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9. ABSTRACT

At this conference, the six overseas laboratories (Southeast Asia, South America, Central America, and Mexico, West Africa, East Africa, and Brazil) were represented by a regional investigator who reported on his particular area. Several consultants and scientists from the U.S. reviewed the basic aspects of the genus, such as taxonomy, morphology, host studies, ecology, diseases, and breeding. These reports and technical discussions are reproduced here. These cooperative research efforts should provide a better understanding of the biological behavior of one of the world's major crop pests. Such behavior is influenced not only by the genetic and biochemical make-up of the organism, but by pressures imposed by various environmental influences. The more known about the nematode and these environmental influences on disease development, the more likely conditions can be provided favorable to the host plant and less or unfavorable to the nematode.

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INTERNATIONAL MELOIDOGYNE PROJECT

IMP

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# PROCEEDINGS OF THE RESEARCH PLANNING CONFERENCE

# ON

# ROOT-KNOT NEMATODES, *MELOIDOGYNE*, SPP.

**JANUARY 12-16, 1976**

NOTE: These proceedings represent Phase II of the International *Meloidogyne* project (IMP), entitled "Research on Integrated Crop Protection Systems with Emphasis on the Root-Knot Nematodes (*Meloidogyne* spp.) affecting Economic Food Crops in Developing Nations." This project has been funded by the United States Agency for International Development and is being implemented through a contract (AID/ta-c-1234) with North Carolina State University. The conference was hosted by the Department of Plant Pathology.

**NORTH CAROLINA STATE UNIVERSITY**
**RALEIGH, NORTH CAROLINA 27607**

Scientists participating in the research planning conference of the International Meloidogyne Project (IMP), North Carolina State University, Raleigh, N. C., January 12-16, 1976.

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# I. PREFACE

This report summarizes the proceedings of the research planning conference on root-knot nematodes, *Meloidogyne* spp., held at North Carolina State University, Raleigh, N. C., January 12-16, 1976. This international effort involves, in addition to North Carolina State University, cooperative research with scientists from 40 to 60 foreign countries working through six overseas regional laboratories. Prior to initiating research within the six geographical regions, an attempt was made to review the basic aspects of the genus *Meloidogyne* and to get some idea of the state of knowledge concerning this group of nematodes in each of the regions involved.

The six overseas laboratories are represented by a regional investigator. At this planning conference, each regional investigator reviewed the state of knowledge for his particular region. Several consultants from the United States, as well as scientists associated with the project at North Carolina State University, reviewed the basic aspects of the genus. Considerable discussions were held concerning the overall goals and objectives of the project. These reports and technical discussions which followed have been of immeasurable help in assessing the present situation with reference to what is known about the genus and in determining research priorities. Furthermore, implementation of the project can now go forth with greater confidence having had the benefit of the ideas and encouragement of several authorities in the field. We wish to thank all who participated, and trust that this report includes some of the most essential information concerning this very important group of plant pathogens and that it can serve as a basis for further research, leading to control of these pests in developing countries, thus increasing food production.

## II. OVERVIEW OF THE PROJECT - PRINCIPAL GOALS AND OBJECTIVES AND IMPLEMENTATION SCHEDULE - DR. J. N. SASSER

One of the major obstacles to the production of adequate supplies of food in developing nations is the damage caused by plant-parasitic nematodes, especially the "root-knot group", Meloidogyne spp. The most effective management practice for control of these pests, especially for developing nations, is through the use of certain cultural practices, including crop rotation, the use of available resistant crop cultivars, and development of other resistant cultivars. Crop rotation sequences capable of reducing soil populations of the root-knot nematode below the economic injury threshold can be developed. It will be necessary, however, to test the resistance and/or susceptibility of the economic crop plants grown in the regions covered by this project to the nematode species present. Furthermore, to develop resistant crop cultivars, it will be necessary to discover sources of resistant germ plasm and to determine the extent of pathogenic variation in the nematode. With this knowledge, effective crop rotation programs can be designed and resistant or tolerant cultivars developed on a regional basis. This proposal is to initiate research on the root-knot nematodes at a U. S. university and to link the research with international and national research institutions for the purpose of gaining information on the biology of these pests that will enable developing countries to cope with crop diseases caused by this group of nematodes.

PRINCIPAL GOALS:

1. To increase production of economic food crops in developing nations.

2. To upgrade crop protection capabilities of developing nations.

3. To advance knowledge about one of the world's most important group of plant-parasitic nematodes.

OBJECTIVES:

1. To determine the species and biotypes of the root-knot nematodes present within each of the six geographical regions.

2. To determine the susceptibility and/or resistance of the currently grown basic food crops in each of the regions to the root-knot nematodes which are present.

3. To establish a bank of information on cultivars which display resistance to any or all of the species of root-knot nematodes which have been identified in the six proposed regions of study (Central America, South America, Brazil, West Africa, East Africa and Southeast Asia).

WHY WAS ROOT-KNOT SELECTED:

The root-knot nematodes (Meloidogyne spp.) were selected because of their overall importance throughout the world as limiting factors in food production. Their world-wide distribution, extensive host ranges and involvement with fungi, bacteria and viruses in disease complexes, rank them high on the list of disease agents affecting the world's food supply. Collectively, the various species of root-knot nematodes attack nearly every crop that is grown including both annuals and perennials. Not only are crop yields greatly affected, but quality is also reduced, especially for root crops such as potato, yams, yuca and peanuts.

PROJECT IMPLEMENTATION SCHEDULE

PHASE I: Organization of the "Research Center" at North Carolina State University, Raleigh, N.C., and establishment of Regional Laboratories.

The team of principal researchers, cooperators, and consultants and their respective locations is as follows:

A. North Carolina State University, Raleigh, N. C.
Dr. J. N. Sasser, Nematologist, Department of Plant Pathology, Principal Investigator
Dr. A. C. Triantaphyllou, Nematologist-Geneticist, Dept. of Genetics, Cooperator.
Dr. Hedwig Hirschmann Triantaphyllou, Nematologist-Taxonomist, Dept. of Plant Pathology, Cooperator.
Dr. James G. Baldwin, Nematologist-Morphologist, Dept. of Plant Pathology.
Dr. James L. Starr, Nematologist-Physiologist, Dept. of Plant Pathology.
Dr. L. A. Nelson, Statistician, Dept. of Experimental Statistics, Consultant.

B. Regional Investigators:

Central America, Mexico and the Caribbean (Region I)
Dr. Rodrigo Tarte
Universidade de Panama
Facultad de Agronomia
Panama, R. de Panama

Brazil (Region III) not funded by project
Dr. Romero Marinao de Moura
Universidade Federal Rural de Pernambuco
Escola Superior de Agricultura
Dois Irmaos, Recife, Pernambuco
Brazil

East Africa (Region V)
Dr. Benjamin W. Ngundo
East Africa Agriculture & Forestry Res. Org.
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Nairobi, Kenya

South America (Region II)
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ICA - Tibaitata
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Bogota, Colombia

West Africa (Region IV)
Dr. Fields E. Caveness
International Institute of Tropical Agriculture
Oyo Road, P.M.B. 5320
Ibadan, Nigeria

Southeast Asia (Region VI)
Dr. Cesar P. Madamba
Nematologist
Department of Zoology
University of the Philippines
College, Laguna
Philippines

PHASE II: Conference at Raleigh

The regional investigators have been invited to spend this week with us for the purpose of discussing the overall goals and objectives of the international *Meloidogyne* project. Their reports concerning root-knot nematode research in their regions will be of much help in our deliberations. Also attending the conference, in addition to local faculty participating in the project, are six consultants who have been asked to review certain basic aspects of the genus. We feel certain that from the discussions to follow, much will be gained in helping us to decide on research priorities and emphasis.

PHASE III: Regional Research Planning Conferences

Following this one-week conference in Raleigh, a regional research conference will be held in each of the six regions. Participants from the various countries will first give a summary of the state of knowledge concerning the root-knot nema-

todes in their country. Each country will then outline research they can do in keeping with the objectives and goals of the international project. Special consideration will be given to research methodology relating to the genus Meloidogyne including collecting and maintaining inoculum, inoculation techniques, species identification, methods of evaluating for resistance, and interpretation of research results.

PROPOSED SCHEDULE FOR REGIONAL PLANNING CONFERENCES

| Locations | Dates |
|---|---|
| Southeast Asia | February 16-20, 1976 |
| South America | March 22-26, 1976 |
| Central America | April 26-30, 1976 |
| West Africa | June 7-11, 1976 |
| East Africa | To be determined |
| Brazil | July 26-30, 1976 |

PHASE IV:

Research will begin within each region upon the completion of the planning conference in Raleigh and the regional research conference in the 6 regions. Specific activities will include:

1) Collection of nematode populations.
2) Identification of species and/or variants in the field collections.
3) Differential host tests to detect pathogenic variation.
4) Host susceptibility and/or resistance studies of major food crops in the region.
5) Utilization of crop response information in the development and implementation of effective rotation schemes for control of root-knot nematodes in each region.
6) Collection of information on environmental factors which may influence development of new pathogenic races and/or species.
7) Identification and utilization of resistant germ plasm.

Research at North Carolina State University on the genus Meloidogyne is ongoing, but will be expanded and intensified. Specific activities will include:

1) Detailed morphological and taxonomic studies, including descriptions of new species. Emphasis will be to find new and more reliable characters to support species identification and to distinguish between new pathotypes of the nematode.
2) Differential hosts tests of populations from the United States and areas not covered by the 6 regions to detect pathogenic variation.
3) A detailed study of the variants encountered.
4) Cytogenetic studies involving comparisons of populations with regard to chromosome numbers, mode of reproduction, sexuality, and the genetic basis of their ability to reproduce on resistant crops.
5) Biochemical investigations of general protein patterns and specific enzyme systems.

