knobs are sticky and are usually orientated on the hyphae. If the fungus is growing on a surface, such as an agar plate, the knobs stand up vertically from the surface. Thus, they are in a good position for capturing eelworms.

These knobs are very numerous, and they are rather regularly placed apart at considerably less than the eelworm's length. A worm caught upon one knob, in the course of its struggling, will usually manage to stick its tail on to another knob, and thus be held doubly. These adhesive knobs are rather less efficient than the networks and branches, in that they do not seem to be able, in most cases, to deal with very large eelworms. The normal limit of the knobs is an eelworm, I should say, 500 microns in length, although larger ones are occasionally captured. I have seen fungi with sticky networks holding eelworms more than one millimeter long. All these traps depend upon the same principle, the secretion of a sticky fluid.

We come now to the mechanical type trap, of which there are two kinds. The simplest is the non-constricting ring, which consists of a ring of three cells. The hypha bears a branch which elongates and curls around, joining to form a ring composed of three cells. If an eelworm pushes its head into a ring of that sort and tries to pass through it, it gets

stuck. There is no question, I think, of any sticky substance being secreted. It is just that the eelworm has not enough sense to withdraw. It just tries to force its way through the ring with the obvious result; it gets well and truly wedged.

The supporting stalk of the ring is rather slender, and it quite often happens that a captured eelworm will tear the ring away from its moorings and thus get away. I have seen eelworms in cultures with as many as half a dozen rings encircling them like dog collars, showing that they had been caught half a dozen times and escaped. Actually, the escape is only temporary, because, even from a detached ring, hyphae will ultimately grow out, penetrating into the eelworm and killing it. Where it has happened that the ring has been detached from the mycelium and the eelworm has been killed, a new mycelium grows out from the ring. Thus, these rings can actually serve as a means for reproduction, as well as for capturing prey.

Now we come to the most dramatic type of eelworm trap, the constricting ring. This is formed in exactly the same way as the non-constricting ring, but it is rather more stout in construction and has a shorter, sturdier stalk. In this type of trap, the fungus does not depend upon the efforts of the eelworm to get it wedged into the ring. The three cells of the ring are sensitive to touch and to friction. The friction of an eelworm's body that has put its head into a ring is sufficient to trigger off the reaction. The three cells suddenly expand inwards, increasing to about as much as three times their previous volume. The opening in the ring becomes completely occluded. The eelworm caught in the constricting loop quite rapidly dies. Hyphae develop from the cells of the ring and grow into the body of the eelworm, consuming its contents and passing them back to the main part of the fungus.

The closure time for the ring is very rapid, being about 1/10 of a second from beginning to complete closure. Immediately after closure there is the formation of numerous small vacuoles which run together and form a globule of glistening, highly refractive material. We do not know what this material is or what is the mechanism for ring closure. I do not think it is osmotic.

A curious thing that I have observed in agar cultures is that these rings are often formed just beneath the surface of the agar and are oriented in a vertical plane. The eelworms move in this area of the surface so that the rings are in a good position to capture the eelworms. What mechanism insures that the rings shall be perpendicular, I just do not know. It is certainly not gravity, because the agar plates can be stored in any position, and the rings will still orient themselves perpendicularly to the surface.

We have discussed the principle types of eelworm trapping fungi. They belong to four genera: *Arthrobotrys*, *Tricothecium*, *Dactylella*, and *Dactylaria*; four very closely related genera which differ only in the shape, septation, and mode of formation in their conidia.

In Arthrobotrys, as in Tricothecium, there is a two-cell spore. In Arthrobotrys the spores are formed in a whorl at the apex of the erect conidiophore. The arrangement is definitely capitate, that is, a definite group at the tip. There may be successive whorls down the conidiophore. Tricothecium has similar spores, but in Tricothecium, unlike Arthrobotrys, which has a terminal spore and then more spores formed down the conidiophore, spores are evidenced more as though on an ear of corn than as a series of whorls.

Dactylella and Dactylaria both have spores with more than two cells; and in both genera the spores may be cigar-shaped, or they may be top-shaped, in which case they always have one cell very much bigger than the others. To distinguish between Dactylella and Dactylaria is quite impossible. I have been trying to do it for twenty years, and I have not succeeded yet. Theoretically, and mind you, I say theoretically, in Dactylella there is a single spore at the tip of the conidiophore, whereas in Dactylaria there is a group. Unfortunately, every Dactylella I have ever examined forms two or three conidia on its conidiophores; and, on the other hand, in many Dactylaria the whorl arrangement is very lax. It is, therefore, impossible to really distinguish it from a Dactylella having more than one spore. Personally, I think they should have been the same genera, and no doubt they will be one day.

Now just one or two points about the physiology of these fungi which might be of importance if they are to be used for eelworm control. First of all, they can all be grown easily in culture, like any other saprophytic fungus. Most of them, when grown in pure culture, do not form the characteristic eelworm traps; but they do so if they are brought into contact with eelworms, or if they are given a little sterile water in which eelworms have lived. They are perfectly able to exist as saprophytes. We do not know yet in what way they mainly exist in nature, whether they occur in the soil as saprophytes and only occasionally capture an eelworm, or whether they depend mainly upon eelworms as a diet. My own personal opinion is, judging from indirect observation in cultures, that it is a question of available food.

There seems to be quite a delicate balance between the predacious and the saprophytic phases of the activity of these fungi. I do not know yet exactly what it is that effects that balance and decides which type of activity they are going to show, but one of the things that can effect it is the presence of eelworms. If one takes a culture of the predacious fungi and feeds it eelworms, usually the fungus will simply remain in the saprophytic state. On the other hand, if one proceeds in the converse way, the result is different. If one has a very good culture of saprophytic eelworms, say on rabbit dung agar, and if it is really so filled with eelworms that no ordinary mold could grow on it, and if one puts on such a plate a very small piece of inoculum of a predacious fungus, then one finds that the fungus will thrive on the eelworms. It seems to me quite clear that there must be something in

the presence of eelworms that, so to speak, stirs up the blood lust of the fungus. That, I think, is a very important point when we come to consider eelworm control, which we are going to do later.

Now, if I may turn just for a moment to the internally parasitic fungi, fungi in such genera as *Harposporium* and *Acrostalagmus*. I am not going to spend much time on them, because they are much less important for our purpose. These attack eelworms, usually through the agency of a sticky spore. They produce very small spores which stick to the cuticle of eelworms, and when the spore germinates, it sends a germ tube through the cuticle and forms a mycelium inside the victim. The mycelium then grows at the expense of the internal organs of the eelworm until the body of the worm is completely filled with hyphae. It does not do the eelworm any good at all, of course. After the host has been consumed, the fungus pushes fertile hyphae out to the exterior. On these fertile hyphae the spores are formed.

These fungi are very common, but whether or not they would ever be of any practical use for eelworm control, I rather doubt, because of the difficulty in handling them. They have mostly been reported as being obligate parasites, but whether that is so, or whether it is merely that attempts have not been made to culture them, I could not tell you. Their isolation, certainly in most cases, would be difficult.

Discussion

- Q. Do these nematode trapping fungi show any specificity as to kinds of nematodes for prey?
- A. The nematode trapping fungi show very little specificity, if any. The internal parasites appear to be much more specific; most of them go for more than one species of eelworm, but actually their range of hosts has not been worked out. I would say the internal parasitic fungi show quite a lot of specificity, and the trappers show very little.
- Q. What is the stimulus for closing of the rings in some of the ring forms? Is it any mechanical stimulus, or is it something more specific?
- A. Purely mechanical. The rings can be made to close by stroking them with a needle, and they can also be made to close by heat. However, I do not think heat is operative in nature, unless, perhaps, the eelworm is running a fever.
- Q. What technique do you use for isolation of the fungi from the soil?
- A. Well, I use the same technique for whatever material I take the fungi from; that is, I first get a mixed culture and then isolate the desired forms from it. As a general technique, I put the material

to be examined for fungi in the middle of agar plates and leave it to fester for quite a long time. It gets into a disgusting state, full of mites, eelworms, and so on. Then I observe under the dissecting microscope if there are predacious fungi present. They will be seen pushing up erect conidiophores with the spores at the end. I take a very small piece of agar on the tip of a sterile needle and simply pick off the spores individually until the agar is filled. It is very simple and effective.

For recovery of the fungi from soil, I use another method. I take a little of the soil and place it in the bottom of a sterile petri dish. I put in with it a small piece of an agar culture of saprophytic nematodes. Then I pour sterile rabbit dung agar onto this, after having first allowed the agar to cool nearly to the temperature at which it jells. I shake the dish a little to distribute the soil. The eelworms from the small peice of agar culture serve to supply prey in case nematodes are deficient in the soil. In this way, one easily gets predacious fungi from the soil. They are then isolated for subcultures in the way previously mentioned.

Q. Do you find these fungi in certain types of soil?

A. I find them very commonly in nearly all types of soil. I have looked at a great many soil plates, but I would not care to attempt drawing any conclusions as yet. One would have to examine many thousands of soil isolation plates before he could get enough data on which to generalize about which soils they occur in specifically or most abundantly. I will merely say that I have found them in nearly all types of soil. There are two types of soil from which I have failed to get these fungi, the very acidic peat and very sterile mineral soil.

Q. How often do you find the fungi capturing plant-parasitic nematodes in the soil?

A. I have never myself made direct observation of them capturing plant-parasitic nematodes, probably because I am, I am afraid, unable to identify the nematodes. Dr. Goodey has recorded an Arthrobotrys capturing the stem eelworm on wheat.

Q. Is there any evidence of attraction of the nematodes to the trapping organs, or is it purely chance that the nematodes come in contact with them?

A. I have never seen any evidence of attractiveness. I think it is purely chance.

Q. Have you ever observed fungi in the genus Fusarium attacking nematodes?

A. No, not yet. I have found that there are some Fusaria that attack the contents of nematode cysts.

Q. You talked about *Dactylellas* that form constricting rings and sticky knobs. Apparently the nematode trapping apparatus does not enter into the classification picture. Is this so?

A. No, the nematode trapping apparatus is in common with various genera, and one cannot distinguish the traps from each other morphologically, except in a few cases. Were you to show me any culture of a fungus with constricting rings, I could not tell what genus it is without seeing the spores. The same thing holds for those fungi with networks.

Q. How often do you find nematodes in nature attacked by these fungi?

A. Well, as for observations firsthand in nature, I cannot answer you. My observations are always secondary, in that I put the material in agar. I have, on one or two occasions, gone out into the field with a microscope to try and examine material directly for predacious fungi, but I have never had any luck. I also tried to develop a technique for making microscopic preparations of soil in situ and have found predacious fungi in the soil actually capturing eelworms. As far as I know, that is the only labor from nature.

Q. At the Salt Lake City station, we have over 36,000 specimens of nematodes, and there are less than a dozen that have fungi attacking them. They are practically unheard of in that district. Perhaps this is due to our soil conditions.

A. Yes. Personally, I think it probable that, in the soil, the fungi do not bother much about the eelworms, unless they are stimulated to go to the predacious mode of feeding.

Q. Have you noticed in your work that any organisms attack the predacious fungi in the cultures?

A. No, I have never observed anything attacking predacious fungi. However, I have noticed that they are, in many cases, sensitive to antibiotics.

Q. I would like to ask, if there were any nematodes in nature attacked by fungi, would you recover them by the standard nematological techniques? It seems to me that they would be washed out or settle out.

A. I think that is exceedingly likely. I think also that the techniques of soil microbiologists are such that these fungi are not recovered. If you go one step less than the agar plate method I spoke of, and simply bring nematodes into the laboratory, leaving them in a dish with sterile water, many of the fungi can be obtained. Apparently, nematodes come into the dishes already infected with these fungi, which begin to grow at the expense of the nematodes' tissues.

Q. I have one observation that is related to what was just said. In

1949 I worked with Pratylenchus affecting boxwoods. At that time we used to put chopped roots in the Baermann funnel and leave them for about two weeks. Several times we recovered Arthrobotrys growing on the water at the surface.

Q. You mentioned that you had never observed nematodes feeding on fungi. I wonder if a turnabout thing can happen. That is, what would happen if you built up large colonies of Aphelenchoides or Aphelenchus, which can subsist on fungi, and introduced trapping fungi into the situation. Which would win out?

A. I think I would take the fungi. I do admit to not knowing whether or not I have dealt with such nematodes in my cultures. As I have before, I am not qualified to identify the eelworms. No doubt, I mixed kinds of nematodes in the cultures, but they always end up food for the fungi.

Q. Have you tried to correlate the numbers of eelworm catching fungi with the number of nematodes in the soil? Do they have a very definite effect on any particular nematode or on a very high population of a certain nematode?

A. I have a strong suspicion that if the nematode population is high, there would be an increase in the fungi. This is a topic I would like to talk more about. In answer to the question, I would like to go on to work with which I think you are familiar.

Linford worked in Hawaii on pineapples in which he first tried to control root-knot nematodes by inoculating the soil directly with predacious fungi. He found practically no beneficial results. He then tried turning into the soil quantities of chopped pineapple tops. He found that he had much better control of the root-knot eelworm. Linford noted that although the root-knot eelworms were reduced, there had also been a very large increase in the saprophytic nematode population. This increase was followed by a great increase in the activity of the predacious fungi which were already present. This was in turn followed by a drastic decrease in the populations of both the saprophytic and root-knot eelworms.

I have confirmed this kind of result in experiments on oats. We used chopped cabbage leaves with manure in applications for control of the cereal-root eelworm. We found a very high degree of protection against the eelworm. This was judged on the basis of actual nematode counts in the roots of seedlings. The protection obtained was very significant. At the same time, the soil samples showed that the naturally occurring predacious fungi were much more abundant.

In all the experiments involving soil inoculations with predacious fungi, I have found that when there were significant results, there was some form of organic matter added along with the fungus. Those experiments in which soil was inoculated with only cultures of the

fungi produced, almost invariably, negative results. My opinion is that for the fungi to become effective there must be some kind of a stimulus which will shift the balance of the fungus from the saprophytic to its predacious phase. I think the presence of eelworms is one of those factors. It is quite possible that the effect of the organic matter is that it increases the numbers of saprophytic eelworms, which, in turn, stimulate the predacious fungi.

In closing, I would like to mention an excellent book dealing with biological control. It is the French book, Parasites (animaux et vegetaux) des Helminthes by Robert Dollfuss.* It is a very good account of all the nematode-attacking fungi known up to the beginning of 1946. As far as I can make out, it is quite complete, and nothing has been left out.

* (Editor's note) The book by Dollfuss is part XXXVII of the Encyclopedie Biologique. It may be available directly from France from Paul Lechevalier, Editeur, 12 Rue de Tournon, Paris VI. The price used to be 2500 fr. It can also be obtained through Stechert-Hafner, Inc., 31 East 10th Street, New York 3, New York, for about \$15.00.

Dr. Duddington has written a book on this subject called The Friendly Fungi. It is printed by Faber and Faber, 24 Russell Square, London, W. C. 1, England. The book is to be published in this country by Macmillan Company, New York. The price of the book purchased from the publisher in England is about \$3.00. This is a very interesting account of the various predacious fungi, how to find and culture them, and their potential as a nematode control. The book is illustrated with drawings and photographs.

There is also a review written by Dr. Duddington which can be purchased for about \$1.00 from the Botanical Review, Box 749, Lancaster, Pennsylvania. The citation is Fungi that attack microscopic animals. Bot. Rev. 21(7):377-439, July 1955.

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(Editor's note) On another day at the Workshop Dr. Duddington was invited to speak concerning biological control experiments. Much of the material he presented is available now, in a more detailed manner than can be presented in these Proceedings, in his book, The Friendly Fungi. However, some of the other parts of the discussion session are printed here.

There seems to be a very definite tie-up between organic matter in the soil and the action of the fungi. Organic matter seems, in some way, to act as a stimulus to the fungus. I think Dr. Linford's explanation, that the increase of organic matter produces an increase in the population of saprophytic nematodes and that in turn reacts on the fungus, is as good an explanation as yet has been found. It certainly ties up

with what happens in laboratory cultures. If one adds a small piece of the fungus to a very vigorous eelworm culture, the fungus becomes extremely predacious. Whereas, if conversely, eelworms are added to an active fungus culture, the fungus normally does not become predacious. It does seem to me that the presence of eelworms is a very strong stimulus to the fungus to change it to the predacious form or habit. I think that, in any further work, the presence of organic matter will have to be carefully considered.

Another point, on which I would like to conclude, is this question of the increase in eelworm populations as a result of the fungal treatment. Obviously, we do not want to go about increasing eelworm populations. My own feeling is that this is not going to be too difficult to get around. It seems to me that the most likely way is to increase the period of predacious activity of the fungi. This could be done in various ways. We have experimented with mixed cultures; mixing in a ring-forming fungus which grows slowly with the more rapidly growing reticulate types. If that can be done, and results have shown some promise, I think that will much increase the period over which the activity of the fungus can be spread. Another thing we are trying is the use of dried fungus cultures. These have an uneven germination period, thus, again spreading the time of application of the fungus over a longer period.

In regards to the question of applying so much inoculum that there is difficulty in distinguishing between a manuring effect and the controlling effect of the fungi, I am very much against putting in more fungus than is necessary. There is a definite disadvantage to overdoing. It goes back to the situation in the cultures where the fungus is in excess of the nematode supply; the fungus won't remain in the predacious condition.

Actually, perhaps, what we should really be aiming at is not introducing more organisms into the soil, but rather to change the soil so that those beneficial organisms which are there become more active. These would include all the predators of the nematodes, including fungi, other nematodes, mites, and so on. I know this is what Linford tried to do, and I think it is very possible.

(Editor's note): After Dr. Duddington mentioned that he would like to do some studies on the effect of mulching, several observations were pointed out by participants in the workshop. In Riverside, California, mulching has increased citrus nematodes in citrus groves. Some early work on nematode control in tobacco, working straw into the soil and making a bedding of the straw gave very effective control. Tea plantations in Ceylon are being brought back into production by using a mulch for root-knot nematode control. One acre is used to produce enough mulch grass to keep nine acres of tea plantings going, and remarkable improvements have been noted after only a couple of years.

SOME FACTORS IN THE MICROBIOLOGY OF THE RHIZOSPHERE

Francis E. Clark

My knowledge of the nematodes living in soil or in association with plant roots can best be summed up in the admission that I am not at all sure that I can distinguish the south end of a nematode from the north end. Obviously then, it would not be fitting for me to attempt to discuss the rhizosphere microbiology with any special reference to the nematodes. What I propose to do is to discuss the microflora of the rhizosphere, and I hope that you will make such additional entries and interpretations as are needed with respect to the microfauna.

In preparing this discussion, I have scanned through several reviews on the microbiology of the rhizosphere. Several such reviews do exist (1,2,3). I do not intend to abstract that literature in any complete or orderly manner. I do intend to exercise considerable freedom in selection of material in order to show some of the general problems faced by the students of the rhizosphere. My point of view, at times, may appear to be that of a soil micro-ecologist rather than that of a soil bacteriologist.

As a beginning, let us briefly orient our thinking about what we mean by the rhizosphere. By definition the rhizosphere is that region of the soil under the immediate influences of plant roots (4). Microbiologically, the plant roots are in their net effect stimulatory, so much so that there occurs perhaps ten times, possibly even one hundred times, as many bacteria in the soil adjacent to roots as in soil apart from roots. This, of course, should not be surprising. The soil bacteria are not uniformly distributed throughout the soil, but are localized largely according to available food supply. In the rhizosphere, where the root, together with its excretory products and debris, constitutes a food source, the site of most intensive localization can be expected to occur at the surface of the plant root.

This fact immediately leads us into a technical difficulty. The rhizosphere, as already noted, is defined as soil under the influence of plant roots. Rhizosphere samples, however, most commonly include the plant roots with their closely adhering soil. Increasingly pronounced rhizosphere effects upon the microbial populations can be demonstrated by including less and less soil and more and more roots in the bulk samples taken for study. The frequent employment in the literature of the phrase "the microorganisms associated with the root surfaces," and of the terms "outer rhizosphere" and "closer rhizosphere" indicates an unwillingness by many workers to ascribe to the surrounding soil the microbes present on root surfaces. Inasmuch as the root surfaces are in fact responsible for the bulk of the microorganisms found present in combined soil and root samples, it would appear more logical to speak of this microflora actually on the roots in terms of the root surfaces and not in terms of the surrounding soil. I have previously

proposed (1) that this be accomplished by use of the term rhizoplane. The rhizoplane is defined as the external surfaces of plant roots together with any closely adhering particles of soil or debris.

Because of differences in terminology and in documentation of results, as well as because of marked differences in laboratory procedures and techniques, it often is very difficult to compare the rhizosphere data of one laboratory with that of another. In the literature one finds that rhizosphere populations are variously expressed. Such data may be given as (a) bacteria per gram of gross sample, that is, the roots plus adhering soil, (b) per gram of soil in the gross sample, (c) per gram of root in the gross sample, (d) per gram of root surface scrapings, or (e) as bacterial numbers per milliliter in successive washings of the root sample. Webley and associates (5) have pointed out that the choice of a basis for expressing results, even when using the same laboratory data, may determine whether twofold or sevenfold differences are shown for microbial populations in the rhizospheres of two plant species.

Today we need not concern ourselves with the very considerable information concerning microbial populations in the rhizosphere. I am assuming that most of you are not interested in census-type information concerning families, genera, and species of bacteria found in the rhizospheres of various plants. At the beginning I did make the remark that microbes are commonly ten to a hundred times more numerous in the rhizosphere than in the surrounding soil. This means that bacterial counts as high as several billions per gram of gross sample material can, at times, be encountered.

We might add that gravimetrically the microbes do not constitute a very important part of the total root sample. On the basis of presently available information, it can be estimated that even at the highest level of rhizoplantal populations thus far encountered, the bacteria present constitute somewhat less than one percent by weight of the gross sample of roots and microbes. Elsewhere I have estimated that the total bacterial mass in soil ranges from .03 to .28 percent, or to make an even rougher approximation, that it amounts to something like one thousand pounds of microbial tissue per acre.

Certain workers have evaluated the relative importance of the microbial population in the formation of carbon dioxide by plant roots. Lundegardh (6) found that nearly one-half the carbon dioxide arising from plant roots growing in unsterilized sand is produced by their accompanying microorganisms. Other workers (7,8) comparing carbon dioxide production of sterile roots with that of roots possessing their normal complement of microflora have presented quite similar estimates.

Root Materials Available as Nutrients

What root materials are available as nutrients to the microorganisms congregated in the rhizosphere?

There are two groups of substances involved, namely, (a) cellular debris, including sloughed-off root hairs, root caps, and cortical and epidermal cells, and (b) excreted materials, as for example amino nitrogen compounds. Information concerning the amounts of organic materials released in the rhizosphere is limited.

Lyon and Wilson (9) measured the release of organic matter from roots of plants grown in sterile nutrient solutions and found it to be of the order of 3 percent of the total weight of the plants at maturity. Whether or not this value represents a good estimate of the loss to the rhizosphere by plants growing under natural soil conditions, I am not prepared to answer.

Another problem concerning the natural rhizosphere is the satisfactory differentiation between excretory products as such and the decomposition products arising either from cell autolysis or as a consequence of microbial attack upon cellular debris. Roviar (10) has presented some measurements of the amounts of material given off by roots of pea and oat plants during early stages of growth in quartz sand. For these plants, after both 10 and 21 days of growth, the soluble organic material formed the bulk of the material coming from the roots. He assumed that the bulk of the soluble exudate was true excretion, not produced by lysis of cells, because of the observation that the amount of cellular debris was doubled between 10 and 21 days, while the amount of soluble material only increased by half. At 10 days the dry weight of material excreted was three to fourfold the dry weight of root debris; at three weeks, the amount of exudate was little more than double that of the sloughed-off cells. He concluded that in older plants true root excretions probably are not as important in supporting the rhizosphere population as is the cast-off cellular material.

It is becoming apparent that the compounds that can be excreted from roots are many and varied. Not many years ago, we were content to think primarily in terms of carbohydrate materials of varying complexity and of amino nitrogen compounds. There was even some disagreement about the excretion of amino acids. Recently Winter (11) has stated that in addition to sugars and amino acids, such compounds as the following come from plant roots: phosphatides, alkaloids, tannins, thiamin, boitin, mesoinositol, and para-amino benzoic acid. Currently, many specific additions are being made to this list, as chromatographic techniques permit the isolation and identification of hitherto unknown chemical compounds. A concurrent development, stemming largely from interest in systemic pesticides for plants, is the recognition that complex organic molecules can be transported, excreted, and taken up by plants.

Having noted some of the nutrient materials that arise from roots, we may now consider briefly a question of origin of the rhizosphere microflora. There have been some recent suggestions that certain components of rhizosphere population arise from specific sources. Gyllenberg (12),

for example, states that there are three components in the soil and root microbial population, namely, (a) that of the surface of plant roots, (b) that of the rhizosphere soil, and (c) that of the soil proper. Observations of Rovia (13) and others are cited to the effect that the root surface population mainly originates from the seed coat, while the rhizosphere flora is of mixed origin, arising partly from the seed coat and partly from the soil.

Without doubt, root surfaces of seedling plants grown in sand and from seeds not surface sterilized do secure many of their colonizing bacteria from the seed coat. However, I am not willing to agree that the seed coat is of any special importance in determining the rhizoplanal microflora of plants as they commonly are found in the ordinary soil. I am convinced that with few exceptions the root surface microfloras of seedlings more than a few days old, in short, of older plants generally, will be found indistinguishable regardless of whether the plants were grown from unsterilized or from surface-sterilized seed. Placing any undue emphasis on the flora of the seed coat as the source of the root surface microflora is an over-simplification of a difficult problem.

Dissimilarities between Root and Soil Microflora

Does the rhizosphere microflora differ appreciably from that found in soil apart from plant roots? This question can unhesitatingly be answered yes. The rhizosphere is not simply a site of profuse development of the soil flora *en masse*. It is rather a site wherein some species are much more numerous than in soil, and other species much less numerous. To illustrate with some specific data, we have observed that in soil one to two inches distant from the cotton root, the aerobic spore-forming bacteria of the genus Bacillus comprised 37 percent of the total bacterial population, while at the root surface this genus comprised only one-tenth of one percent of the total. In contrast, the gram-negative dye-tolerant bacteria amounted to only 16 percent of the total bacterial flora one to two inches distant, and 86 percent of the flora at the root surface.

For the most part, published information concerning differences between soil and root floras is given in terms of morphological and taxonomic types. Lochhead and associates (14,15), however, have made extensive use of a nutritional grouping of bacteria in characterizing the rhizosphere. They have determined the growth responses of bacterial isolates on seven cultural media of increasing complexity. They have found that the rhizosphere contains an unusually high proportion of bacteria that require amino acids for their nutrition. Bacteria requiring growth factors such as are provided by yeast extract either were proportionately no more numerous, or were proportionately less numerous, at the root surface than in the soil.

A nutritional grouping has some merit in that it attempts to relate

microbial types present to environmental factors. It has the shortcoming that it is only a partial characterization of the root microflora. More complete characterization, and translation of the results to binomial terminology, are needed. Perhaps it should be pointed out that physiological methods of studying the rhizosphere microflora are currently in a state of flux. Apparently the use of seven cultural media as originally proposed proved to be unnecessarily cumbersome. Recently, workers have been attempting to develop nutritional grouping schemes based on fewer substrates. Katznelson et al. (16), for example, have reported studies using the following three media: (a) basal medium consisting of inorganic salts and glucose; (b) this medium plus amino acids; and (c) the basic medium plus yeast and soil extracts. Gyllenberg (12) has made a different simplification; he used (a) basal medium, (b) basal medium plus amino acids, and (c) basal medium plus both amino acids and B vitamins. Very recently, Katznelson et al. (17) have employed manometric techniques for characterizing the rhizosphere microflora.

Rhizosphere microfloras differ between plants. Not only is this true between species, but it is also true for differing varieties within species, as well as for plants differing in age and in conditions of growth. Indeed even for individual plants there are differences depending upon part of the root system examined.

The rhizosphere differences between species as well as between the higher taxonomic categories of plant life are extremely kaleidoscopic. Our knowledge of the rhizosphere microbiology is as yet too inadequate to permit any satisfactory discussion of the microfloras of different plant species. In order to emphasize that such differences do exist, let us for the moment speak somewhat superficially of the legumes.

It is well known that many leguminous species, even though by no means the majority, form root symbioses with the legume-nodule bacteria. Among the nodulating legumes, there is considerable specificity between the two partners in the symbiosis. Thus the soybean rhizobia do not nodulate alfalfa and clover even though they are capable of proliferating on the roots of these legumes. Koreniako (18) has noted that rhizobia grow abundantly in the rhizospheres of some non-leguminous plants, for example, wheat and cotton, but not in those of others, for example, corn and flax. In brief, rhizobia can grow on roots of legumes, but they may or may not form nodules thereon. They cannot nodulate non-legumes, but they reportedly can grow on the roots of some non-leguminous plants.