SUMMARY:

These cooperative research efforts involving a network of scientists, and institutions representing major georgraphical regions of the developing world, should enable us to better understand the biological behavior of one of the world's major crop pests. Such behavior is influenced not only by the genetic and biochemical makeup of the organism, but by pressures imposed by various environmental influences. The more we know about the nematode and these environmental influences on disease development, the more likely we will be able to provide conditions favorable to the host plant and less favorable or unfavorable to the nematode. The thrust of this research proposal is to provide this information.

## III. REPORTS BY REGIONAL INVESTIGATORS ON THE STATE OF KNOWLEDGE CONCERNING ROOT-KNOT NEMATODES IN THEIR REGIONS.

### A. SOUTHEAST ASIA - Dr. Cesar P. Madamba

In Southeast Asia there has been little research on basic problems until recently; most of the research effort has been on applied problems. Even this work has been of a preliminary nature; surveys, species identification, and some work on chemical control.

Southeast Asia is a region of diverse climates, cultures, and agriculture. The 10-12 countries within this region are highly nationalistic. Because of this nationalistic attitude, much of the research that has been done on root-knot nematodes is difficult to find, mostly published in national journals of limited circulation.

There have been only a few surveys, to date, to determine which *Meloidogyne* spp. are present in Southeast Asia. An extensive survey has been conducted in the Philippines and in India (in the early 1960's). Thailand has conducted only limited regional surveys. All major *Meloidogyne* spp. appear to be present in Southeast Asia with *M. incognita* more widespread than *M. javanica*. A few new species have been identified, *M. graminicola* on cereal grains in Thailand, *M. mali* on apples in Japan, and a new species (not named) on citrus in Taiwan.

In the Philippines there are several trained nematologists (5 Ph.D.'s) and most of the major crops have been surveyed. These include rice, sugarcane, banana, coconut, and field legumes. Other crops that have been surveyed to a lesser extent include vegetables, tobacco, corn and ramie. In addition to the surveys, the nematology program in the Philippines includes work on biology, pathogenicity, population dynamics, and some work on control and sex development on different crops.

The IMP objectives and goals fit in very well with the current work being done in the Philippines.

DISCUSSION:

Sasser - Which countries in the region will participate in the program? Should have 8-10 countries, but not completely restricted to developing countries.

Madamba - Eight countries in addition to the Philippines have been invited to participate in the regional workshop, including 12 participants.

Sasser - Should India and Australia be included?

Madamba - No, This would make the region too broad.

Van Gundy - These two countries offer different ecological niches and diversified agriculture. There are also reports of interesting fauna being present in these countries. Workers in South Korea should have a good level of expertise in taxonomy due to the efforts of Dr. Sher.

Fassuliotis - There is a great deal of work being reported from India which should not be ignored.

Van Gundy - Could invite workers from India and Australia to the regional workshop without being formally involved in the program. Possibly could have only one formal participant from each country, others could be involved on an informal basis.

B. SOUTH AMERICA - Dr. Rodolfo Barriga

Dr. Barriga proposed to include all the countries of South America in his regional program, excluding Brazil, and including Trinidad. He discussed the status of root-knot nematode research in several countries where such work is relatively advanced. In Venezuela there have been some international nematology courses which have helped to produce trained people. Research in Venezuela has included studies on control of root-knot of tobacco, tomato, and cotton by using trap crops. In Peru, the University of Lima has contributed to knowledge of root-knot nematodes,

particularly on cotton and tobacco. Under the direction of Dr. Shepherd, some work has been done on root-knot of potato during the past three or four years. In Colombia, considerable progress has been made since 1965, including work on chemical control and genetics, particularly on tobacco. Work has also been done on chemical control of red ring disease, D. dipsaci on garlic, Radophilus similus on banana, and other nematode species. Some research has been conducted on the potato cyst nematode since it was reported in 1961. Root-knot nematodes, including M. incognica, M. javanica, M. arenaria, M. hapla, and M. exigua have been described on about seventeen cultivated crops, at altitudes as high as 2600 meters (M. hapla on ornamentals). The most prevalent species are M. javanica and M. incognita. Meloidogyne exigua and M. javanica cause an estimated $600 million to $800 million damage per year on coffee.

Dr. Barriga emphasized that this project is very important to South America. More knowledge is needed on root-knot in South America, particularly in temperate zones. He suggested that the prospect for supplementing AID funds with funds from other sources, for example the Dutch government, is good.

DISCUSSION

Sasser - What type of workshop should be held in each region? Because of time and monetary restrictions, workshops probably should deal with standardization of techniques required for working with root-knot nematodes rather than elementary nematology training.

Van Gundy - Agreed that elementary training not required. Workshops should include people with some training in nematology and discussion of special techniques required for this program. Also should include discussions concerning the research program for each region.

Dropkin - Should obtain a list of trained nematologists for each region.

Tarte - For the Central America-Mexico region, may have to have some preliminary training in nematology because of the scarcity of trained nematologists within the region.

Mai - Although basics should be covered, the emphasis should be on root-knot nematodes.

Madamba - No known reports of M. hapla in the Philippines, even at higher elevations.

C. CENTRAL AMERICA AND MEXICO - Dr. Rodrigo Tarte

This region will include Mexico, Puerto Rico, Jamaica, El Salvador, Nicaragua, Guatemala, Panama, Costa Rica, Trinidad, Guadeloupe, St. Lucia, and the Dominican Republic. The major crops of this region include tomato, rice, sugarcane, coffee and corn. Other minor crops are cowpea, potato, onion, pineapple, banana, tobacco, citrus, lettuce, cabbage, carrot, soybean, plantain, papaya, pigeon pea, and cassava.

To date, there are approximately 88 publications dealing with Meloidogyne from this region. M. arenaria, M. exigua, M. hapla, M. incognita, M. incognita acrita, and M. javanica are the most prevalent species. In many of these publicationsthe Meloidogyne spp. was not identified, and before 1950-51, the reports referred to root-knot as Heterodera marioni.

In this region, there are several countries that do not have trained nematologists, however other agriculturists may be involved in the project by way of collecting samples for the survey work. In Panama, the current work on Meloidogyne involves screening for resistance to root-knot in tomato in conjunction with a bacterial wilt resistance breeding program. A source of resistance (probably to M. incognita) has been identified. This resistance was derived from Hawaiian sources of resistances.

DISCUSSION

Van Gundy - Should *Hypsoperine* (and other closely related genera) be considered as root-knot in this project?

Golden - Yes.

Sasser - Would it be advisable to combine the South and Central American workshops and hold a joint workshop at CIAT in Colombia?

D. WEST AFRICA - Dr. Fields E. Caveness

This region was surveyed in the early 1960's and 48% of the samples were found to contain *Meloidogyne*. *M. arenaria*, *M. incognita*, *M. incognita acrita* and *M. javanica* are known to occur in Nigeria.

A major portion of the current program at the International Institute for Tropical Agriculture has involved screening cowpea lines for root-knot resistance. Out of the more than 200 cowpea lines examined, there were no cases of immunity, 4 cases of resistance, and 52 lines were moderately resistant. The 4 resistant lines are being tested in the field and appear to reduce root-knot nematode population levels. Other work on *Meloidogyne* includes a project to develop viable yam seeds so as to reduce loss through use of infested seed pieces, and the use of microplots for studies on control of root-knot on African spinach. Root-knot nematodes are a problem on tobacco. Survival of the nematodes through the dry season is an important area that needs research. It was also noted that because several different crops may be harvested from a single plot of land in one year the nematode problems are complex.

DISCUSSION

Mai - Is there evidence of breakdown of resistance to root-knot in your field trials?

Caveness - Yes, breakdown of resistance has been observed in tomato after 8-9 crops.

E. EAST AFRICA - Dr. B. W. Ngundo

The east Africa region includes Kenya, Uganda, and Tanzania. Nematology as a science in this region (Kenya) was started about 20 years ago by Dr. Whitehead (1956-1963). Other workers include Dr. Shoemaker (1964-1967), Dr. Taylor (1971), and Dr. Ngundo (1968-present). In the early surveys by Whitehead, *M. hapla*, *M. incognita*, *M. javanica*, *M. africana*, and *M. coffeicola* were found to be present. Most of the nematology in this region has centered on *Meloidogyne* spp. with some work on nematode problems of sugarcane (other than root-knot), *Aphelenchoides besseyi* on rice, and *Radopholus similis* on banana. Dr. Ngundo has demonstrated damage on beans due to *M. incognita* and has been working on identification of sources of resistance. He has found some lines that are susceptible to heavy galling but show little growth reduction. This may be due in part to removal of water stress in his field plots through application of irrigation water. Other work by Ngundo has shown that *M. hapla* appears to be present only at the higher altitudes and that there appears to be no root-knot populations in Kenya that will attack maize.

It was noted that plant breeders in the region will probably be available for cooperative work on breeding for root-knot resistance in the future.

DISCUSSION

Fassuliotis - The irrigation of your plots may indeed be affecting the noted growth responses. Even heavily galled plants will produce good yields if there is sufficient fertilizer and moisture. The importance of preplant population densities on yield responses needs to be stressed and should be included in regional programs if possible.

Van Gundy - What is the acreage devoted to beans in Kenya?