Different varieties of the same plant species have been shown to have differing rhizosphere microfloras. Lochhead, Timonin, and West (19) noted that the bacteria associated with wilt-resistant and wilt-susceptible varieties of tobacco and flax differed qualitatively and quantitatively, even when both varieties were grown entirely free of disease. Timonin (20) extended their work and showed striking differences in the fungal flora in the rhizospheres of wilt-resistant susceptible plants.

Coming, lastly, to individual plants within the same variety of species--these also are known to have differing root microfloras, particularly if the plants are of different ages, or if they are grown in dissimilar environments (2). Much of the information that does exist concerning rhizosphere differences between individual plants is in connection with the root-destroying fungi. Knowledge, insofar as the bacterial saprophytes are concerned, is very limited.

Why Do Root Microfloras Differ?

Why do root microfloras differ? This constitutes a basic question in any discussion of the rhizosphere. We may start with some very broad generalizations. One is that certain microbes are present on root surfaces because those roots provide some special nutritional or growth-promoting factors. For many microbial species on roots, this may simply be a matter of luxurious food supply and an ability to compete for that supply. Other microbial species may fail to colonize these same roots, even though they are nominally capable of using the food supply present, because they are unable to grow in the presence of toxic or inhibitory substances produced by the roots. Finally, microbial interactions may influence the quality of the microflora, in that the growth or absence of growth of some microbes may encourage the growth of others, or inhibit them. We will return in a moment to some further discussion of these synergistic and antibiotic factors.

At the present, let us consider the possibility that substances produced by plant roots can be toxic to certain microorganisms and harmless to others. Many higher plants produce substances that are variously inhibitory to other plants. Some developments in this field have been reviewed by Bonner (21). He suggested that these specific toxic substances could be responsible for ecological phenomena such as the composition of plant communities or the sequence of particular species in a succession.

Phytopathologists also have recognized the importance of plant-produced materials as determining factors in the varietal susceptibility of crop plants to fungal root pathogens. That chemicals produced by the host plant influence the host's resistance to disease was first shown by Link and associates (22,23,24). The resistance of colored onions to smudge and neck rot diseases caused by fungal parasites was found due to toxic phenolic substances identified as protocatechuic acid and catechol.

Timonin (20), noting that wilt-resistant and wilt-susceptible varieties of flax showed striking differences in the fungal flora of their rhizospheres, studied the two varieties under sterile culture conditions. The nutrient solution in which the resistant variety had grown contained appreciable amounts of cyanide. When he allowed the solutions in which the plants had grown to diffuse from colloidal sacs into the surrounding soil, thus creating artificial rhizospheres, differences in the

fungal flora were established that were very similar to those found in the natural rhizospheres.

Turner (25), working with the fungus *Ophiobolus* that causes the take-all disease of wheat but that ordinarily does not attack oat plants, found that the resistance of oat roots was due to an unidentified antibiotic substance. The material, even when diluted to one part in a hundred thousand, was effective in reducing the growth of *Ophiobolus* in laboratory culture. Kirkham (26) found that apple and pear leaves contained phenolic compounds that strongly inhibit the fungi causing black spot diseases of apple and pear. That plants also may produce compounds that are inhibitory to the microfauna, was shown by Ellenby (27). Roots of cruciferous plants that were resistant to the potato root nematode were found to contain a mustard oil, allyl isothiocyanate, that prevented the eggs of the parasite from hatching and that was lethal to its larvae.

Especially interesting is the recent work of Virtanen and Hietala (28,29). In the course of investigating differences in rye seedlings to susceptibility to the snow-mold fungus, *Fusarium nivale*, these workers isolated a substance strongly inhibitory to the fungus. The substance was separated by cellulose column chromatography, crystallized in pure form, and identified as 2(3)-benzoxazolinone. It could not be detected in ungerminated rye seeds, but after 5 to 6 days of germination in light at room temperature, the anti-*Fusarium* factor was strong. The fact that appearance of the compound in the seedling roots could be correlated to conditions of seedling development makes the discovery appear an important step forward in explaining the environmental effects on seedling susceptibility to root diseases. In the words of Virtanen and Hietala, the discovery "opens new prospects about the resistance of plants to fungal diseases."

It would now appear that we can confidently look forward to much further information concerning compounds secreted by the roots of different plants or of the same plant at different times and under different conditions. Not only will such information be of great value in plant breeding programs and in achieving effective control of soilborne parasites, but it will also enable us to achieve a much better understanding of the saprophytic microfloras of plant roots.

How Important Is Antibiosis in Determining the Microflora of the Rhizosphere?

The importance of microbial inter-actions or antagonisms in restraining the fungal parasites of plant roots was recognized by soil and plant scientists many years before the antibiotic wonder drugs became as well known as aspirin, even though, as most of us are painfully aware, by no means as inexpensive. The pioneer work of Sanford (30), Millard and Taylor (31), and Sanford and Broadfoot (32) showed that scab on potatoes and root rot of wheat could be measurably reduced or controlled, under appropriate conditions, by the antagonistic action of various soil

saprophytes. Weindling (33,34,35) demonstrated the parasitism of the pathogenic fungus Rhizoctonia solani by the saprophytic fungus Trichoderma viride, the production of the antibiotic gliotoxin, by Trichoderma, and the fact that antibiotics by Trichoderma could be secured only in an acid soil. These several contributors, together with other workers, thus showed that the saprophytic microflora was an important factor in the ecology of the root-invading fungi. In speaking of this earlier work, Garret (36) has summed it up admirably in the following paragraph:

"The marriage between plant pathology and soil microbiology, now consummated, was followed by a honeymoon period of unbounded optimism, during which the most extravagant hopes were entertained for the progeny of the union, to be christened 'Biological Control.' The first optimistic forecasts of the prospective god-parents....were dimmed by anxiety when it was eventually realized that the pregnancy would be unexpectedly long and difficult. One child, indeed, was still-born, and was quietly laid to rest under a tombstone inscribed 'Biological Control by Inoculation of the Soil with Antagonistic Microorganisms,' even though some of the bereaved claim that it was buried alive and is, in fact, still faintly breathing. Other children of the marriage, though at first puny, are still alive and slowly progressing, and more promising ones will assuredly be born in due course."

It is not necessary for us here to discuss antibiotics generally or the principles of antibiotic action. We need only to pause sufficiently long to note the extent to which microbial interactions have been found to affect the microflora at the root surface. Some work already has been cited showing that root-invading fungi are less destructive in their attacks on plants in the presence of a vigorous saprophytic microflora. A statement should be interjected here that in the following paragraphs, almost without exception, examples cited will have a strong phytopathological flavor. There are both economic and procedural reasons why most of the work thus far performed has been concerned with the root parasites.

Selecting one example, among many, of antibiotics at the root surface, we may mention the observation of Eaton and Rigler (37) that the corn plant, never known to be attacked by the cotton root rot fungus in normal culture, succumbs readily when grown in sterile culture completely lacking root surface bacteria. We could turn to the observations of numerous other workers who have concerned themselves with the Streptomyces scabies scab on potatoes, with the Rhizoctonia seedling and root diseases of a number of plants, with the many similar damping-off and root diseases caused by Phytophthora and Pythium species, or with other diseases caused by Ophiostoma, Helminthosporium, or Fusarium. For each, there are reports that antibiotics presumably plays an important role in protecting plants from soil-borne parasites. For those of

you who are interested, may I suggest the excellent review by Wood and Tveit (38). They conclude that no clear picture emerges from the very considerable work which has been done with the several root pathogens just named. In brief, there is no doubt but that the saprophytic microflora of roots is antagonistic to various pathogens. This antibiosis has been demonstrated in numerous experiments. Nevertheless, the real importance of this antibiosis at the root surface is still to be assessed.

Some recent work by Harley and Waid (39) illustrates the need for caution in the interpretation of rhizosphere data. They determined numbers of Rhizoctonia and of Trichoderma on root surfaces of beech seedlings grown in varying intensities of daylight. A plot of their data shows Rhizoctonia to decrease linearly at roughly a 45 degree angle with increasing percentage of daylight, and Trichoderma, to increase linearly, also at roughly a 45 degree angle. Because Trichoderma is well-known as a soil fungus which can inhibit many other fungi in culture, one can interpret the inverse relationship between numbers of Rhizoctonia and Trichoderma as antibiosis. Harley and Waid recognized that sampling and growth problems could well be involved, as Trichoderma over-grows very rapidly in culture. They then determined how greatly the presence of Trichoderma led to underestimation of Rhizoctonia by enumerating this latter fungus on plates not bearing Trichoderma. They found that Trichoderma had only slight influence on growth of Rhizoctonia. They believed that the only permissible conclusion was that the condition of the beech seedlings determined the nature of the root surface populations.

Such data serve to temper our enthusiasm when we look at information concerning root microfloras and root parasites. I do not mean to infer that biological control does not function at the root surface. There are a number of excellent reports showing that it does. There is much left to be discovered. Garrett (40) has made a forceful summarizing statement, adroitly side-stepping the unknown:

"Interference by other soil microorganisms with the parasitic activity of a root-inhabiting fungus is greatest at the root surface, where the soil microflora is greatly intensified, and is also changed in composition, by root excretion and other activities of the living root. The rhizosphere and the root surface microfloras thus appear to constitute the root's outermost barrier against invasion by pathogenic fungi."

To What Extent Can We Alter the Root Surface Microflora?

Some further insight into the ecology of the rhizosphere can be gained by noting the influence of soil, seed, or plant treatments on the quality or quantity of the rhizopanal microflora. I shall end my discussion by commenting briefly upon the extent to which the root

surface microflora can be altered by experimental or agronomic procedures. Before discussing these individually, I wish to emphasize that the root microflora is known to vary according to age and condition of plant growth (41); therefore, any treatment that produces in net effect a dissimilar plant will undoubtedly affect the root microflora. I mention this point now in order that I need not continually repeat it later when speaking of various soil and plant treatments.

Firstly, manipulation of the physical environment--light, moisture, pH, temperature--influences the root microflora. Harley and Waid (39) have shown that different levels of daylight radiation affect the growth and nature of the mycorrhizal and rhizoplanal microfloras of beech roots. They concluded that the condition of the host plant was the major factor in determining the nature of the root population. Also, it has recently been shown that length of photoperiod affects the nodulation response of legume roots (42).

Moisture, as one might expect, markedly influences the number of microorganisms in the rhizosphere. Both Timonin (20,43) and myself (44) have shown that drier soils have larger microbial populations in the rhizosphere than do wet soils. I have found that this is largely a sampling effect, due to decreased adherence of soil particles to roots in the drier soils.

Liming of soils was reported, by Obraztsova (45), to increase the density of microorganisms in the rhizosphere; and Pohlman (46) noted that in soil initially of pH 5, nodules on roots of alfalfa were largely concentrated in the soil layer receiving high-lime treatment, regardless of whether this layer was 8 to 16 or 16 to 24 inches deep.

Temperature also influences the root microflora, at least insofar as the root-invading pathogens are concerned. The effect of temperature differs with the crop and the parasite. Sugar beet seedlings are less susceptible to damping-off at soil temperatures below 60° F. than at 75 or 80°, but tomato seedlings are more susceptible. In common bunt of wheat, the greatest infection occurs at soil temperatures of 40 to 60° F. If fall wheat in the northern states is planted early while warm soil temperatures still prevail, the wheat seedlings largely escape infection.

Plant mutilation treatments may be used to modify the root microflora. I have noted (1) that stem and root girdling procedures affect the incidence of saprophytic fungi in the rhizosphere of cotton; and Mitchell and myself (37) have shown that with cotton, cutting half the leaves from flowering plants and deflowering of fully-leaved plants provide plants whose roots differ in their bacterial floras and in their susceptibility to the cotton root-rot fungus.

Soil treatment, either with inorganic fertilizers or with organic manures, does not appear directly to affect the rhizosphere microflora,

even though organic fertilizations almost invariably produce large increases in the soil population. In some work (47) in which manured, untreated, and steam-sterilized soils were variously layered, one above another, and then cropped to wheat, the root microflora was found to be largely independent of the soil flora. Other workers also have noted the independence of the root and soil floras, excepting insofar as manuring may effect growth rate and vigor of the plants. Possibly the indirect effects account for the finding of Hildebrand and West (48) that manurial treatment affecting the incidence of Ontario rot of strawberries also affected the relative incidence of nutritional groups within the rhizosphere.

That root microfloras have been found largely independent of soil treatment is not surprising. Stanier (49) has pointed out that the microbial environment is a micro-environment, "hundreds or even thousands or which lie concealed from the gross ecological eye in any gram of soil. A single cellulose fibre provides a specialized environment with its own characteristic microflora, yet may occupy a volume of not more than a cubic millimeter."

The root surface is likewise a micro-environment. Whether or not additional micro-environments, with a wealth of microorganisms, are set up in the soil by the addition of the numerous bits and pieces of an organic manure is largely immaterial to the microbes in the rhizoplane.

Soil bacteriologists long have attempted to control the root surface microflora by seed inoculations. Notable success has, of course, been achieved in the inoculation of legumes with rhizobia. Inoculations with non-parasitic bacteria other than rhizobia have, for the most part, been without success. This failure can be blamed neither on any paucity of attempts by serious-minded workers, nor on any lack of enthusiasm or sanguine statements on the part of those who have attempted to market various miracle inoculants. Quite apart from any personal profit motive, a great number of inoculation attempts have been made in efforts to secure root rot control. Our previous citation from Garrett (36), concerning the stillborn child quietly laid to rest under the inscription of "Biological Control by Inoculation of the Soil with Antagonistic Microorganisms," emphasizes that, in Garrett's opinion, most such inoculation attempts have been unsuccessful.

There are some contrary views. Morrow and associates (50) were of the opinion that either fungi or bacteria could be established on cotton roots by use of proper inocula. In my opinion, they failed to demonstrate that their recoveries represented more than chance occurrence or perhaps a passive survival. More convincing and more sharply limited data have recently been presented by Wright (51) who has found that Pythium damage to mustard plant seedlings can, to some extent, be controlled by inoculation of the seeds with some common soil saprophytes.

Perhaps that supposedly stillborn child was, indeed, buried alive and will yet eventually overcome his dyspnea.

Application of pesticides to plants can conceivably alter root surface microfloras. Most of the investigations conducted to date with pesticides have been concerned with effects on the soil, rather than on the rhizosphere population. There are a few reports (52,53) that sublethal doses of 2,4-D reduce the nodulation of legumes. There are other reports (54,55,56) that chlorinated insecticides applied at field rates do not interfere with the nodulation of legumes. Data concerning the effects of herbicides and pesticides on the rhizosphere populations are too limited for any satisfactory evaluation of such effects.

Yet, another way in which the microbial population of the soil may be altered is by the use of soil fumigants. I am sure that, in a meeting of this type, soil fumigants have been, or will be, discussed in far greater detail than I can possibly hope to duplicate. In closing, I will simply note that soil fumigants, designed to rid the soil of undesirable microorganisms and noxious weed seeds, do have drastic and long-lasting effect on the soil microbial population. Fumigation treatments may variously influence the root microfloras. Martin and co-workers (57,58) and Bliss (59) have given some special attention to these effects. Bliss has shown that control of *Armillaria* on citrus roots by use of carbon disulfide is not due to direct fungicidal action of the disulfide on the parasite, but rather is due to the killing of many species of soil fungi, allowing *Trichoderma* to become dominant. *Trichoderma* then invades the boundaries of infected host root tissues and attacks *Armillaria*, which is not directly killed by the carbon disulfide. Like so many problems connected with the rhizosphere, the part fumigation plays in controlling the root surface microflora deserves further study.

REFERENCES

1. Clark, F. E. *Adv. Agron.* 1: 241-288. 1949.
2. Katznelson, H., Lochhead, A. G., and Timonin, M. I. *Botan. Rev.* 14: 543-587. 1948.
3. Harley, J. L. *Biol. Rev.* 23: 127-158. 1948.
4. Hiltner, L. *Arb. deut. landw. Gesell.* 98: 59-78. 1904.
5. Webley, D. M., Eastwood, D. J., and Gimmingham, C. H. *J. Ecol.* 40: 169-178. 1952.
6. Lundegardh, H. *Der Kreislauf der Kohlensäure in der Natur.* G. Fischer, Jena. 1924.
7. Barker, H. A., and Broyer, T. C. *Soil Sci.* 53: 467-477. 1942.
8. Stille, B. *Arch. Mikrobiol.* 9: 477-485. 1938.
9. Lyon, T. L., and Wilson, J. K. *N. Y. (Cornell) Agr. Expt. Sta. Mem.* 40: 1-44. 1921.
10. Rovira, A. D. *Plant and Soil* 7: 178-194. 1956.
11. Winter, G. *Orion* 7: 377-381. 1952.
12. Gyllenberg, H. G. *Canad. J. Microbiol.* 3: 131-134. 1957.

13. Rovira, A. D. *J. Appl. Bact.* 19: 72-79. 1956.
14. Lochhead, A. G. and Chase, F. E. *Soil Sci.* 55: 185-195. 1943.
15. Lochhead, A. G. and Thexton, R. H. *Can. J. Res., C*, 25: 20-26. 1947.
16. Katznelson, H., Rouatt, J. W., and Payne, T. M. B. Sixth Internat. Congr. Soil Science, Paris, III: 151-156. 1956.
17. Katznelson, H., Rouatt, J. W. and Zagallo, A. C. *Bact. Proceedings* 1957, p. 10. 1957.
18. Koreniako, A. I. *Microbiology (USSR)* 11: 105-108. 1942.
19. Lochhead, A. G., Timonin, M. F., and West, P. M. *Sci. Agr.* 20: 414-418. 1940.
20. Timonin, M. F. *Canad. J. Res., B*, 18: 444-456. 1940.
21. Bonner, J. *Bot. Review* 16: 51-65. 1950.
22. Link, K. P., Angell, H. R., and Walker, J. C. *J. Biol. Chem.* 81: 369-375. 1929.
23. Link, K. P., Dickson, A. D., and Walker, J. C. *J. Biol. Chem.* 84: 719-725. 1929.
24. Link, K. P. and Walter, J. C. *J. Biol. Chem.* 100: 379-383. 1933.
25. Turner, E. M. *J. Exp. Bot.* 4: 264. 1953.
26. Kirkham, D. S. *Nature* 173: 690-691. 1954.
27. Ellenby, C. *Min. of Agric. Ent.* 53: 219. 1946.
28. Virtanen, A. I. and Hietala, P. K. *Acta Chem. Scand.* 9: 1543-1544. 1955.
29. Virtanen, A. I. and Hietala, P. K. *Suomen Kemistilehti B* 28: 165-166. 1955.
30. Sanford, G. B. *Phytopath.* 16: 525-547. 1926
31. Millard, W. A. and Taylor, C. G. *Ann. Appl. Biol.* 14: 202-216. 1927.
32. Sanford, G. B. and Broadfoot, W. C. *Sci. Agric.* 11: 512-528. 1931.
33. Weindling R. *Phytopath.* 22: 837-845. 1932.
34. Weindling, R. *Phytopath.* 27: 1175-1177. 1937.
35. Weindling, R. and Fawcett, H. S. *Hilgardia* 10: 1-16. 1936.
36. Garrett, S. D. *Ann. Appl. Biol.* 42: 211-219. 1955.
37. Eaton, F. M. and Rigler, N. E. *J. Agr. Research* 72: 137-161. 1946.
38. Wood, R. K. S. and Tveit, M. *Botan. Rev.* 21: 441-492. 1955.
39. Harley, J. L. and Waid, J. S. *Plant and Soil* 7: 96-112. 1955.
40. Garrett, S. D. *Biol. Rev.* 25: 220-254. 1950.
41. Starkey, R. L. *Soil Sci.* 27: 355-379; 433-444. 1929.
42. Bonnier, C. and Sironval, C. *Nature* 177: 93-94. 1956.
43. Timonin, M. I. *Soil Sci.* 52: 395-413. 1941.
44. Clark, F. E. *Soil Sci. Soc. Amer. Proc.* 12: 239-242. 1948.
45. Obraztsova, A. A. *Doklady Akad. Nauk. USSR. IX (1-2)*: 70-71. 1935.
46. Pohlman, G. G. *Soil Sci.* 62: 255-266. 1946.
47. Clark, F. E. *Trans. Kansas Acad. Sci.* 42: 91-96. 1939.
48. Hildebrand, A. A. and West, P. M. *Canad. J. Res., C*, 19: 183-198.
49. Stanier, R. Y. 3rd Symp. Soc. Gen. Microbiol., Cambridge, 1953.
50. Morrow, M. B., Roberts, J. L., Adams, J. E., Jordon, H. V., and Guest, P. *J. Agr. Research* 56: 197-208. 1938.
51. Wright, J. M. *Plant and Soil* 8: 132-140. 1956.

52. Payne, M. G. and Fults, J. L. J. Amer. Soc. Agron. 39: 52-55.
1947.
53. Carlyle, R. E. and Thorpe, J. O. J. Amer. Soc. Agron. 39:
929-936. 1947.
54. Wilson, J. K. and Choudhri, R. S. J. Econ. Entomol. 39: 537-538.
1946.
55. Appleman, M. D. and Sears, O. H. J. Amer. Soc. Agron. 38: 545-
550. 1946.
56. Wilson, J. K. and Choudhri, R. S. J. Agr. Reserach 77: 25-32.
1948.
57. Martin, J. P. Soil Sci. 69: 107-122. 1950.
58. Martin, J. P., Baines, R. C., and Ervin, J. O. Soil Sci. Soc. Amer.
Proc. 21: 163-166. 1957.
59. Bliss, D. E. Phytopath. 41: 665-683. 1951.

SOIL ENVIRONMENT AND MICROBIOLOGICAL ACTIVITY

W. V. Bartholomew

It has been announced that you would be meeting someone to properly introduce you to the subject of soil organic matter and physical factors in the soil. With your permission, I would like to deal particularly with the factors in the soil environment that influence microbiological activity. I want to admit that there is a dearth of information, but there is some which may be of interest.

To begin, I want to say that the soil properties and the soil environment determine to a large extent the kinds of organisms which will inhabit the soil and also, in turn, the activities of these organisms. I would like to use some very simple illustrations in explaining some of the things occurring in the soil.*

The soils are a mixture of things: solid materials, liquids, and gases. The solids are very often of a mineral nature. One also finds all kinds and shapes of pore spaces, and it is chiefly the pore spaces in which we shall be interested. Some pores are large; some, very small. The kind of pore space is mainly determined by the kind of solid particles present. If there are large, solid particles like sandy materials, in general, the pore spaces will be of the larger kind. In clay soils, where the solid particles are very small, there is a different kind of pore space distribution and a different type of space in which the microorganisms live. The solid particles have a density, in general, of about 2.6-2.75, and we may presume there are deviations on both ends of the scale. The soil unit density per unit volume is a good deal less. Table 1 illustrates some of the ranges in bulk density of the soil. There is not much deviation in the specific gravity of the solid materials; they are usually limited to quite a narrow range. The differences one finds in the bulk density, or the weight per unit volume of soil, arises chiefly from the differences in packing of the soil particles.

You will note that the bulk density of soils range in this table all the way from the neighborhood of 1 (one gram per cc.) up to perhaps 1.7. This last value would be for a rather dense soil. What all this means is that you have a volume of soil with about 50% of it being made of solid particles and another 50% being space. When the soil is dry, this pore space would be filled with air. When the soil is wet, the spaces would be filled to various degrees with water. As the moisture fluctuates in the soil, these pores are filled and drained again.

* Editor's note: Because of the large number of figures, they are printed as a group at the end of Dr. Bartholomew's talk. Tables are presented in the body of the text.

Table 1. Bulk Density for Certain Wisconsin Soils^{1/}

<u>Horizon</u>	Marathon	Miami	Spencer	Superior
	silt <u>loam</u>	silt <u>loam</u>	silt <u>loam</u>	clay <u>loam</u>
Plow layer	1.34	1.28	1.38	1.46
Upper subsoil	1.49	1.41	1.55	
Lower subsoil	1.59	1.43	1.66	1.66
Parent material	1.72	1.49	1.63	
	<u>Cropped soil</u>		<u>Uncropped soil</u>	
Hagerstown loam	1.25		1.07	
Marshall silt loam	1.13		0.93	
Nappanee silt loam	1.31		1.05	
Ave 19 Georgia soils	1.45		1.14	

Specific gravity of soil minerals: 2.6-2.75.

^{1/} From Lyon, Buckman and Brady. The nature and properties of soils. 5th Ed. 1952.

Soils differ markedly in the kind of pore space they have. I have illustrated in Figure 1 some information about pore size and distribution. I have to admit that soil physicists will say that there is not a good method of measuring or evaluating pore size. One method we do have is to find out how much tension is necessary to drain a certain amount of water out of the soil. This can be equated to the amount of tension required to draw capillaries of known diameters. This method does give some idea of pore size and distribution, although it has a lot of shortcomings.

In the first graph in Figure 1 you will notice data for fine, sandy loam are expressed on the basis of the total soil volume (100 cc.) being occupied with solids (50 cc.) and with pore space (50 cc.). In the sandy loam soil there are very few pores smaller than 0.02 microns in diameter. The large pores in the soil greater than 20 microns in diameter constitute quite a large portion of all the pore spaces. In the other illustration, Loam Soil, there are very few pores that are greater than 20 microns in diameter. These are only two illustrations; in between them are to be found all gradations and some that, perhaps, are different.

I think the next illustration (Figure 2) will give you another idea about pore sizes. The author of these particular data did not provide enough information to enable us to use the same type of illustration that we had in the first figure, but these diagrams will give an idea

of pore space distribution. On the vertical axis is expressed pore space per 100 grams of soil. In the fine sandy loam, only a small portion of the total pore space is less than 0.02 microns in diameter and a large portion is greater than 20 microns in diameter. In the clays, a large portion of the pore space is very small in diameter. In many of the clays, the total volume of pore space of large-sized pores will be small. Thus, there are all gradations of the sizes of pore spaces.

The size of the pore spaces and the other physical properties accompanying pore size distribution have a lot to do with the kinds and types of organisms that live in the soil and the environment they find to live in. Let us consider the environment these microorganisms encounter in the soil. Consider the water of the environment first. The pores of the soils may fill up with water and then, as the water subsides, the pore spaces fill with air. As water is added to the soil, the first result is absorbed water as films on the soil particles. The first increments of water that are added are absorbed very strongly. Succeeding increments of water are held less tightly to the soil particles, and, as water is added, the surface films begin to fill the small capillaries. As more water is added, successively larger pore spaces become filled with water until, perhaps, only a few of the larger pore spaces remain unfilled.

The soil physicist characterizes the water in the soil differently than stating the total quantity of water present. He is probably more interested in the forces that hold water in the soil and finds a number of systems of measuring these forces. Physicists may characterize the situation by determining the amount of tension it takes to pull the water out of the soil, and they may use a number of methods of expressing that value. They may use "atmospheres tension" which is a unit of force expressed in centimeters of height of a column of water. Or, they may use another term, "pF," which is the logarithm of this force in centimeters of water. Or, still another term is used here as this force is related to the relative humidity of the soil air within the pore spaces.

Figure 3 shows the relationships between several of these measures of the force that holds water in the soils. The first column gives the appearance of the soil. When the soil is dry, the tensions are very high; and when the soil is moist, the tensions may go nearly to zero. The second column shows tensions in centimeters of water. The third column is the log of this tension; and so on. The column for "pore size filled" refers to the largest diameters of pore sizes which are filled at particular tensions. The normal range of water in the soil at which plants will grow lies somewhere between the wilting range and what is called "field capacity" down to about a tension of 100 cm. of water.

You will note the pore sizes that remain filled with water. In general, soils do not remain at field capacity very long; so you see, there are not many large pores filled with water to permit the microorganisms to swim freely about all the time. Most likely, they are restricted to a

thin moisture film which will limit movement and other activities of the microorganisms. That is the moisture environment the microbes find in the soil. Only when the soil is quite wet is there enough pore space filled with water to provide for free movement through the soil.

Figure 4 illustrates the quantity of water held in the soil at particular tensions in two soils. One is a sandy soil; and the other, silt loam. They do not represent extremes in range, but they will give some idea of the ranges we deal with. You will note from the previous figure that the range of soil moisture where plants grow is from about log soil moisture tension of 2.2-2.5 to 4.0. From the graph you can obtain an idea of the range of the moisture content between the two soils over the growing range of tensions for plants.