Ngundo - Most beans grown are for commercial processing--however, there are a large number of individuals with low acreage of beans for personal consumption. In this program, most effort should be directed toward the latter.

F. BRAZIL - Dr. Romero M. de Moura

Dr. de Moura summarized the history of nematology in Brazil. In the 1940's - 1950's, several zoologists did general studies of root-knot nematodes. In 1950, Dr. Steiner conducted a survey of nematodes on coffee and taught a nematology course in Brazil. The first post-graduate program in nematology was initiated in 1965. Presently there are about fifteen nematologists distributed throughout Brazil. However, a good bibliography of research on *Meloidogyne* spp. in Brazil is not yet available. Numerous root-knot nematode species are present: *M. exigua*, *M. coffeicola*, *M. javanica*, *M. incognita*, *M. hapla* (in southern Brazil), *M. inornata*, *M. brevicauda*. Root-knot nematodes occur on most important crops, including sugarcane.

There is now a federal organization for work on sugarcane which includes nematodes in its research program. Some chemical control and identification studies have been done on *Meloidogyne* spp.

DISCUSSION

Barker - Notes that root-knot nematodes are a major problem on soybeans in Brazil.

Sasser - In most of the regions the state of knowledge appears to be about the same. Also, it is apparent that *M. javanica* and *M. incognita* are present in all regions, and are generally the most important species. The reason for this association is not known, may be due to common development or dissemination by man.

## IV. A REVIEW OF THE BASIC ASPECTS OF THE GENUS MELOIDOGYNE.

### A. MELOIDOGYNE TAXONOMY - CURRENT STATUS, SOME PROBLEMS, AND NEEDS - Dr. A. Morgan Golden

As we all are aware, root-knot nematodes have been recognized and recorded by man for only about 120 years. Though they have no doubt been around for millions of years, our first record of their existence was made by Berkeley in 1855. He reported "cysts" and "vibrios" on cucumber roots from a greenhouse in England. Within the next 30 years or so, Cornu (1879) described Anguillula marioni in France, Goeldi (1887) established Meloidogyne exigua on coffee in Brazil, Treub (1885) named Heterodera javanica from sugar cane in Java, and Neal (1889) described Anguillula arenaria from peanut in Florida. Thus, root knot was beginning to be recognized in different parts of the world though referred to by different names. Soon however, the name Heterodera marioni became widely accepted by most workers for root-knot nematodes although Cobb in 1924 had proposed Caconema radicicola to differentiate root knot from cyst forms in Heterodera (genus established by Schmidt in 1871 for sugar beet nematode). Unfortunately, the widespread use of H. marioni or some other binomial to designate root knot apparently fostered the erroneous idea that only one species was involved. As a result the term, the root-knot nematode, became prevalent, but I hope the idea of just a single root-knot species has been permanently laid to rest by now.

In 1949 Chitwood re-established our present genus Meloidogyne and at the same time described five species and one variety. He based these actions on the excellent host relations research of Christie in the mid 1940's. Later, in 1952, Chitwood established M. thamesi as a new subspecies and this was followed in 1953 with a description of M. brevicauda by Clive Loos. So in 1953 we had six species and two subspecies instead of just "the root-knot nematode" as we had thought for almost 100 years.

During the 20 years or so since 1953, there has been a massive increase in the number of Meloidogyne species. They have been described from various hosts in many parts of the world, ranging through Europe, the United States, Africa, South America,

Russia, India, and Japan. In his review of Meloidogyne in 1968, Whitehead described four new species and listed a total of 23 species which he considered valid. However, these included certain new combinations and synonomies, some of which have not been accepted by all workers. Since then several additional new species have been named, bringing the present total to well above 30 species. In the latest compilation of root knot by Esser, Perry and Taylor (1976) a total of 35 species is listed. This includes acrita and bauruensis as full species but both of these were not recognized by Whitehead though already described at the time. (List of these 35 are attached plus another new species, M. microtyla just described by Mulvey, November, 1975.)

With such relatively large number of species already on hand, how do we go about identifying them? What characters can we draw upon, hopefully with reasonable reliability? (In the good old days, prior to 1949, identification was no problem - if the nematodes made knots on the roots, it was the root knot nematode, H. marioni; if it formed a cyst, it was H. schachtii or essentially some variety of it.)

FEMALES

1. Perineal pattern - good for overall grouping - O.K. for identity of certain species locally but not conclusive alone worldwide.
   A. Presence or absence of lateral field and its nature (javanica and africana).
   B. Shape of total pattern - rectangular as in brevicauda, circular and large (coffeicola) with tail whorl, or just circular (ovalis).
   C. Presence or absence of punctation or stippling (good for some pop. of hapla).
   D. Presence, absence, and nature of dorsal arch (incognita versus hapla) and arenaria with "shoulders".
   E. Nature of striae - Faint, as in acronea; continuous or wavy as in incognita vs broken and straight to forked in acrita; or arranged in distinctive manner as in thamesi -- or dog-eared as in graminicola.
2. Stylet and DGO - Length useful for certain species, hapla vs graminicola et al.
3. Excretory pore - Location useful for certain species (at or near base of stylet

in incognita, acrita, naasi, graminicola et al but far back on neck in hapla, arenaria, javanica et al).

LARVAE

Tail length and total length of larvae are very worthwhile characters.

1. Tail length - very useful, especially to get into groups.

   Very short - (17-23) - artiellia, brevicauda, indica)

   Short tail - (about 30) - inornata, coffeicola, kikuyensis.

   Intermediate - (42-53) - arenaria, javanica, incognita, acrita, hapla, acronea et al)

   Long - (70) - graminis, naasi, graminicola.

   Longer - (100) - spartinae.

2. Tail tip - useful in certain species (mucron, bifid, clavate)

3. Total length - helpful, especially with tail lengths. hapla larvae over 400, incognita group much less (about 375).

4. Inflated (or dilated) rectum - of limited use - may be helpful with certain specie but needs further evaluation with all species.

MALES

1. Stylet length - perhaps most reliable male character. Useful for a number of species, such as hapla and graminicola (about 17) vs. incognita, arenaria, et al well above 20.

2. Head annules - Somewhat variable in exact numbers, but useful as supplemental character in several species.

3. Lateral field - number of lines useful, as decalineata (10), africana and litoralis (5), some others 4-6 as ovalis, et al with 4-5. Also, areolation might be useful in comparing two individual species.

The above are the most important and most widely used characters for Meloidogyne species identification today. Some other characters are used to a lesser extent in

certain species, such as location of hemizonid vs. excretory pore in M. graminis larvae, size of female phasmids (naasi). In general there is, unfortunately, no one particular character that can be used alone for accurate species identification on all specimens from worldwide sources. Thus, identification of a species involves a combination of characters and with their weight and value varying with different species.

SOME MELOIDOGYNE PROBLEMS

Easiest part in preparation of this talk - there are all kinds of problems in most all directions.

1. Present species complexes and races. Widespread occurrence of certain forms, soil - inhabitating nature, little natural movement, et al, all contribute to development of geographic populations which in time might differ in morphology (subspp.), physiology and host response (races).

   A. incognita group

   Most widespread and perhaps most important overall of any of the Meloidogyne forms. Not a homogeneous species. Instead, apparently contains four recognizable subspecies plus one or more races (NC 95 tobacco).

SLIDES (on races or subspecies in incognita group)

1. check - Iris speculatrix - almost 3 years growth
2. acrita - no damage - no attack
3. incognita - severe damage - perineal pattern same after 3 years
4. incognita pattern - fine, distinct, wavy lines continuous - attacks azalea but not Christmas pepper.
5. acrita pattern - coarse, discontinuous lines - attacks Christmas pepper but not azalea - most common form.
6. new form in incognita group - fine, distinct, wavy lines - from Hong Kong in 1967 and 1968 on I. speculatrix - obtained plants and grew in greenhouse - incognita attacked it but in 3 years pattern did not change. Acrita - no attack.

7. Another specimen from Hong Kong.

8. Another specimen from Hong Kong

These Hong Kong specimens make a third form in the incognita group but is known only from that area.

Now lets take a look at a fourth form in this group. This is the Wartelle race of incognita that is a severe probelm on soybeans in certain areas of Louisiana. It attacks the varieties having resistance to other root knot, with only Bragg being resistant to this form. Recently, I have studied the various stages of the Wartelle race and feel there are differentiating characters present.

9. Wartelle female - excretory pore - far back

10. incognita female - excretory pore at or near base of stylet (Salisbury, MD)

11. acrita female - excretory pore at or near base of stylet (Beltsville)

12. Wartelle male - no distinct annules

13. acrita male - distinct annules (3 usually)

I am planning to set this form aside as a new subspecies of incognita. The important point here is not whether this Wartelle form is called a race, subspecies, or whatever, but that we have characters that we think will identify it, thus pinpointing its distribution, etc. There are other species complexes which should be discussed here also.

B. javanica group (includes bauruensis)

javanica is very widespread and when studied in greater detail, might be found far less homogeneous than we now think. Peanut race in Georgia - Also, the closely related bauruensis must be examined critically for its relationship and sound differentiating characters.

C. hapla group -

I am not convinced that hapla is a simple, homogeneous species in all areas of its occurrence.

- patterns of populations with: stippling, no stippling, circular, or wing-type.
- also, Chitwood's measurements seem accurate - but Whitehead's larvae are too short (337 vs 425); stylet of larvae and females also too short (9 and 11 vs 10 and 12-14); but his male stylet too long (20 vs 17-18). Did Whitehead have "true" hapla? Will all these populations respond the same on same host at all times, especially resistant varieties?

D. ethiopica - why does this form have patterns typical for both arenaria and incognita? Was there a mixture in the initial population as described? Was there crossing of the above two species, giving rise to ethiopica as a fertile hybrid? (Am looking into this to some extent but the matter deserves thorough, in-depth investigation. Apparently in Turkey also.)

2. Undescribed species.

Several new species are known to me now and others are being found, and will continue so. Must be alert to any new forms; these in turn must be accurately identified and described in order to use and maximize biological and other experimental data. ("acrita" on rice - "rostoc" in Yugoslavia).

3. Species variation

"Normal" inherent morphological and physiological variation within the species - how much variation before considering a new species? One must know and rely on the valid, specific characters for the particular taxon and not be misled by insig-

nificant and highly variable characters (as a, b, c and total body length in many cases). Also, one must be aware of and evaluate changes or variation resulting from selection pressures, such as host, environment, etc. These may bring about so-called races, or over a long time, perhaps development of new subspecies. These are some of the problems we face in taxonomy of Meloidogyne.

## SOME NEEDS IN MELOIDOGYNE TAXONOMY

1. A basic need is for a reasonably quick but fully reliable and accurate means of identifying species, races, and other populations which might react differently on a given host in different areas. In discussing some of our problems above, I have at the same time pinpointed some of our other needs also.
2. Resolve and clarify the various species complexes and races. Otherwise, the development and use of resistant crop varieties might well be jeopardized or completely nullified.
3. New species as they are discovered should be thoroughly and accurately described so that their identity can be quickly determined wherever they are found in the future. Similarly identifying described forms also will develop excellent information on hosts, occurence, and distribution of all these forms. Concurrent with these activities and resulting in part from them would be the development of data providing a better understanding of the kind, amount, and nature of variation occurring in and between species.
4. As a result of and as a part of these activities, the numbers and kinds of root knot forms which actually exists, and their true relationships, would be revealed to a much greater extent than we now know. Also, this in turn would tend to advance and clarify the classification of this important group of world-wide pests and place present and future biological and experimental research on a firmer basis.

How to accomplish these needs? Obviously this is a huge task requiring the latest and most modern techniques and equipment, highly trained personnel, and the cooperation of colleagues on a world wide basis. The International Meloidogyne Project, as it has been conceived and initiated here, is a major step toward bringing about solutions to our needs in identifying root-knot nematodes. I am pleased to be associated with this excellent undertaking.

DISCUSSION:

Golden - Reasonably quick methods for identifying different species are needed. Accurate species descriptions and developmental studies are also important areas for further work.

Van Gundy - It will be necessary to have a check list of characters to use as a screening device for selecting odd populations for further study. Much of this screening could probably be done in the regional centers before sending the populations to Raleigh. Should standard techniques and procedures be used for IMP? This would include preparation of samples for identification and the number of samples examined.

Dropkin - Will two species (described by different individuals) from separate geographic areas have very similar behavior? If so, should attempts be made to closely identify the different populations?

Sasser - Most evidence suggests that these widely separated species do have very similar behaviors, but there are exceptions.

LIST OF MELOIDOGYNE SPECIES (From Esser, Perry and Taylor - to be published in 1976.

Species

1. acrita - Chitwood, 1949
2. acronea - Coetzee, 1956
3. africana - Whitehead, 1960
4. ardenensis - De A. Santos, 1968
5. arenaria - (Neal, 1889) Chitwood, 1949
6. artiellia - Franklin, 1961
7. bauruensis - Lordello, 1956
8. brevicauda - Loos, 1953
9. coffeicola - Lordello & Zamith, 1961
10. decalineata - Whitehead, 1968
11. deconincki - Elmiligy, 1968
12. ethiopica - Whitehead, 1968
13. exigua - Goeldi, 1887
14. graminicola - Golden & Birchfield, 1965
15. graminis-(Sledge & Golden, 1964) Whitehead, 1968
16. hapla - Chitwood, 1949
17. incognita - (Kofoid & White, 1919) Chitwood, 1949
18. indica - Whitehead, 1968
19. inornata - Lordello, 1956
20. javanica - (Treub, 1885) Chitwood, 1949
21. kikuyensis - De Grisse, 1960
22. kirjanovae - Terenteva, 1965
23. litoralis - Elmiligy, 1968
24. lordelloi - Da Ponte, 1969
25. lucknowica - Singh, 1969
26. mali - Itoh, Ohshima & Ichinohe, 1969
27. megadora - Whitehead, 1968
28. megriensis - (Pogosyan, 1971), Esser, Perry & Taylor, 1976
29. naasi - Franklin, 1965
30. oteifae - Elmiligy, 1968
31. ottersoni - (Thorne, 1969) Triantaphyllou, 1973
32. ovalis - Riffle, 1963
33. spartinae - (Rau & Esuliotis, 1965) Whitehead, 1968
34. tadshikistanica - Kirjanova & Ivanova 1965
35. thamesi - Chitwood in Chitwood, Specht & Havis, 1952
36. microtyla - Mulvey, 1975 (described in Nov., 1975)

B. UTILITY OF PERINEAL PATTERN MORPHOLOGY IN IDENTIFICATION OF MELOIDOGYNE SPECIES - Dr. J. N. Sasser

Females of Meloidogyne spp. are currently of greater value than males or larvae for species identification, and the most characteristic feature is the perineal or posterior cuticular pattern first observed by Chitwood (1). In the region of the tail terminus, the cuticle is ridged and furrowed in a fingerprint-like pattern. This area comprises terminus, phasmids, lateral fields, anus, and vulva which is surrounded by cuticular folds or striae making a pattern characteristic for some species. The patterns of individuals and populations within species often vary but there are basic species characteristics underlying the variations. Such characteristics include the overall shape of the pattern which may be circular, oval (with the long axis either parallel or perpendicular to the vulval slit), pear-shaped, or hourglass-shaped; the relative positions of the vulva, anus, tail terminus, and phasmids; the presence or absence of markings in the lateral field area and of punctations on the terminus; the form of the striae (smooth, wavy, or zigzag). Whitehead (4) studied the possibility of using measurements of certain features of the pattern for characterizing species. In the limited number of species he studied there were differences in vulval width, distance between phasmids, between anus and tail terminus, and between anus and vulva. These characteristics could be useful for species identification, but the extent of their variability in a number of populations of a species must be known before they can be used with confidence. Dropkin (2) studied variability in the patterns of two species and found that although the general characteristics of the pattern were inherited, the coefficients of variability of pattern shape within two lines derived from single larvae were about 20%, and in one M. arenaria population, 40%. The progeny of a single female M. incognita may vary greatly, but special characteristics of an isolate are often inherited (3). There have been no other intensive morphometric studies on several populations of a single species, but figures given by Whitehead (4) show that much variation can exist between populations of the same species.

Even though considerable variability has been reported, it seems worthwhile to further test and hopefully improve the utility of perineal pattern morphology in identification of Meloidogyne spp. The method is rapid and, in spite of variation, a high percentage of the populations encountered can be reliably identified to species by this method. There is little question that additional studies of the range of "normal" variation should be conducted. Also, any specific markers in the perineal pattern morphology, which are repeated from generation to generation should be identified. Such studies might render this method of identification more reliable or it might indicate that this character is of limited value. In the latter case, we will have to rely on other characters or a combination of characters. In considering alternative approaches to identification, one would have to conclude that considerable effort should be directed toward refinement and better utilization of the perineal pattern method. We are particularly interested in this method here at North Carolina State University, where efforts are underway to study this genus of nematodes on an international basis. We will have access to many populations from widely separated geographical regions and from diverse habitats and hosts. While we have already had considerable experience with populations from other parts of the world, in the future more emphasis will be in identification of a large number of species from other states in the United States and from many foreign countries. Our university is frequently called upon to make identifications of materials sent from other states and other countries and requests for this service will undoubtedly increase over the next decade. Progress in improving the reliability of identification through observations of perineal pattern morphology will be of great benefit to us as well as all others engaged in the control of this group of pests.

Although considerable progress may be made in improving the reliability of the perineal pattern morphology as a basis of species identification, I feel that this character should be one of several used in making final identification. Other

criteria of equal or perhaps even more importance in identification would include additional morphological measurements, cytological make-up, ability to infect and reproduce on selected differential hosts and, in some instances, biochemical analysis and data from serological studies. In other words, through a multi-pronged approach to the morphological and behavioral characteristics of a population, we should be able to more accurately identify the organisms in question. This will be our goal in the international program.

LITERATURE CITED

1. Chitwood, B. G. 1949. Root-knot nematodes - Part I. A revision of the genus Meloidogyne Goeldi, 1887. Proc. Helminth. Soc. Wash. 16:90-104.

2. Dropkin, V. H. 1953. Studies on the variability of the anal plate patterns in pure lines of Meloidogyne spp., the root-knot nematode. Proc. Helminth. Soc. Wash. 20:32-39.

3. Triantaphyllou, A. C. and J. N. Sasser. 1960. Variation in perineal patterns and host specificity of Meloidogyne incognita. Phytopathology 50:724-735.

4. Whitehead, A. G. 1968. Taxonomy of Meloidogyne (Nematodea: Heteroderidae) with descriptions of four new species. Trans Zool. Soc. Lond. 31:263-401.

DISCUSSION

Tarte - What are the morphological distinctions between M. incognita incognita and M. incognita acrita?