The illustrations in Figure 5 relate to water and air movement and moisture content of the soil. As water moves into or out of the pore space of the soil it influences the movement of gas in the pores. Water moves more freely in the large pores, and so does the air, anytime there is mass movement. The relationship between the water and air content is not a linear one, because movement of air is by mass movement, when larger pore spaces are present, and by diffusion, which is not a function of the large pores. It makes little difference to the diffusion rate whether the pores are large or small, because diffusion is a function of the total pore space per unit area.

We are interested here in some of these environmental factors as they influence microbial activity. Before we get into that, I would like to have you look at Table 2 which gives data concerning composition of the air in the soil.

Table 2. Composition of Soil Air^{1/}

Per cent CO₂ and O₂ by volume in soil air

	1st foot		2nd foot		3rd foot		4th foot	
	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂
Nov. 14, 1937	1.2	19.4	2.4	11.6	9.6	0.7	15.5	0.2
Mar. 23, 1938	0.15	20.15	2.1	0.15	5.6	0.3	----	---
May 24, 1938	3.7	15.2	3.95	13.95	5.6	13.4	----	---
July 25, 1938	2.0	19.8	3.1	19.1	5.2	17.5	9.1	14.5
Sept. 22, 1938	3.0	15.3	4.8	11.0	5.8	10.0	8.5	9.0

^{1/} From Bayer, Soil Physics, 2nd Ed., 1948.

Supposedly, the air in the soil is in equilibrium with the air of the atmosphere above. Up near the surface of the soil, the composition of

the air in the soil pores will be largely that of the atmosphere above. Deeper in the soil, higher concentrations of carbon dioxide and lower concentrations of oxygen are found, and probably there are other gaseous products formed by the biological processes. Seldom, even at great depths, does one find all of the oxygen being used or very high concentrations of carbon dioxide. Diffusion seems to be rather rapid. When the soil is filled with water, the oxygen can be used up rather rapidly with resulting anaerobic conditions and high concentrations of carbon dioxide. As soon as the soil pores drain, the gas diffusion is rapid and an equilibrium system becomes set up quickly. We shall want to refer to some of the oxygen and carbon dioxide values a little later.

Now, as to the influence of these factors on the overall microbiological activity. The measurements that have been made have generally dealt with the overall activity, not with the activity of specific groups of organisms. Little information is available on specific groups. It may be presumed, and I am quite sure it would be all right, that all organisms would not act alike. Some organisms would be more sensitive to these changes in the environment than would others. I wish we had more information on this particular topic.

In Figure 6 is plotted microbiological activity, measured by carbon dioxide production, as a function of moisture content of the plant material. This is not in the soil but in plant materials. You will note that moisture is given in a number of ways. Maximum activity occurs at high moisture content, unless anaerobic conditions develop. Then the microflora would be changed and, in so doing, alter the overall activity. As the moisture content is decreased, there is less and less activity until a threshold point is reached at which the organisms are just barely active, at least, activity can be just barely measured.

You will also note from the graph that microbes are quite active at moisture levels below that at which plants will grow. I have indicated on the chart the wilting point and the field capacity values. You will note on the relative humidity scale of Figure 3 that in the range in which plants will grow, the relative humidity is around 99 to 100% and the air is well saturated. There are reports of microbial activity in beet tops when the relative humidity has been down as low as 75%. Thus, microbes can grow under stringent moisture conditions.

Figure 7 gives an idea again of microbiological activity in the soil under the influence of varying soil moisture. The information is plotted in two ways: one as percentage moisture and the other as logarithm of centimeters of water tension. You will note that, in general, there is a wide range of moisture conditions in which the overall population of microorganisms is rather active. Only at the low moisture contents does the activity go down. Although there might be specific members of the soil population that would be affected a good deal differently, it is likely that the overall population activity represents the mean. Thus, one does not reduce microbial activity markedly until reaching moisture contents below that at which plants will grow.

We were interested sometime ago in what were the moisture conditions which are limiting to microbiological activity. The soil physicist, in studying moisture, as far as plant growth is concerned, has pretty well come to the conclusion that moisture tension is the best measure of available water to the plants. This moisture tension, I am sure, has a lot to do with how fast moisture will migrate from one place in the soil to the place where plant roots are and where it is taken up. There is this translocation process of water uptake and movement through the plant which is a limiting factor. The microbes, however, live in the film of water apparently unrestricted by the factors which affect the higher plants. We are, therefore, interested in finding out if tension is the important thing about soil moisture; or is there some other factor? In Tables 3 and 4 I have given some figures which will help to illustrate, at least, the conclusions we came to.

Table 3. Relative Humidity, Moisture Content,
and Rate of Decomposition (25° C.)

Plant Material	Relative Humidity %	Moisture Content %	Mg. CO ₂ in 192 hours
Alfalfa	94.4	45	144
Sudan Grass	94.4	34	92
Oat Straw	94.4	34	33
Hemp Bark	94.4	35	20
Pine Needles	94.4	21	12
Sudan Grass Roots	94.4	18	10
Alfalfa	83.5	24	90
Oat Straw	89.4	24	11
Oat Straw	80.8	16	0
Sudan Grass Roots	92.0	16	10
Alfalfa	80.8	21	2
Sudan Grass	85.9	22	42
Sudan Grass Roots	97.1	21	17

In Table 3 we have the situation where a uniform relative humidity was maintained and various plant residues were incubated in equilibrium with this particular relative humidity. You will note, however, that the moisture content of the plant materials varied widely although the relative humidity was kept constant. Note that also the overall activity of the microflora inhabiting these residues was proportional somewhat to the moisture content; it was not a direct function of the moisture tension (relative humidity). Those plant materials that absorbed more

water supported the larger microbial activity, as measured by carbon dioxide production. One cannot say that moisture tension is the single factor that limits microbiological activity.

In the same Table the data are grouped showing equal moisture contents but in different plant materials. Consider the data for Alfalfa and Oat Straw. Each was 24% moisture content. Alfalfa was at about 84% R.H. and the Oat Straw at about 89% R.H. The greater microbiological activity was found in the Alfalfa. Thus, one cannot say that moisture content alone is involved; there is a difference in the kind of plant materials too. So it may be that there are other things besides moisture involved.

Table 4. Threshold Moisture Conditions Indicated
for a Number of Plant Materials

<u>Plant Material</u>	<u>Moisture Range %</u>	<u>Relative Humidity Range %</u>
Alfalfa	20-22	75-80
Sudan Grass	14-16	77-82
Oat Straw	15-17	77-82
Hemp Bark	15-17	77-82
Pine Needles	15-17	80-86
Sudan Grass Roots	10-12	80-86

Table 4 illustrates again that it is difficult to say that overall microbiological activity is a function of total moisture. There seems to be a combination of the tenacity with which moisture is held to particle surfaces and the total moisture. Both factors are needed to explain limiting moisture conditions for microbes.

Table 5 presents data relating microbial activity to moisture tension. As the moisture present was decreased, the numbers of all the organisms increased. Now we want to know whether or not overall microbiological activity is a straight function of moisture content or whether there was some temperature interaction.

In Table 6 we have some data which illustrate that, as far as these studies were concerned, it seemed that each factor operated fairly independently, and we did not find any interaction between the two. That is, as we increased the temperature between certain limits, there resulted an increase in activity regardless of the moisture content. Increased moisture content resulted in increased activity regardless of temperature, and there was no interaction. I suspect in some cases one would find an interaction.

Table 5. Microbial Numbers Determined for Webster Silt Loam
When Incubated at Selected Moisture Tensions

Moisture Tension Equivalent in Om. of Water	Fungi		Actinomycetes		Bacteria	
	5 days	15 days	5 days	15 days	5 days	15 days
	thousands per gram		millions per gram		millions per gram	
0	25	25	7.3	6.5	16	28
	26	39	7.7	3.5	11	21
1	26	40	7.0	4.0	12	26
	21	34	5.0	3.7	9	18
10	35	74	13.3	8.3	66	60
	31	73	7.0	7.0	63	30
50	58	287	10.0	8.0	97	70
	62	250	8.0	4.0	82	66
500	113	463	41.5	28.0	85	105
	104	517	38.8	27.0	131	110
3160	107	593	76.1	67.0	105	97
	110	517	55.0	50.0	140	110

* * * * *

Table 6. Mg CO₂ Evolved from 1 Gram Samples of Straw Incubated
at Varying Temperatures and Moisture Contents

<u>C°</u>	<u>60%</u> <u>Moisture</u>	<u>150%</u> <u>Moisture</u>	<u>250%</u> <u>Moisture</u>
50	174	182	197
37	104	138	165
25	79	139	152
12	45	67	79

Another interesting thing about the environment in the soil is desiccation. If one takes a sample of soil and brings it into the laboratory, dries it, and then remoistens it, one finds that there is a marked increase in microbiological activity. There is this increased overall activity for a period of time, and then it gradually subsides to normal. There is such a stimulation that some people wonder if on alternate

drying and wetting of the soil there might not actually be a higher total activity than if the soil remains continually moist. In some cases, I think this has actually been demonstrated. I will not try to explain how this stimulation comes about.

Table 7. Effect of Alternative Wetting and Drying on Decomposition of Plant Material and Loss of Soil Carbon

Muscatine Silt Loam (20 Gram Samples)

	Rate of corn residue addition	Carbon Dioxide evolution from 20 g. soil during 48 days	
		Plant	Soil
Continuously moist	0	-	165.3
Continuously moist	2.5%	264.6	173.6
Alternately wet and dry*	0	-	150.1
Alternately wet and dry	2.5%	239.5	159.3

* Wet to 150% of moisture equivalent, left wet for 3 days, dried with dry air for 6 days, left dry for 3 days, re-wet at 12 day intervals.

Table 7 compares samples that were kept continuously moist with samples that were alternately wet and dry. In this particular study there was not a great deal of difference in the carbon dioxide evolution or, shall we say, in the total microbiological activity in the two systems. Dessication is a peculiar phenomenon and a very interesting one. It is probably one that has quite a marked influence on not only the total microbial activity but on specific members of populations.

We have heard a lot from various sources about the influences of the gaseous components in the soil on the overall activity of the microorganisms. A number of studies have been made. Figure 8 shows the influence of three levels of oxygen content upon the microbiological activity expressed in terms of carbon dioxide production for a period of 52 days. It was found that going from 21% oxygen in the air stream, which is about the normal content of the atmosphere, down to the low percentage had very little influence on the carbon dioxide evolution. The oxygen content has to get to a rather low amount before a marked effect is evident. Also, one would expect to still get carbon dioxide production in an anaerobic condition, so this does not tell much about the activity except that it is not reduced a great deal by reducing the atmospheric oxygen. Another consideration is that from the normal

oxygen content of the atmosphere of about 20%, it has to go to a rather low value before anaerobic conditions exist. In normal, well-drained soils, except when filled with water for short periods of time, it is not likely that the oxygen content is sufficiently low to have any marked effect on the overall microbiological population. Aeration, as far as the microbes are concerned, is not very much of a problem. Figure 9 illustrates the same thing: reduction of the oxygen to a low percentage had only a minor effect on overall activity.

Figure 10 illustrates the influence of oxygen in its gaseous phase on a strictly aerobic process, that of oxidation of ammonia to nitrates. In going from the normal oxygen content of the air down to the neighborhood of less than 2%, the rate of oxidation is reduced by only a little more than 50%. Reducing the oxygen content down to about 5% only reduces the oxidation rate about 30%. In other words, to inhibit the oxidation of ammonia to nitrate, the oxygen has to get well down below 1% in the gaseous phase. Seldom do we find it so low in well-drained soils, except for short periods of time.

The last of the factors that I want to stress a little bit is temperature. In the soil, there is a lot of temperature fluctuation. If one plots, for example, soil temperature against time, one finds that, in general, the upper soil levels have minimum temperatures along in the early morning hours. During the day, the temperatures climb and reach maxima sometime in the afternoon and then begin to diminish. Layers beneath the soil surface do not fluctuate in temperature this much. They do not get temperatures as low in the morning or as high in the afternoon. If the soil is covered, in general, the uppermost layer does not get as high in the afternoon as does the air temperature, whereas, in bare soil, the temperatures may exceed the air temperature. Covered soil will also not get as cool as the air temperature during the night. Likewise, there is a seasonal pattern to the soil temperatures. All these pose a problem as to just how does one characterize soil temperature to somehow relate it to soil microbiological activity.

There is no uniform system at all. There are a series of fluctuations from day to day, differing at varying depths and to varying extents, depending on such things as soil cover, weather, and season. We tried one time to come up with some particular temperature reading which would characterize the temperature condition for the upper six inches of soil. This was done by taking the fluctuation patterns for various depths in the upper six inches of soil. A mean was taken for the several horizons at each hour during the day. Then taking the mean of all these means, we came out with a value that perhaps has some significance. At least, it is a single figure that can be used, whereas one cannot use a whole group of mean values for anything. For what it may be worth, we found that this particular mean was very close to the soil temperature reading at four inches depth at about noon. Thus, when we wanted to get something to characterize the soil temperatures for a full day, we merely took a noon reading of the temperature at four inches depth. This does a fairly good job, but, of course, it does not tell anything about the fluctuations which probably are very important.

We also had some studies on the influence of temperature on the oxidation of ammonia. Again, this was a practical problem, but, in all probability, a large majority of the other microorganisms in the soil would react like the nitrifiers. In the last graph, Figure 11, is plotted the influence of temperature on the rate of oxidation of ammonia to nitrates in a number of soils. The influence varies between soils and is not well defined. In general, one can say that within the limits of the range of soil temperatures (8° to 25° C.) there is a direct relationship between soil temperature and microbial activity. Other data support this thesis that nearly complete inhibition of these activities does not result until the temperature gets down to the freezing point. Again, this is the characteristic of the microflora in general, the overall, not the specific, members of the microflora.

In conclusion, the data which have been presented are of the overall activity of the gross microflora. The influence of the individual factors on specific members I do not know, nor would I know where to find the information that would help out in that respect. I do presume that various members of the soil micro-populations do act quite differently.