Sasser - The wisdom of this separation is doubtful since a given female can produce progeny of both pattern types.

A. C. Triantaphyllou - 95% of the M. incognita populations in the U.S.A. are of the acrita type.

Tarte - Does the host determine whether a pattern is of the M. incognita acrita type or of the M. incognita incognita?

Sasser - Investigations, thus far, seem to indicate that host has little influence on perineal patterns.

## C. MORPHOLOGY OF MELOIDOGYNE SPP. - SUMMARY AND PROSPECTS FOR FURTHER INVESTIGATION. - Dr. J. G. Baldwin

The justification for research on the morphology of root-knot nematodes is apparent. Chemical treatment for root-knot control is often not economical; thus practices such as use of resistant cultivars and crop rotation must be employed. Many resistant cultivars have resistance to only one or a few species of root-knot nematodes, and their value for control is limited to where these species are dominant. Crop rotation, for example with corn, would be effective in fields infested with M. hapla, but would have little effect on other species of root-knot nematodes. Thus, successful crop rotation and use of resistant cultivars is dependent on a knowledge of the species present. It follows that rapid and reliable methods for identification of Meloidogyne species will enhance the ability to control these pests.

We have been reminded that there are many approaches to root-knot nematode identification including cytology, host differentials, and biochemistry; every available method must be used. However, routine identification based on morphology is relatively quick, and it is a method which is accessible to modestly equipped laboratories.

What can be done to improve our method of identification based on morphology? Dr. Sasser has described the important role of the perineal pattern. However, this character is variable within species, and some of its features overlap among the large number of species. The value of the perineal pattern in identification would be greatly enhanced if we could identify sources of variation and eliminate them where possible. Triantaphyllou and Sasser (1960) found no evidence, in M. incognita, that this variation was affected by host species, or that a given isolate became less variable with successive generations. However, the perineal pattern was affected by age of the female, and it was suggested that variation might be attributed to the rate of development of the nematode, its

position in the host tissue, and other factors. Investigations are needed to determine how the perineal pattern develops, and particularly at what point in development it is most species specific.

In addition, current identification utilizing perineal patterns are often qualitative and subjective. Further attempts should be made to more objectively describe the pattern using quantitative measurements. Some quantitative measurments from the perineal region have been used, including interphasmidial distance, vulval width, anus to vulva and anus to tail terminus distance, and ratios which tend to describe the height of the arch. These characters are of some value in distinguishing among certain species. However, Franklin (1972) has indicated that the value of such measurements in perineal patterns is limited because of variation in size of females of a given species. Thus, ratios may be most useful in quantitative descriptions of perineal patterns. Whitehead compared several measurements from the anterior region of females and found length of stylet to be the most valuable character.

Males of root-knot nematodes are of limited value in morphology studies because they are extremely variable, and under some conditions dwarf males are produced. However, stylet length, form of knobs, position of cephalids, position of phasmids, and form of spicules is of some value. Spicules are likely to be variable among root-knot nematode species, but their detail is particularly difficult to see. These structures should be examined more carefully, possibly by developing a method for excising them. Such excised structures could be more accurately examined and studied by light and scanning electron microscopy for comparison, among Meloidogyne spp.

Second stage larvae may be found to be particularly valuable in the taxonomy of Meloidogyne spp. Total length, tail length, and tail form are currently the most valuable characters, although Franklin (1972) has shown these to overlap considerably among species. Whitehead has recommended further research on the anterior ends

because "... measurement differences in this region have been found between the larvae of nearly all the species studied." Whitehead suggests that identification might be possible with second stage larvae alone. Research which would facilitate distinguishing among common species by second stage larval characteristics would be particularly valuable to nematode assay programs. Soil assays may include only second stage larvae, and yet it is often essential to make precise crop rotation or resistant variety recommendations on the basis of such assays.

A major objective for morphological studies with this project, is to identify (genetically based) quantitative and qualitative characters in females, males and larvae which are most reliable for identification of Meloidogyne spp. Furthermore, additional effort will be made to correlate variation in chromosome number and mode of reproduction with morphology. Correlations might also be possible between intraspecific variation in physiological response to differential hosts, and morphology. A second objective is to facilitate identification of Meloidogyne spp. through discovering, and perhaps learning to regulate, sources of nongenetic intraspecific variation in the perineal pattern. It is our primary concern that these studies not only expand our basic knowledge of Meloidogyne spp., but that they elucidate practical methods for rapid, accurate species identification.

DISCUSSION:

Dropkin - Might not behavioral studies be used to identify different Meloidogyne spp., i.e. sensory responses?

A. C. Triantaphyllou - Would identify only single gene effects, often brought about by mutagenic treatments.

Dropkin - Physiological differences are as important as morphological differences in species identification.

Mai - Environmental influences can result in morphological variation.

A. C. Triantaphyllou - What about numerical taxonomy?

Fassuliotis - Numerical taxonomy, use of curves to evaluate correlations, and utilization of the computer may be valuable in this project.

Caveness - Can artifacts of nematode death be characterized? They may be species specific.

Sasser - In all work to characterize the different populations what will be the initial working material? It is important to determine whether field samples or populations derived from a single egg mass will be used.

## D. ULTRASTRUCTURE OF MELOIDOGYNE AND ITS POSSIBLE VALUE IN SPECIES IDENTIFICATION - Dr. H. HirschmannTriantaphyllou

We have learned from the earlier discussions that the light microscope should play the primary role in the search for stable morphological characters in Meloidogyne species. However, some structures that might be helpful in identification may be so small as to be at the limit of the resolution of the light microscope. Therefore, the higher resolution of the electron microscope may be necessary to find differences or similarities in structure. Although the basic fine-structural make up of organs is expected to be similar in species of the same genus, any functional changes may also be expressed in structural changes. Thus, differences may exist on the fine-structural level that we are not able to detect with the light microscope. Furthermore, fine-structural studies may also "sharpen" our eyes and clarify basic organization, so that we will be able in turn to see more details with the light microscope after having studied the ultrastructure.

In recent years, there have been numerous publications on the fine structure of plant-parasitic nematodes. Most of these papers are of a purely descriptive nature and do not compare organs or structures in members of the same genus, except for some studies with the genus Heterodera. Only a few among these investigations deal with species of the genus Meloidogyne. These studies include transmission as well as scanning electron microscopy.

The scanning electron microscope bridges the range of magnification between the light microscope and the transmission electron microscope and reveals details of surface structure combined with great depth of focus. It is a useful tool in a study of the nematode exoskeleton. The scanning electron microscope has been successfully employed in comparative morphological studies of various species of Heterodera by several investigators. We believe similar investigations can be carried out with the genus Meloidogyne. Since the scanning electron microscope

concerns surface morphology, a study of the perineal pattern of the female Meloidogyne comes to mind immediately. We have initiated such a study with a few populations of different species of Meloidogyne and found that the scanning electron microscope reveals features of the perineal pattern, such as details of the anal and vulval areas and the type of striation, that are not observed in the light microscope. Furthermore, the cuticular annules or folds of the pattern appear very pronounced and differences in size and depth of these cuticular folds appear to vary among species. The surface morphology around vulva, anus and tail tip exhibits more details than in the light microscope. Any existing vulval depression or protuberance is more obvious, and the surface expression of the phasmids can be clarified. Measurements are more easily made due to higher magnification. However, we must consider, when comparing the same perineal pattern with light and scanning electron microscope, that the light microscope reveals more than surface details, whereas the scanning electron microscope is confined to surface structures. Therefore, when using the scanning electron microscope, new criteria may have to be developed for the description of the perineal pattern. The development of the perineal pattern during postembryogenesis, as proposed earlier by Dr. Baldwin, seems to be easier to study with the scanning electron microscope due to the higher magnification available.

Other surface structures that might be of taxonomic significance are present in the anterior region of females, larvae and males. Face views of these various life stages have been examined with the scanning electron microscope in other nematode genera and have been found particularly helpful in the classification of the species of the genus Heterodera. In the latter genus, the head morphology of the second-stage larvae could be used exclusively to support and complement our present classification of species within this genus. The second-stage larvae of Meloidogyne are much smaller than those of Heterodera. Head structures are at the limit of light microscope resolution and the larvae appear to deteriorate easily under the

electron beam. However, after the development of proper techniques the scanning electron microscope should give us more information about surface structures. This would include shape of stoma opening and oral disc, shape and position of amphidial openings, position of labial papillae, number of head annules, etc., all characters that may differ among the species. The same could apply to female and male face views. In the posterior region of the nematode, the external manifestation of phasmids in larvae and males might be helpful in species differentiation. In the male, the structure of the lateral field could be observed with more clarity in the scanning electron microscope, and as Dr. Baldwin mentioned, the spicule shape may differ among species. The spicule tips which could be examined protruded in whole males may differ in structure as has been found e.g. in males of Heterodera vs. those of Globodera. To examine their entire morphology, the spicules would have to be isolated for viewing under the scanning electron microscope and special methods will have to be developed to dissect them.

With respect to transmission electron microscopy, the majority of the studies in plant-parasitic nematodes deal with the basic structures of certain organs of different genera. Comparative fine-structural investigations of species within a genus are very limited. Only species of Heterodera have been studied comparatively with respect to the fine structure of various organs. Some differences have been found e.g. in cuticle structure between species with lemon-shaped and those with round cysts which supports our existing placement of the round-cyst species into the separate genus Globodera. In addition, within the lemon-shaped cyst nematodes, the species can be distinguished by certain ultrastructural features of their cuticle. Little use has been made of transmission electron microscope for discovering characters of taxonomic value in Meloidogyne species. However, some structures that we have recently described by transmission electron microscopy in M. incognita males may be variable among species. These include the cephalic sensory organs, such as the nature of amphids and papillae, the stomatal region, esophageal glands

and presence and extent of intestinal caeca. Other structures that might prove helpful in species identification are the number of layers of the cuticle and their thickness in larvae, females and males, the shape of the head skeleton and the internal structure of phasmids, as well as the fine structure of the male sperm.

E. HOST RANGE, DIFFERENTIAL HOST STUDIES, AND PATHOGENIC VARIATION - Dr. J. N. Sasser

The first evidence that root-knot nematodes differ in their host preference was probably that presented by Sherbakoff (1939). He reported considerable root-knot injury to cotton, Gossypium hirsutum, grown on land previously planted with cotton but observed no injury to cotton grown on land previously planted with tomatoes, Lycopersicum esculentum, even though the tomatoes had been severely injured by root-knot. At that time, all root-knot nematodes were taxonomically classified as Heterodera marioni (Cornu) T. Goodey. Christie and Albin (1944) and Christie (1946) established experimentally that populations of H. marioni differed in their host-parasite relationships. Among 14 populations studied, these investigators demonstrated that host specialization may be shown by: 1) distinct host preference, or 2) the character of root galling on susceptible hosts. Chitwood (1949), after a morphological study, removed the root-knot nematodes from the genus Heterodera and reassigned them to Meloidogyne. Although emphasis was on morphology, Chitwood made important observations with reference to host differences. For example, he observed that M. hapla Chitwood was the only root-knot nematode known to reproduce on strawberry and that M. incognita (Kofoid and White) Chitwood failed to infest peanut but reproduced readily on cotton.

Sasser (1954) using single species cultures identified according to Chitwood's morphological characters, studied the suitability of 50 plant species and varieties as hosts for the known root-knot nematode species at that time, except for M. exigua Goeldi. These investigations showed that some species were more host specific than others and that for each species there were certain crops which were non-hosts. These differences made it possible to devise a scheme of identification based on the response of certain plant species when grown in soil infested with a single root-knot nematode species. This method, which employed the use of differential hosts, proved useful in identifying the common species known

to occur in a given geographical region. This method of identification was found to be less reliable however, when populations of the various species were obtained from widely separated geographical regions (Sasser, 1966).

Some of the early evidence of variation within a species was that presented by Martin (1954), who found differences in parasitism on cotton among isolates of M. incognita, ranging from non-parasitism to severe parasitism. Other investigators (Allen, 1952; Linde, 1956; Sasser and Nusbaum, 1955; Powell, 1957; Goplen, et al, 1959) found that within a species, certain populations react differently on a given host. These have been referred to as races, biotypes or pathotypes. To date, such within-species variation has been shown for M. incognita, M. javanica (Treub) Chitwood, M. hapla and M. arenaria (Neal Chitwood).

Because of this pathogenic variation within and among species, it became evident that progress in understanding this genus would be more rapid if populations could be studied from widely separated geographical regions. Thus, a worldwide collection of Meloidogyne species and populations was begun at North Carolina State University about 15 years ago and at present we have more than 400 populations representing about half of the known species. A determination of the extent of pathogenic variation within and among species of M. incognita, M. javanica, M. hapla, M. arenaria and to a limited extent, M. exigua, has been a major objective.

To study such variation it was necessary to pick a set of "differential hosts" for testing against the various species and populations within a species. Host-range studies conducted by Sasser (1954) revealed that there were one or more crop plants which were not attacked by some root-knot nematode species and that the non-hosts varied with the nematode species. Thus, a set of differential hosts was available for use in separating the species, based on host reaction. This method has been used somewhat successfully in distinguishing between species of M. incognita, M. javanica, M. arenaria, and M. hapla. As other species are included it will undoubtedly be necessary to add additional differential hosts. This method is based on the assumption that species and populations within a species will always react

on given hosts according to previous tests. When reactions are different, this constitutes evidence of pathogenic variation within the species being tested. The selected crop plants used and their usual response to differential hosts are shown in Table 1.

When populations of a given species are collected from another part of the world, different host responses may occur (Sasser, 1972). For example, populations of *M. incognita* from Peru and Colombia readily attack the tobacco variety NC-95 which is practically immune to the *M. incognita* populations tested from various parts of the United States. Some populations of *M. incognita* from Peru, however, as well as populations from Taiwan, Greece, Nigeria, Belgium, and the Ivory Coast, do not infect the resistant NC-95 tobacco. Furthermore, all populations of *M. incognita*, regardless of source, failed to infect peanut and strawberry. Populations of *M. javanica* used in similar studies failed to infect peanut, pepper, strawberry and Puerto Rico sweet potato. More recently, Martin (1958) and Minton *et al.* (1969) report peanut as a host for *M. javanica*. Populations of *M. hapla* failed to infect corn, cotton and watermelon and all populations of *M. arenaria* failed to infect cotton, strawberry, and Puerto Rico sweet potato. Thus, while there is some variation in most species, there is also considerable uniformity in host response. Populations of *M. arenaria* vary in their ability to infect and reproduce on peanut, yet this nematode is commonly known as the peanut root-knot nematode. In fact, about half of the populations tested thus far reproduce and cause severe galling on peanut; others do not. Aside from these two exceptions, many of the populations of the four species worked with thus far react in a "normal" manner; that is, according to the results shown earlier in Table 1.

LITERATURE CITED

Allen, M. W. 1952. Observation on the genus Meloidogyne Goeldi 1887. Proc. Helm. Soc. Wash. 19:44-51.

Chitwood, B. G. 1949. Root-knot nematodes -- Part 1. A revision of the genus Meloidogyne Goeldi 1887. Proc. Helm. Soc. Wash. 16:90-104.

Christie, J. R. 1946. Host-parasite relationships of the root-knot nematode, Heterodera marioni. II. Some effects of the host. Phytopathol. 36:340-352.

Christie, J. R. and F. E. Albin. 1944. Host parasite relationships of the root-knot nematode, Heterodera marioni. I. The question of races. Proc. Helm. Soc. Wash. 11:31-37.

Goplen, B. P., et al. 1959. Demonstration of physiological races within three root-knot nematode species attacking alfalfa. Phytopathol. 49:653-656.

Linde, W. J. van der. 1956. The Meloidogyne problem in South Africa. Nematologica 1:177-183.

Martin, G. C. 1958. Root-knot nematodes (Meloidogyne spp.) in the Federation of Rhodesia and Nyasaland. Nematologica 3:332-349.

Martin, W. J. 1954. Parasitic races of Meloidogyne incognita and M. incognita var. acrita. Plant Disease Reptr. Suppl. 227-86-88.

Minton, N. A., et al. 1969. Meloidogyne javanica attacks peanuts in Georgia. Plant Disease Reptr. 53:668.

Powell, W. M. 1957. Variation in host-parasite relationships of the root-knot nematode, Meloidogyne hapla Chitwood. Unpublished Masters Thesis, N. C. State College, Raleigh.

Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne spp.) Univ. of Maryland Agr. Exp. Sta. Tech. Bull. A-77, 30 p.

Sasser, J. N. 1966. Behavior of Meloidogyne spp. from various geographical locations on ten host differentials. (Abstr.) Nematologica 12:97-98.

Sasser, J. N. 1972. Physiological variation in the genus Meloidogyne as determined by differential hosts. EPPO Bull. 6:41-48.

Sasser, J. N. and C. J. Nusbaum. 1955. Season fluctuations and host specificity of root-knot nematode populations in two-year tobacco rotation plots. Phytopathol. 45:540-545.

SHERBAKOFF, C. D. 1939. Root-knot nematodes on cotton and tomatoes in Tennessee. (Abstr.) Phytopathol. 29:751-752.

DISCUSSION

Caveness - Does temperature affect the differential host response?

Sasser - I don't know, but results using this procedure do indicate biological differences within a species or a species group.

Dropkin - Why base the system on galling response?

Sasser - Results are not based on galling alone but on galling and production of egg masses. Not all species have the same type of galls. M. hapla and others, to a lesser extent, have distinctive galls; this may be important. This definitely indicates different physiological effects due to different species.

A. C. Triantaphyllou - It is not known, and there is some doubt, if the NCSU differentials will be useful in the different regions.

Madamba - Some of the differential hosts may not grow well, or survive, under the environmental conditions of the different regions.

Caveness - M. javanica on cabbage induces little or no galling but there is abundant egg production.

Sasser - This phenomenon is not uncommon.

TABLE 1. Usual response of plant species to attack by the more common *Meloidogyne* spp.

| *Meloidogyne* species | Differential Hosts 1/ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tobacco | Cotton | Pepper | Water-melon | Peanut | Corn | Straw-berry | S. Potato All Gold | S. Potato P. Rico | Tomato |
| *M. incognita* | - | ± 2/ | + | + | - | + | - | + | ± | + |
| *M. javanica* | + | - | - | + | - | + | - | + | - | + |
| *M. hapla* | + | - | + | - | + | - | + | + | + | + |
| *M. arenaria* | + | - | + | + | ± | + | - | ± | - | + |
| *M. exigua* | - | - | + | + | - | - | - | - | - | - |

1/ Plant varieties include: Tobacco, N.C. 95; Cotton, Delta Pine 16; Pepper, California Wonder; Watermelon, Charleston Grey; Peanut, Florunner; Corn, Minn A401; Strawberry, Allbritton; Sweet Potato, All Gold and Puerto Rico; Tomato, Rutgers.