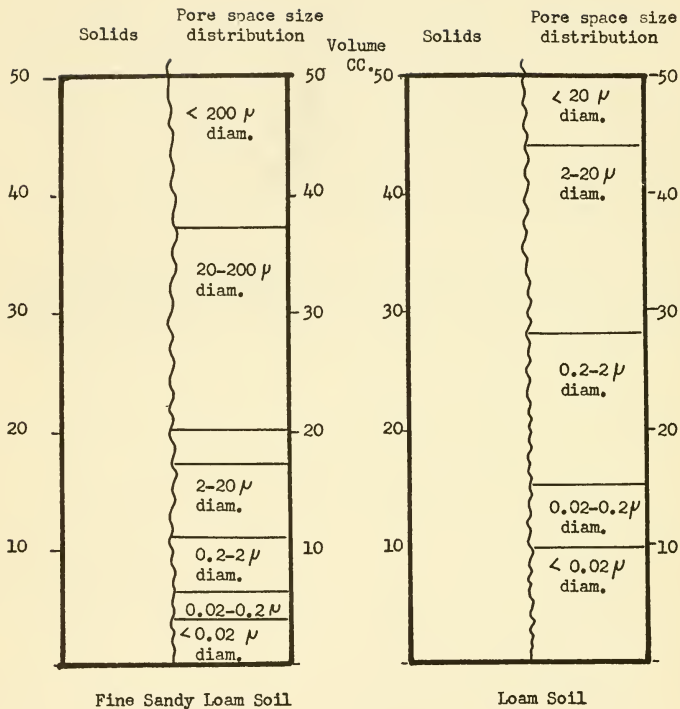


Figure 1. Pore size distribution

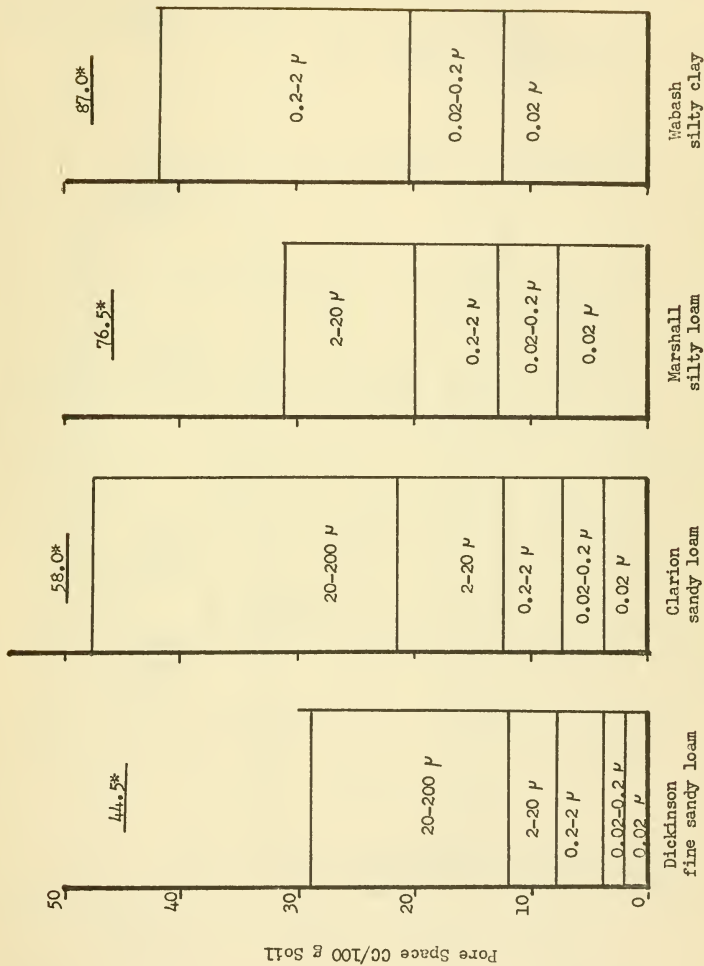


Figure 2. Pore Space Distribution In Soils.
 (*Maximum Water Holding Capacity - % dry Wt. Basis)

Soil appearance	Tension cm. water	Log Tension cm. water	Tension atmospheres	Rel. humid. 25°C %	Core sized filled μ dia.	Availability ranges	
						Plants	Microbes
DRY	10,000,000	7	10,000				Range of limiting moisture for microbes ????????????? RANGE OF MICROBIOLOGICAL ACTIVITY
		6.5		10			
	1,000,000	6	1000	50		Water unavailable to plants	
		5.6		75			
	100,000	5.15	100	93	0.02		
		5					
MOIST	31,623	4.5	30.6	98		Wilting range	
		4.15	13.6	99	0.2		
	10,000	4	10				
		3.15		99.9	2		
	1000	3	1.0				
	501	2.7	0.5	99.96	5.8	Field capacity	
WET		2.15		99.99	20		
	100	2	0.1			Water removed by gravity	
		1.15		99.999	200		
	1	0	0.001				

Figure 3. Soil Moisture Relationships

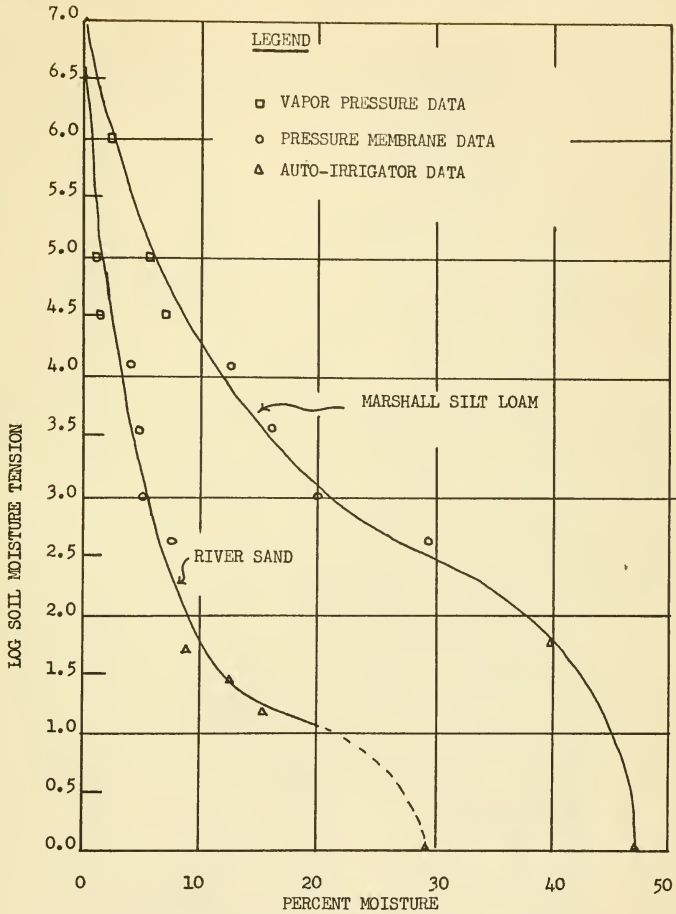


Figure 4. Complete Moisture Sorption Curves For Marshall Silt Loam and River Sand.

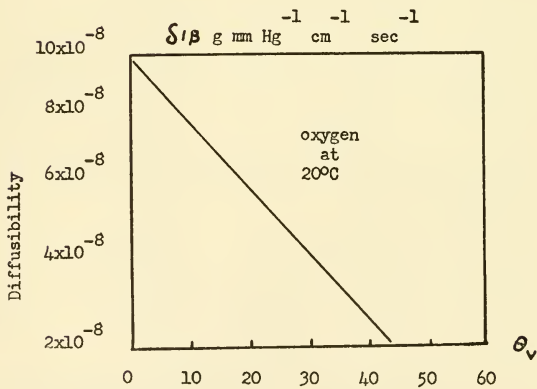
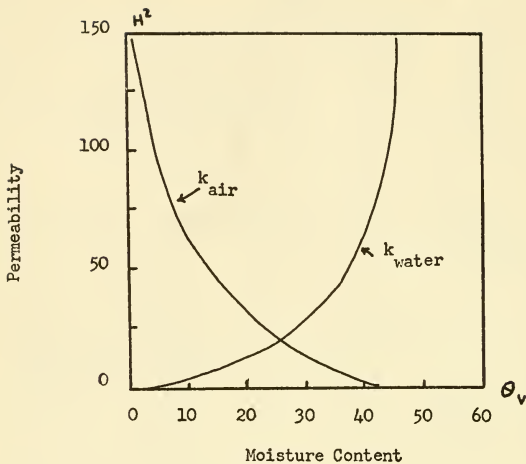


Figure 5. Water and Air Movement and Moisture Content.

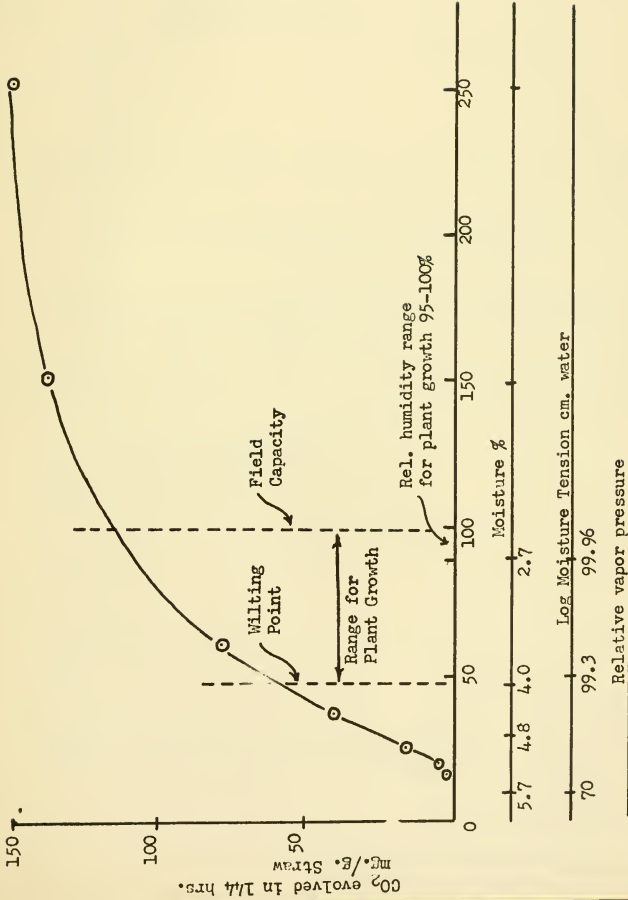


Figure 6. Moisture Influences on Decomposition of Straw.

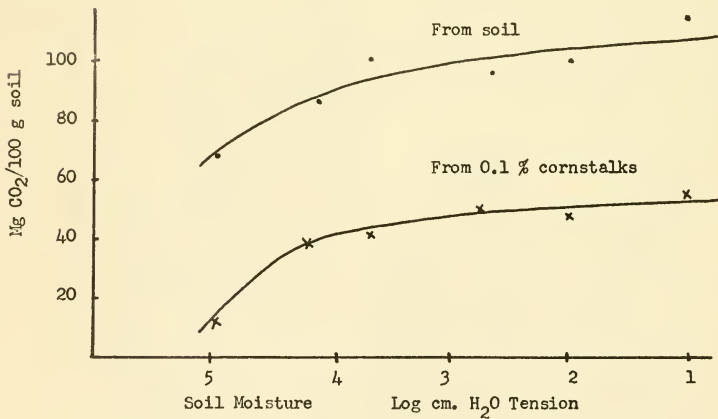
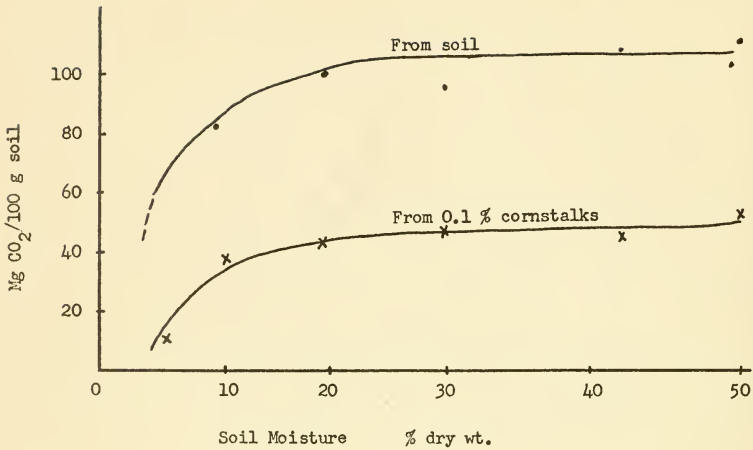


Figure 7. Moisture content and CO₂ evolution in 53 days from Monona sil. loam. (Hallam Ph D. Thesis. Iowa State College. 1952.)

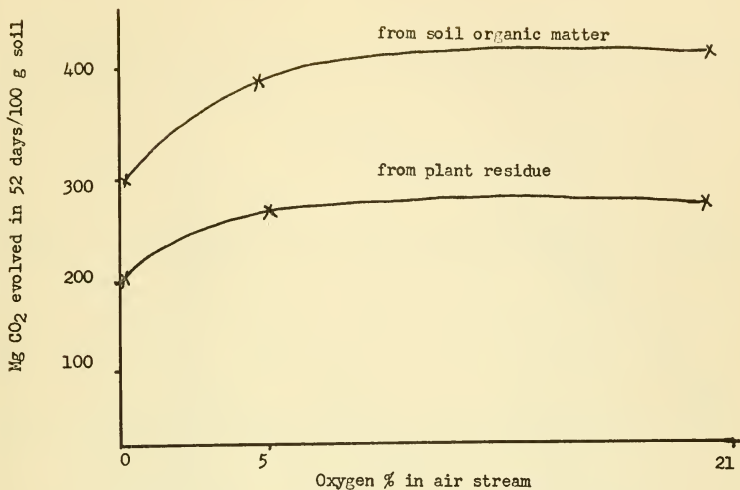


Figure 8 . Influence of Oxygen in Air Stream on CO Production in Decomposition of Crop Residues (residue added at rate of 0.5% by wt.)

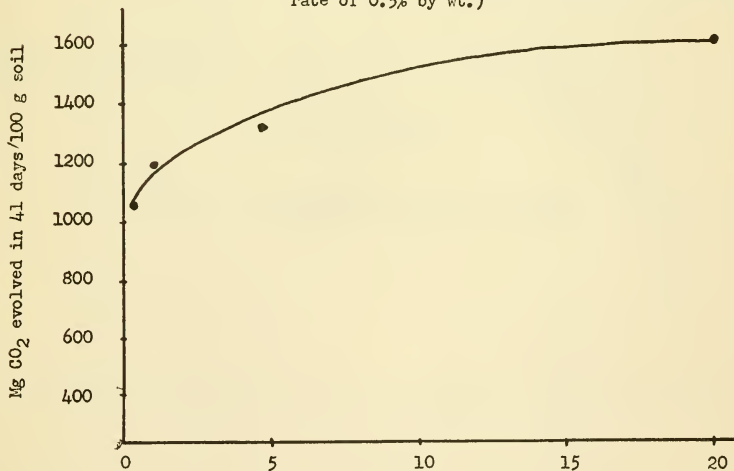


Figure 9 . Influences of Oxygen % in Air Stream on CO² Production in Decomposition of Crop Residues (residue added at rate of 2.0% by wt.)