2/ Some populations attack the differential hosts while others do not.

F. CYTOGENETICS OF ROOT-KNOT NEMATODES - Dr. A. C. Triantaphyllou

Much of the taxonomic and biological complexity of root-knot nematodes may be the result of their cytogenetic variation. There are few animal groups that can match the cytogenetic diversification of root-knot nematodes. Recent cytogenetic studies have demonstrated that evolution of these nematodes has been influenced extensively by changes of their chromosomal complement, including the establishment of polyploidy and aneuploidy, in association with the establishment of various types of parthenogenetic reproduction.

With regard to mode of reproduction it has been found that some species reproduce by cross-fertilization, but most species reproduce by parthenogenesis. Two types of parthenogenesis have been observed, meiotic and mitotic. In meiotic parthenogenesis synapsis of homologous chromosomes takes place during early prophase of the first maturation division of the oocytes. This is followed by two normal maturation divisions during which two polar bodies are given off and then the egg pronucleus is formed containing the haploid chromosomal complement. The diploid or somatic chromosome number is re-established later, not by fusion of the egg pronucleus with a sperm, as it would be in the case of cross-fertilization, but by fusion of the egg pronucleus with one of the polar nuclei. Since synapsis takes place, the chromosomes at metaphase of the first maturation division are bivalent and in the haploid (n) number. Each bivalent consists of two homologous chromosomes which are associated end to end. Each homologue consists of two chromatids that lie parallel to each other. Thus each bivalent appears as a "tetrad." This type of reproduction is characteristic of many species of root-knot nematodes (Table 1) including most populations of Meloidogyne hapla. In mitotic parthenogenesis no synapsis of homologous chromosomes takes place during prophase, and at metaphase of the first maturation division the oocytes contain univalent chromosomes in the diploid (2n) number. Each univalent chromosome consists of two chromatids that lie parallel to each other and form a "dyad". There is only one maturation division with a single polar body being given off. The egg pronucleus is then formed with the diploid chromosome number and proceeds

with the first cleavage division without the need of fertilization.

It is reasonable to assume that cross-fertilization has evolved to facultative meiotic parthenogenesis, i.e. meiotic parthenogenesis with occasional cross-fertilization, then to obligatory meiotic parthenogenesis and finally to obligatory mitotic parthenogenesis. This pattern of evolution of reproduction is very instructive in interpreting phylogenetic relationships among species of root-knot nematodes.

The study of the chromosomes of these organisms during gametogenesis and in a few cases during embryogenesis has been quite productive because it so happens that various species or forms within a species have different chromosomal complements. They differ with regard to chromosome number, chromosome size, DNA content and chromosomal behavior during the maturation divisions.

It appears that the basic chromosome number of the genus Meloidogyne is n=18, observed in the amphimictic M. carolinensis and the facultatively parthenogenetic M. graminicola, M. naasi, M. graminis, M. ottersoni and M. exigua (Table 1). A reduction of the basic number has occurred in M. hapla which exists in various chromosomal forms with n=17, 16 or 15. Other populations of M. hapla have evolved toward mitotic parthenogenesis and have become polyploid (probably triploid) with a somatic chromosome number of 45. M. arenaria also exists in two chromosomal forms, a diploid with 2n=36 and a triploid with about 51 to 54 chromosomes. M. incognita, the most important species of root-knot nematodes, can easily be distinguished from all other species on the basis of the behavior of its chromosomes during maturation of the oocytes (unpublished information). It is obligatorily parthenogenetic and shows extensive variation in chromosome numbers. M. javanica is also obligatorily parthenogenetic, and shows some variation in chromosome numbers, although most populations have 46 to 48 chromosomes. M. spartinae with n=7 chromosomes and amphimictic mode of reproduction probably should be transferred again to the genus Hypsoperine because it does not fit cytogenetically to the genus Meloidogyne.

It is hoped that the study of many populations of root-knot nematodes from many parts of the world in connection with the International *Meloidogyne* Program will provide a more complete picture of the cytogenetic diversification in the genus. Such information will help clarify the biological and taxonomic status of root-knot nematodes, one of the major objectives of this project.

## References

Triantaphyllou, A. C. 1971. Genetics and cytology. *In* "Plant Nematology" (B. F. Zuckerman, R. A. Rohde and W. F. Mai, eds.). Vol. 2, pp. 1-34. Academic Press, New York.

## DISCUSSION

Golden - With a mitotic parthenogenetic species is there any possibility of amphimixis?

A. C. Triantaphyllou - No.

Van Gundy - On the basis of your work would you recommend starting with single egg masses or a larger field population?

Triantaphyllou - That depends on your objectives. Generally, there are many genes, or alleles, within a population that are not carried by each individual within that population.

Table 1. Cytogenetic relationships among root-knot nematodes.

| | | | |
|---|---|---|---|
| | | *carolinensis* | Amphimictic (n=18) |
| | | *graminicola*<br>*naasi*<br>*graminis*<br>*ottersoni*<br>*exigua* | Amphimictic (n=18)<br>Meiotic Parthenogenetic |
| *Meloidogyne* | | *hapla* | Amphimictic (n=17,16,15)<br>Meiotic Parthenogenetic |
| | | hapla | Mitotic Parthenogenetic (2n=45) |
| | | *arenaria* | Mitotic Parthenogenetic (2n=36, 51-54) |
| | | *incognita* | Mitotic Parthenogenetic (2n=36-44) |
| | | *javanica* | Mitotic Parthenogenetic (2n=43-48) |
| | | sp. (undescribed) | Mitotic Parthenogenetic (2n=30) |
| (*Meloidogyne*?)<br>*Hypsoperine* | | *spartinae* | Amphimictin (n=7) |

G. PLANT RESPONSE TO ROOT-KNOT NEMATODES AT THE CELLULAR LEVEL. - Dr. Victor H. Dropkin

I. Introduction:

The central theme of this paper is that the host-parasite interaction between Meloidogyne and plants is a complex system subject to genetic control at many stages in both host and parasite.

All sedentary nematode parasites feed for weeks in one position in the host plant. They must therefore not kill tissues. The plant cells experience continual drain upon their resources as nematodes slowly remove their contents. Unlike aphids, nematodes cannot withdraw nutrients directly from xylem or phloem vessels. The cells must respond in some way to maintain a steady supply of nutrients to the growing and reproducing parasites.

Meloidogyne induces spectacular changes in host anatomy - galls and giant cells, as well as other effects. An infected root system develops abnormally and plant growth is disturbed. At the same time, the infected root system is subject to attack by other organisms, thus complicating the problem. We shall examine the life cycle of Meloidogyne with special emphasis on plant response and show that at every step, species and varietal differences exist which may contribute to resistance. These differences reflect genetic factors in both hosts and parasites.

II. Infective larvae.

Single-celled eggs of adult female Meloidogyne are deposited into a gelatinous matrix secreted by rectal gland cells. This matrix usually loses water, turns brown, and probably has a protective function. After about 9 days at 27C, each egg contains a 2nd-stage infective larva (Tyler, 1933). With adequate moisture and aeration, the larva breaks out of the egg shell and moves about as the infective stage. In many plants, egg sacs protrude from the root surface, but in certain fleshy roots or underground stems, egg sacs may be entirely enclosed within host

tissues. Larvae hatched internally may inhabit the same host, other larvae may move about in soil, or be carried in soil water (or irrigation water) to distant places.

Infective larvae must penetrate plant tissues or starve. They may survive for weeks in soil, or as eggs for longer, especially under dry conditions. Infected pieces of roots serve as sources of inoculum for long periods even after soil fumigation (Raski and Lear, 1962). Fallow or flooding is often ineffective unless carefully done.

Infective larvae are attracted to growing roots. The exact stimuli which lead nematodes to find growing roots are not known. In artificial systems, root knot larvae migrate toward a variety of stimuli: $CO_2$, some oxidizing and reducing agents, amino acids, and unspecified components of root exudates (see Green, 1971 for summary of attractants). It is clear, however, that roots differ in their attractiveness and ease of penetration. The same root may be less attractive to one population and more attractive to another. Some hosts are penetrated by large numbers of larvae whereas others receive smaller infections under the same conditions (Dropkin and Webb, 1967). Some plants are believed to release toxic compounds that repel or kill nematodes (Pangola grass, asparagus, Tagetes and others) (see Gommers, 1973 for review).

Although the initial barrier to attraction and penetration is not usually employed in breeding for resistance, I believe it operates in some cases and should be exploited.

Larvae may penetrate through the root tip or farther back. Incidentally, larvae will also invade other plant tissues in contact with soil. Once inside, the nematodes migrate through the cortex to the region of differentiating xylem, i.e. just behind the root tip. I have seen cleared roots with root knot larvae turning around at the root tip, as if they had migrated toward the tip from above

and turned back to find the region of differentiation. The larvae may leave a trail of destruction in the root cortex or not, depending on the particular host and parasite.

III. Giant cells.

In the region of differentiating xylem, several changes occur in the first 24-48 hours after inoculation. Developing tracheids show loss of orientation of their wall structure (shown by loss of birefringence in polarized light); nuclear multiplication without cell division, increase in cell size. In whole mounts of seedlings penetrated by Meloidogyne, there may be areas without cells, as if there had been a general maceration of the tissues.