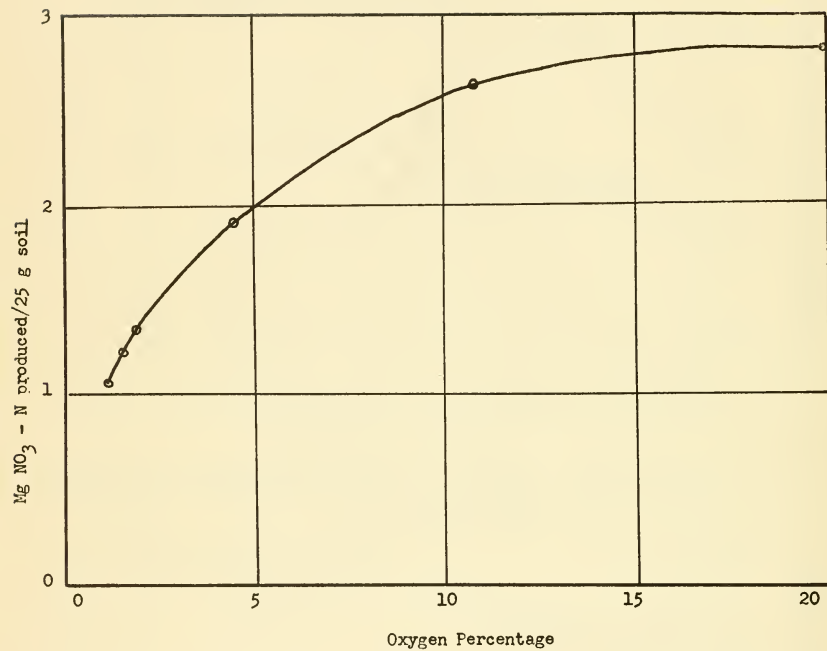


Figure 10. Amounts of Nitrates After 21 Days of Incubation at Different Oxygen Percentages.

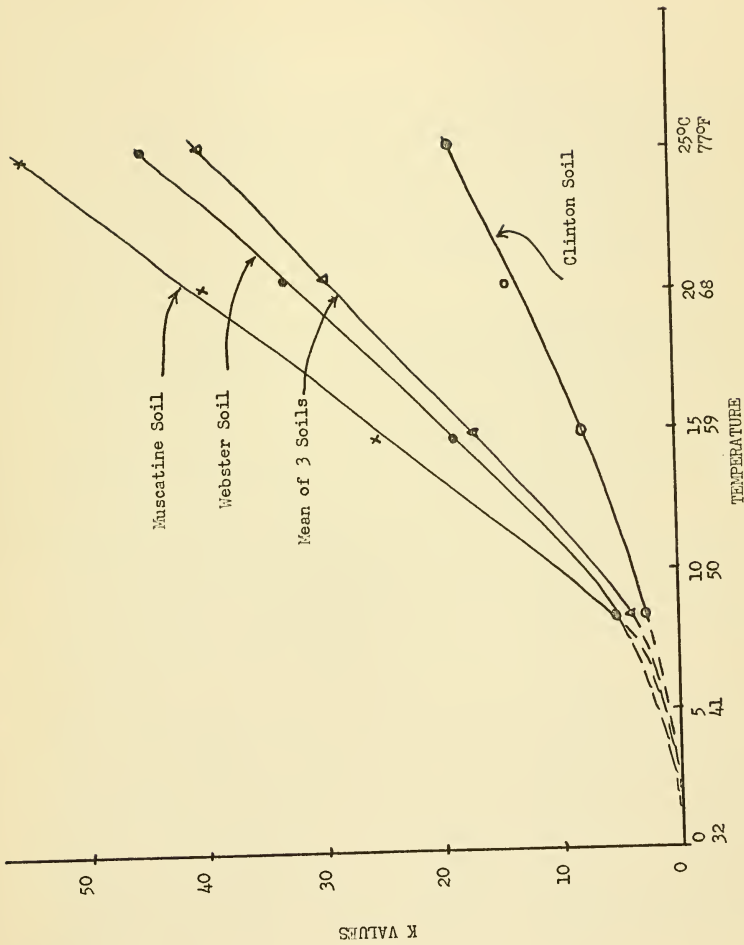


Figure 11. Influence of Soil Temperature on Rate of Microbiological Oxidation of Ammonia to Nitrate.



PHYSICS OF VAPORS AND GASES IN THE SOIL

Lloyd F. Seatz

The nature of the soil -

The soil is made up of a framework of solid matter; this solid matter being minerals and organic material. This framework is separated by voids that we call pore spaces. In an average soil, the soil framework makes up roughly 50% of the total soil volume, and the pore space makes up the remaining 50%. This pore space is filled with either liquids or gases. We call liquids the soil solution. The gas in the soil atmosphere. Obviously, the amount of gas that will be present in the soil will depend on the amount of moisture that is present. In other words, the amount of pore space that is not filled with liquids, naturally would be filled with gases.

The size and distribution of this mineral and organic material is referred to as the texture of the soil. This material is usually in some sort of arrangement that we refer to as soil structure, whether it be single grained, crumb, or whatever types of aggregates that might be present.

It is the textural and structural relations in the soil that are most closely related to the type of pore space and the size of pores found in the soil. In particular, the size and continuity of this pore space will determine the movement of liquids and gases in the soil.

There may be many dead-end pores brought about by certain types of soil arrangement. These dead-end pores are not effective in allowing movement to occur. It is only those pores that are continuous that allow for the movement of liquids and gases. So we see that the size, shape, continuity, and distribution of the pores, as a reflection of the textural and structural arrangement in the soil, are the really important things governing the air and water relations in soil.

Another term I think we should review is this one called "bulk density." This is defined as a mass per unit of bulk volume. In other words, we have a certain volume, including both the solid material and the pore space. Bulk density differs from the absolute specific gravity, which refers to weight per unit mass of the solid material only. Other things being equal, if we assume that we are using only mineral materials which Dr. Bartholomew said had a specific gravity of about 2.65, the bulk density will be a reflection of the percentage pore space. If the bulk density is low, then that means there is a higher per cent of pore space in the soil than if the bulk is high, again assuming that the same percentage of minerals and organic material are present. If the per cent of organic material, having absolute specific gravity of .9 varies, you see that the bulk density would vary with the percentage mineral and organic materials,

even though the percentage pore space does not vary. So much for some of the things about the soil and its atmosphere and the factors in the soil which will effect pore space.

Kinetic theory of gases -

I think if we are going to consider gaseous movement, we perhaps should review very briefly something about the kinetic theory. It is the kinetic energy that the gas molecules possess which will affect to a great extent their movement. The gas molecules are in constant motion and, at a constant temperature, have a constant average velocity. The amount of kinetic energy, or this motion, is a direct function of the absolute temperature. Theoretically, if we reached zero degrees, Kelvin, the kinetic energy, would be zero, and the molecules would cease to have motion. But under normal situations, as the temperature goes up, the average velocity of the molecules increases accordingly.

The molecules of the gas, theoretically, also behave as elastic spheres, and the impacts that they have with one another are instantaneous and thoroughly elastic. They lose none of their energy through the impact of molecules with one another. It is also assumed that the mean free path of the molecules between impacts is much greater than the time for the instantaneous impacts that they have with one another. They exert no forces upon one another that would cause them to stick together; each impact is, as I say, thoroughly elastic. We assume that the duration of the impact is instantaneous or negligible compared to the time interval between these impacts.

Gases, as you know, also have the property of unlimited and indefinite expansibility. That means that they will fill completely any volume into which they are introduced. They exert a pressure on the walls of enclosing vessels, due to the impacts the molecules have against the walls of the vessel. The molecular movement that they possess is a manifestation of the kinetic energy in the system.

It is shown by the kinetic energy formula that the kinetic energy of a system is equal to: $K.E. = \frac{m \cdot v^2}{2}$, in which "m" is the mass of molecules and "v" is the velocity of molecules. So we see that this system possesses a certain quantity of kinetic energy. Then, if we increase the mass of the molecules, the velocity of the molecules will be reduced accordingly. If we have a mixture of gases with a given amount of kinetic energy in that system, the larger gas molecules would be moving at much slower velocity than the smaller molecules. Now this is somewhat important as one goes into consideration of larger and larger molecules.

The process of diffusion -

The rate of diffusion at a given temperature would be reduced according

to this increase in molecular size. The process of diffusion, which is perhaps the main means by which gases move in soils, is one in which the mixing of gases will be due to the kinetic energy that the molecules possess where there is no external force entering into the reaction.

Diffusion always takes place from a zone of greater concentration into a zone of lesser concentration, in other words, according to the concentration gradient.

As I say, most gaseous movement in soil is a result of the diffusion process. There are several factors that affect the rate of diffusion. First, one might mention temperature. As I mentioned a moment ago, the kinetic energy that molecules possess is a direct function of the absolute temperature. As we increase the temperature of the system, if everything else remains constant, the quantity of kinetic energy in that system will increase. If the mass of the molecules remains constant, then the velocity must increase accordingly, and the process of diffusion will increase also. Certain studies concerning gaseous movement have shown that in some instances the rate of diffusion will increase by a factor of about 50% for each 10°C. rise in temperature.

A second factor that will affect diffusion is the difference in pressure. If there is a higher pressure of a gas in one volume than there is in another volume, the rate of diffusion will be greater than if the pressures are essentially the same. This is not only true of the total pressure on the two sides of some membrane, but it is also related to the partial pressures of the gases that make up the total pressure.

You will perhaps recall Dalton's Law of Partial Pressures. In the gaseous system, the total pressure is equal to the sum of the partial pressures, and the partial pressures are proportional to the percentage composition of the various components in the gas. In other words, if you introduce into a vacuum various quantities of gases to make a gaseous mixture, each gas would fill entirely the volume into which it was introduced and would yield a partial pressure. The summation of these partial pressures would equal the total pressure in that volume.

Diffusion not only occurs according to total pressure differentials but also according to partial pressure differentials. If the soil's gas system and the atmosphere above ground each consist of around 80% nitrogen and 20% oxygen, then we can say that in these systems the partial pressure of nitrogen would be 0.8, and the partial pressure of oxygen would be 0.2. If, in the soil through respiration processes, the oxygen was consumed to a certain extent and CO₂ replaced it, the partial pressure of oxygen would be less than 0.2, and the partial pressure of the CO₂ would be greater than .03%, and diffusion would take place from the atmosphere into the soil according to the partial pressures of the various gases. So the relative partial pressures of gases will affect diffusion in to and out of the soil.

The next factor, of course, is the size of the various molecules of the system, as I mentioned awhile ago. As the mass increases, the velocity

at which they move diminishes. Free diffusion might occur between two volumes of gas where free diffusion was allowed to occur. This follows the kinetic equation: $\frac{N}{AT} = D_0 \frac{(dP)}{(dL)}$. Thus the number of molecules that will diffuse is related to the area, the time, and the diffusion coefficient which is characteristic somewhat of the system and the gas with respect to the differential of pressures per unit length change.

But in soils, we are not dealing with free diffusion, because diffusion does not occur in soil as rapidly as it would in a free volume system. This is due to the impedance by the pore system of the soil. So there must be another factor introduced which is the diffusion coefficient of the gas through the pore spaces.

We say that the ratio of the diffusion coefficient in a porous medium to the diffusion coefficient in a free diffusion system is some function of the porosity of the system expressed as $\frac{D}{D_0} = f(S)$.

Curiously enough, this sort of derivation was worked out around 1904 by Buckingham when he was working for the Bureau of Soils at that time. Not a great deal was done concerning diffusion of gases until some time in the 1940's. I am sure that Great Britains are familiar with the work of Penman during the early '40's, in which he re-evaluated some of the earlier work of Buckingham. Since that time, other work has been done in the last ten years or so, more or less confirming some of the same facts that Penman discovered in England.

This term "porosity" is, I think, the actual volume that is occupied by gas. If one takes volume occupied by gas divided by the volume of the soil that is occupied by the solid material, plus the volume that is occupied by water, plus the volume that is occupied by gas; all of which, of course, is the total soil volume that give the percentage porosity in that system ($S = \frac{V_g}{V_g + V_w + V_s}$)

this ratio of $\frac{D}{D_0} = .66S$. $\frac{D}{D_0}$ again is the rate of diffusion through the porous medium with respect to the rate of diffusion in a free volume and is somewhat in relation to the distance through which the diffusion occurs. For example, gas in a free volume might diffuse twice the distance in the time required for gas to diffuse a certain distance in a porous system.

There are other factors, of course, than the porosity of the soil that will affect this rate of diffusion. Not only is the size of pores, which is really some function of the area through which the gases must diffuse, but also the much lengthened path that the gases must follow because of the irregular shapes of the soil pore system. Also, for very fine pores, there seems to be an interrelation between the impact of the molecule against the wall of that pore and with the other molecules in the gas system.

In this situation, the gas diffusing through an exceedingly small pore space would not follow the initial assumptions of free diffusion that I made a moment ago, in that molecules act independently from one another. What factors in the soil then will affect this diffusion rate? One is called compaction. The effect of compaction is essentially to increase the bulk density of the soil. Again assuming that there is no change in the percentage composition of that soil, as the bulk density increases, the value of percentage pore space will decrease, other things being equal. Therefore, the rate of gaseous diffusion will decrease. Some data in the literature indicate that one can decrease the rate of diffusion as much as 40% by merely compacting the soil and thereby reducing the effective pore space through which diffusion occurs.

Naturally, surface compaction will only affect diffusion rates through that surface, whereas the presence of a plow sole or some other impeding structure deeper in the soil profiles could affect diffusion at that point. These compacted zones, perhaps, are the rate limiting factors in the diffusion process, as is well known. Very often some constriction in a pipe, as an example, serves as the rate limiting factor and affects the flow in that pipe. Likewise, the amount of pore space present in the impeding structure would be the rate limiting factor that would affect the total diffusion rates through the entire system.

The second factor which will affect porosity and the diffusion rate is the amount of moisture present in the system. Dr. Bartholomew showed in one of his charts that under various moisture tensions various size pores in the system will drain. If you started with dry soil and added moisture, first the smaller pores would fill, and as more and more moisture was added to the system, larger and larger pores would fill with moisture until the soil became saturated. If, on the other hand, you started with the saturated soil and added increments of tension to the soil system, first the large pores would drain, and as more and more tension was applied, smaller pores would drain under the influence of this greater tension. The total porosity of the soil system will, therefore, be a function of the tension with which the moisture in that soil is held. Starting with a saturated soil, the gas in that system would be zero. Then, as more and more tension was applied to that system, pores would drain, films of water surrounding the soil particles would decrease, and more of the soil volume would be available for gaseous diffusion. Thus, at high moisture tensions or low percentages of water, there would be more volume for diffusion. As the water content increased, the rate of diffusion would decrease.

Another point of Dr. Bartholomew's that I think should receive some emphasis is with respect to size of pores and its affect on diffusion. The size of pores will affect the rate of mass flow, and it is a limiting factor in water movement, because water generally moves by some sort of mass flow arrangement or where there is actually some pressure differential. If gases were moving under some sort of a head, then the gas movement, as well as the water movement, would be regulated by Darcy's or Poiseuille's law, rather than by kinetic diffusion or kinetic energy. In

this instance, the size of pores would be a factor, as well as the total porosity of the system, but where free diffusion is occurring and mass flow is not a factor, then it is total porosity that is governing the amount and rate of diffusion and not necessarily the size of pores through which the diffusion is occurring.

I think I need to differentiate between these two types of movement and how the effects of size of pore would be a controlling factor in the one, whereas it is not a controlling factor in the other. Although Hagan, in some work in California, did indicate that in some instances the size of pore was a factor in carbon disulfide movement, his empirical equation was more closely similar to Poiseuille's law than it was to the diffusion processes. The importance of moisture content and its effect on the gaseous movement, of course, implies that a steady state has been reached. By "steady state" we mean an equilibrium has been established, which may or may not be true in many soil systems. If a gas is introduced into the system and it were to go into solution in the water present in the soil, the state would not be reached and the diffusion rate would be increased, because of the increased differential brought about by solution of the gas. One of the components in the system may be adsorbed by soil particles. This, too, would prevent a steady state condition. If in some instance CO_2 is being produced by biologic action in the system, this, too, would upset a steady state arrangement, and diffusion rates would be affected accordingly.

I think there are certain interests in other factors as indicated in other discussions by this group. One of these would be temperature effects. If one were to add a fumigant to the soil, its partial pressure in the system would be related to the temperature at which it volatilized, assuming it volatilized immediately at ordinary temperature. As the temperature raised, the partial pressure of that gas in the system would increase and there would, of course, be effects on rate of diffusion. This would be in addition to the ordinary kinetic effects that I mentioned previously.

The depth to which gas is placed would also affect the volume of soil through which diffusion would occur. If you place the gas at the surface, diffusion will occur in all directions, according to differential and partial pressures of that gas. If the introduced gas is placed near the surface, naturally, most of it would diffuse into the atmosphere rather than against the steeper gradient in the soil. I think that this would indicate in certain instances you would wish to place the fumigant deeper into the soil to get reduced loss by diffusion into the atmosphere.

Oftentimes some sort of plastic cover or something of that kind is placed over the surface to prevent diffusion losses back into the atmosphere. The amount of moisture in the soil will certainly affect the rate of diffusion. If it is necessary for the introduced gas to go into solution, then one is confronted with a dilemma. Is it more important to have the gas go into solution, or is it more important to have rapid diffusion? These things are reciprocal to one another. As the moisture content of

the soil increases, the rate of diffusion of that gas through the soils decreases as this moisture affects the porosity of the system.

In concluding, let me mention again the matter of compaction. As we compact the soil, for example, into a plow sole, or traffic sole, or if there are some other naturally occurring barrier structures of one sort or another in the soil, all these affect the rate at which diffusion will occur and the depth to which gases might diffuse in the soil. If you wish to have deep penetration, then sub-soiling or some other mechanical measure of this kind might be necessary to allow for deeper penetration of the gas within a reasonable length of time in order to prevent its complete dissipation without having done the job for which it was used.

Discussion

Q. The efficiency of a fumigant seems to be tied directly to the moisture equivalent of the soil. Up to about 20%, the average dosage of D-D or EDB, or whatever it is we are using, is effective. Above that, the rate has to be increased rapidly. Unfortunately, the data are always published without any reference to the moisture equivalent of the soil. I wonder if you would explain that term and something of the techniques that are necessary for individuals to get such a measure.

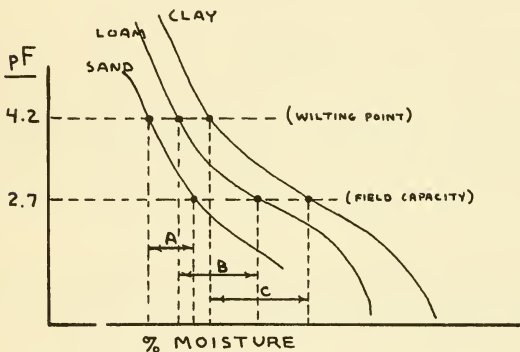
A. This goes back to a point that I overlooked. The amount of moisture in the soil, as I mentioned, will affect the rate of diffusion. There seem to be some conflict in the literature concerning the moisture content at which diffusion will almost cease. Hagan, in the work he did with carbon disulfide, said that the rate of diffusion became almost zero after moisture equivalent was reached. Some later work by Taylor, who was at Cornell at the time, indicates that there is a rather sharp break in the curve between 20 and 30 cm. of water tension. This would be considerably wetter than the moisture equivalent.

To get to the question you asked as to how the moisture equivalent is termed, it is a defined term that has no theoretical implication. It is defined as the percentage moisture in the soil remaining after the soil to 1 cm. depth has been saturated and has been centrifuged at a force of 1,000 times gravity for 30 minutes. This moisture equivalent value is theoretically close to the approximate field capacity which is the tension of a pF value of about 2.7.

To actually state moisture equivalent, it has to be determined according to the technique of Briggs and McLane. According to their technique, and I believe that the definition I gave is correct, it approximates field capacity. Field capacity is approximated by about 500 cm. of water tension.

Dr. Bartholomew showed a chart in which he rated five or six things together, such as relative humidity, pF, and centimeters of water tension. pF and cm. of water tension are, of course, the same thing. pF is a logarithm of the height of a column of water expressed in cm., but certainly

you have to relate it to some energy concepts of water; you cannot relate it to percentage water in the system, for percentage water means absolutely nothing with respect to the forces by which water is held. This is related to the thickness of the film or the size of the pores that will drain under a given tension. They would be related to each other, if you held the texture of the material constant. I would like to draw a graph on the board which may help relate various terms we have been discussing. This is similar to the information given by Dr. Bartholomew. The graph shows more or less typical moisture characteristics for three different soil types:



The values of A, B, and C are the so-called available water. So you see the percentage available water will be much greater for a clay soil than for sandy soil. Also, the amount of water present at wilting will be much greater in the clay soils than for sandy soils. Do you see how, as Dr. Jones has said, the percentage moisture itself has to be related back to some physical factors in order to be really meaningful. You can get these water tensions by putting the soil sample on a so-called tension table, or you may want to go over to more elaborate pressure membrane type of apparatus. It is not too difficult to set up a tension table to get an accurate measure of large pores. Some of you may want to explore that possibility. To get up to higher tension ranges, specialized and expensive equipment would be involved.

Comment: Acknowledging the necessity for precise work in certain circumstances, I would like to point out that in practice there is a certain range of conditions in a particular soil type in which certain fumigants can be expected to be effective. I have had quite a little experience in chemical control in the western states. I know the soil

types for my area fairly well. The Shell Company has determined the moisture equivalents of these soil types for me. I have become able to judge the soil when called to look at a field around planting time as to whether the moisture is about 20% or below its moisture equivalent. If the soil is at 20% or below, 25 gallons of D-D will give good control of the sugar beet nematodes. If the moisture runs up around 25%, I know well enough from past experience that I can tell the grower to go ahead and rotate his crop that year, because he cannot afford to put on the extra 10 gallons per acre necessary for effective control. I would like to suggest that field workers try to get to the point of being able to recognize the soil types and have someone in your locality determine the moisture equivalents for you until you are able to make sound recommendations to the growers. They cannot afford to fumigate if it will not be effective due to unsuitable moisture conditions.

Q. Why not report the condition of the field at the time we apply the fumigant? Do you think that the soil type, compactness, temperature, and some of these other things could be readily obtained for reporting in fumigation experiments?

A. If you are comparing soils with similar mineral and organic contents, then the bulk density value would give some measure of compactness. You have to take bulk density with some precautions, as I pointed out, because they are not universally comparable to each other. In addition to that value, one should also, as Dr. Jones pointed out, have some measure of the tension with which the moisture is held in the soil at that time. The only way to get at that is to run a complete pF versus percent moisture curve on that soil and then determine the percentage moisture in the soil at the time you did your fumigation. Relate that back to the pF value of the soil on a predetermined curve and that would give you a more or less absolute measure of the tensions or the physical forces that were operating over moisture in the soil at that percentage moisture content.

Q. Is there not an instrument of some type that could be put in the soil to give us a reading?

A. There have been several attempts to get devices that will give the measures of soil moisture, and perhaps the most widely known one is the so-called Bouyoucos block. Variations of that have been devised. The nylon cloth and gypsum blocks determine temperature and moisture at the same time, but their readings have very limited value. For comparative purposes for yourselves, I think that Bouyoucos blocks do have value. However, they have to be closely calibrated for every particular soil system. A good many people are for more accurate work, and I understand they are getting away from the use of these moisture blocks and are going back to the method of drying samples to obtain percentage moisture. I am sure you understand percentage moisture in itself is quite meaningless unless it can be referred to something, and that is why one has to go to the pF scale or atmospheres, tensions, or whatever you wish to get your curve. This has to be determined, you see, eventually, long before you put this percentage water on the scale. Then results can be compared

from one place to another.

Q. Can you tell us how much you think we may have been wrong in the past fumigating in the manner in which we have? what I mean by that is, most growers do not work the soil when it is too wet or too dry. A lot of times we use the system of picking up soil, clenching it lightly in our hand; if it barely hangs together, we say it is about the moisture level we want for a good fumigation. Very few of us, I think, would go out and fumigate a very wet or a dry soil. Perhaps the moisture range judged in this manner is fairly critical. In other words, I wonder if our data have not been reasonably comparable in spite of the simple way we have judged the soil moisture. Would you care to comment on this?

A. I know so little about fumigation problems that it would be rather hard for me to answer that question, I am sorry to say. I think that these empirical methods of determining field capacity or less than field capacity are perhaps fairly good. I cannot answer the question, because I really do not know how critical moisture is in the fumigation problem.

Q. I think probably what we are getting at is that we must fumigate somewhere near the point where the soil moisture characteristic is such that the soil has lost its excess water and before it is down to very fine water films. I would like to make this comment. For a long time we were puzzled about what to do in regards to moisture content, but, as you know, out in the field you cannot be too particular. You take the problems the way they are encountered. On our sandy soils we have gradually come to the point where we have fumigated enough of them when the soil has been at or above field capacity that we have decided that this actually, in a sandy type soil, is the best time to fumigate. We get our best control under these conditions. As the soil dries, gas losses through the surface reach the extent that they exceed penetration into the soil spaces. I think there are some data to support this published by the Dow Company concerning diffusion of methyl bromide in sandy soils.

Comment: I would like to make a comment about Dr. Cairns' earlier question about compaction. Actually, under field circumstances you should not be really concerned with compaction, because one of the elements of fumigation is that you work the soil up and get it into good seedbed condition. So what we are concerned with may be a layer down deeper that is packed. My experience is that growers usually take care to be rid of that packed zone, because they know they have to. They break it up some way, if possible in their cultural operation. Any measurement of the soil compaction would have to be made after all the cultivating had taken place and the soil was in seedbed condition, which it should be for fumigation.

Q. We have to differentiate between economic control levels and some kinds of theoretical or exact experimental work. If we are going to

record anything for comparative purposes, how many of these things can one get reasonable measures of for reporting? I do not see why only one single factor is enough to report. There is a good answer in our case, for we do not have the people that can do all these determinations for us. That is why I am trying to find out how many of these determinations can and should a person reasonably do.

A. I think something you could do is to determine the percentage moisture, which is a fairly simple task. You could also report the texture classification of the soil, and you might also report bulk density, if this is not too difficult to determine, although it might be in certain instances. Those simple values are probably the minimum. Then you could make some inferences concerning the other factors from the data.

Q. I am interested in Mr. Throne's statement about moisture equivalent; can it be brought into terms of pF?

A. Moisture equivalent is the percentage water in the soil that is held under a certain gravitational force. Theoretically, this is related to pF value which is the centimeters of water tension which will remove a certain quantity of water leaving that particular amount of water in the soil. Thus, the centrifugal force which you exert on the soil sample in order to get moisture equivalent will be equivalent to a certain number of centimeters of tension which is again related to field capacity. It does have a value on the pF scale which is also related to a certain percentage moisture. Now the percentage moisture held in the soil under certain tensions, of course, is much less for sandy soil than for clay soil.

Comment: One thing we should remember on this field capacity thing as a guide for fumigating is this: It is fine for soils that have a field capacity or a moisture equivalent of somewhere below about 15 ordinarily, but do not apply it for a clay soil that has a field capacity of 30. There is no farmer in the world that can take a tractor or horse out on wet soil to work it up or to draw fumigation equipment. In those cases, somewhere in the order of 50-75% of the field capacity is necessary or no one can work the soil and fumigate it.

Q. Is not determination of the soil type as important as the moisture determination because of porosity factor?

A. Of course, structural effects are also things you cannot ignore.

Comment: Effective fumigation is probably dependent both upon porosity and the moisture content in the pores. All of the fumigants that we generally use are soluble in water at about 1 part per 1,000; so the more water present, the more of the gas can be taken out by this water immediately around the point of injection. Also, the water may slow down the movement of the gas, because it has to diffuse through liquid rather than air in the soil spaces.

Q. Would not adsorption be more of a factor in clay than it would in

sand? Also, will the amount of water present not be critical because with more water there may be less loss of the fumigant by adsorption?

A. Adsorption is a function of surface and, of course, in a clay system the amount of surface is tremendously greater than on coarser soil particles. Naturally, gas adsorption would be much, much greater in a clay soil than in a sandy soil. If your problem is, as Dr. Jones pointed out, one of getting the gas in solution, rather than having it adsorbed on the surface, then the presence of moisture is important. You would have to fumigate at a higher moisture content in clay soil than in a sandy soil, as far as percentage moisture is concerned, in order to prevent the adsorption from occurring.