In a few more days, a group of 5-10 distinct, large, multinucleate cells develop around the head of the nematode. These cells, called "giant cells", surrounc the anterior portion of the nematode and are most probably the food supply for the nematode. The cytoplasm of giant cells is dense, the central vacuole is absent, there are many, large, polyploid nuclei, and the walls of the giant cells increase in thickness and develop wall ingrowths. The latter are finger-like projections toward the cytoplasm lined with plasmalemma. According to their morphology, these cells appear to be in a condition of very active metabolism.

Developing males are associated with small giant cells whereas developing females usually feed on much larger giant cells. Toward the end of the nematode's life, giant cells degenerate, their contents are replaced by parenchyma cells, and only the thickened walls remain. Nematode reproduction takes place only when well-developed giant cells are present.

IV. Galls.

Meloidogyne induces gall formation soon after penetration. Galls also develop in response to invasion by other nematodes, by insects, mites, bacteria, fungi, and viruses. In root-knot, cortex cells swell and pericycle tissues divide.

The reaction is rapid (Dropkin and Boone, 1966). The particular form of the gall is determined by the nematode species and host species or variety. For example, in soybeans, Cv. Amsoy develops large galls with M. incognita but Cv. Capitol has very small or no galls. M. hapla induces small galls with multiple side roots in tomato, but M. incognita induces large galls with fewer side roots in the same host. Galls may be terminal, as in barley with M. naasi, or the root may continue to grow through the gall.

The galling response is separate from giant cell formation. In addition to the variation in gall size and form mentioned above, some combinations result in large galls with small giant cells and no nematode reproduction. Further, the system responds to environmental influences. The gene Mi for resistance of tomato to certain species of Meloidogyne operates at temperatures below 28C. At higher temperatures it is not effective so that resistant tomatoes do not form galls below 28 but do so at higher temperatures.

The mechanism by which nematodes induce galling and giant cells is completely unknown. Plant growth regulators are certainly part of the story. The nematode injects the products of its esophageal glands, and these undoubtedly stimulate some of the changes seen in giant cells. In addition, excretory products are liberated into plant tissues. Many of the characteristics of giant cells result from the steady withdrawal of cell cytoplasm by the nematode. Similar phenomena occur throughout the plant kingdom in the form of "transfer cells" - specialized cells in plants located in regions with high demand for nutrients, such as leaf traces, nectaries, embryo nurse cells, and the like. A typical transfer cell has wall ingrowths, enlarged nucleus, loss of central vacuole, increase in concentration of cell organelles. Meloidogyne apparently stimulates nuclear division and polyploidy and increased cell size while at the same time inhibiting cell wall deposition (Jones and Northcote, 1972).

V. Root-knot disease.

Root-knot disease results from the interplay of several factors:

1) Malformation of roots prevents the normal development of feeder roots so that the plants suffer from inadequate water and nutrients, or even inadequate mechanical support. At the same time,

2) The diversion of growth to galls alters the distribution of hormones, of minerals, and of photosynthetic products so that relatively more of the plant's growth is concentrated in roots to the detriment of flowering or fruit development. This diversion of growth is reflected in lowered photosynthetic rates of leaves (Bird, 1974).

3) Galling and giant cell formation are accompanied by the accumulation of high concentrations of metabolites = amino acids, organic acids, nucleic acids, carbohydrates and proteins. At the same time, there are membrane changes so that nutrients leak out into the soil. This makes galls fine substrates for growth of fungi - and root rots become a problem. Perhaps the defense mechanisms of plants vs. pathogens are also inhibited.

VI. Breeding for resistance.

What can we conclude from this examination of the life cycle of Meloidogyne about strategies for breeding for resistance?

1) Selection for resistance has usually been made with one or a few populations of Meloidogyne. I believe that strong efforts should be directed at setting up collections of local populations from various sources so that primary screening can be made utilizing a wide variety of nematode populations.

2) Selection has often been based on seedling response to invasion - and root galling has been used as the index for finding resistance. We should also pay attention to many other characteristics such as patterns of root growth, resistance to moisture stress, response to intercurrent infection with other organisms, and growth characteristics of the whole plant. This means that we have to develop efficient assay methods for these other characteristics in addition to the scoring systems now in use. Perhaps we should think of inoculating seedlings

in beds of mixed populations of Meloidogyne, then moving the seedlings out-of-doors to develop to maturity.

3) We should strive to find individual genes that affect aspects of the interaction among nematodes, plants, and other organisms. This will necessitate establishment of pure lines of nematodes as well as of plants. In my view there are very many genes affecting attraction, establishment and development of giant cells, patterns or root growth, types and severity of galls, resistance to reduced root function, photosynthetic rates, rates of nematode growth and maturation, -- and many more. We must broaden the genetic base as much as possible. And the most difficult task here is to develop assay methods that will permit the large scale screening that underlies all plant breeding for resistance.

## Literature Cited


1. Bird, A. F. 1974. Plant response to root-knot nematode. Ann. Rev. Phytopathology 12:69-85.

2. Dropkin, V. H. and W. R. Boone. 1966. Analysis of host-parasite relationships of root-knot nematodes by single larva inoculations of excised tomato roots. Nematologica 12:225-236.

3. Dropkin, V. H. and R. E. Webb. 1967. Resistance of axenic seedlings to Meloidogyne incognita acrita and to M. hapla. Phytopathol. 57:584-587.

4. Gommers, F. J. 1973. Nematicidal principles in Compositae. Meded. Landbouuhogeschool Wageningen, Nederland. 73-17:1-71.

5. Green, C. D. 1971. Mating and host finding behaviour of plant nematodes. pp. 247-266 in Plant Parasitic Nematodes. (B. M. Zuckerman, W. F. Mai, R. A. Rohde, eds). Vol. II, Chap. 24. New York-London: Academic Press.

6. Jones, M. G. K. and Northcote, D. H. 1972. Multinucleate transfer cells induced in Coleus roots by the root-knot nematode, Meloidogyne arenaria. Protoplasma 75:381-395.

7. Raski, D. J. and B. Lear. 1962. Influence of rotation and fumigation on root-knot nematode populations on grape replants. Nematologica 8:143-151.

8. Tyler, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:291-413.


DISCUSSION:

Nusbaum - There has recently been work on gall production induced by insects. Has this information been of any help in understanding the root-knot system?

Dropkin - Yes, the entomologists have been able to extract gall inducing materials from the insects. In another study aphid proboseus excretions were duplicated and found to be capable of inducing galls. Excretions composed primarily of amino acids.

## H. MOLECULAR APPROACHES TO TAXONOMY OF MELOIDOGYNE - Dr. R. S. Hussey

We are entering a new era in nematode taxonomy where we do not have to and should not rely on only morphological features to identify and characterize root-knot nematodes. Success of a taxonomy is judged largely by the consistency obtained when different classes of information are employed. New approaches such as cytogenetics and biochemical analyses are providing valuable information and are useful adjuncts in taxonomic investigations of Meloidogyne species (10). Problems encountered in Meloidogyne taxonomy and phylogeny will best be resolved by pooling information obtained from morphological, cytological, and biochemical investigations.

When considering the application of biochemical systematics to the genus Meloidogyne, one is immediately confronted with two general problems. These are discussed below.

### Availability of Nematode Material

Axenic culturing is the most ideal method of propagating nematodes for biochemical analyses because the influence of other organisms on the nematode's metabolism are removed and culture techniques can be standardized.

Because root-knot nematodes are obligate parasites and cannot be cultured axenically, obtaining nematodes in large enough quantities for examination is not always easy. Due to their highly sophisticated host-parasite relationships, members of the important genus Meloidogyne are easiest cultured xenically on greenhouse grown tomatoes. Large numbers of tomatoes can be inoculated easily with root-knot nematode eggs collected with a method using chlorox (8). Another alternative to propagating large numbers of nematodes would be adapting the techniques to measure the smaller amounts of material.

An important problem to overcome after we have nematodes in culture is that of separating the different stages. Certain characteristics are age-dependent. The genus Meloidogyne, however, is particularly adapted to biochemical analyses because living adult females of this genus can readily be separated from other developmental

stages (4), as should be the case with most nematodes exhibiting sexual dimorphism. This permits analyses to be carried out on one developmental stage, thereby eliminating age-dependent variation. Eggs and second-stage larvae could also be collected for biochemical analyses.

Biochemical Analyses

Biochemical systematics depends on elucidation of the subtle molecular differences which underlie taxonomic variation (3). The primary problem is determining which processes will be useful in providing information for taxonomy.

Comparative studies of proteins by electrophoresis and serology have recently provided information that may prove to be helpful in elucidating relationships among *Meloidogyne* species (1,2, 5, 6). Since proteins are the immediate manifestation of an organism's genetic endowment, comparisons of soluble proteins and enzyme patterns may actually reflect direct relationships of the genotypes of the species compared.

Electrophoresis: Polyacrylamide-gel electrophoresis employed for separation of proteins appears to have the greatest potential, compared to other biochemical methods, for characterizing *Meloidogyne* species. However, results of protein analyses by electrophoresis obtained by different investigators will be useful for nematode taxonomic and phylogenetic studies only if standardization of procedures is adopted; otherwise meaningful comparisons cannot be made. Of several enzymes studied by this technique, malate dehydrogenase, a-glycerophosphate dehydogenase, and non-specific esterase patterns appear to be particularly useful in separating *Meloidogyne* species (6). These enzymes occur in multiple forms in various species, and this further facilitates species identification. For example, *M. arenaria* and *M. incognita* can be definitely differentiated on the basis of certain enzyme patterns (6). Four and three isoenzymes were detected for malate dehydrogenase and a-glycerophosphate dehydrogenase, respectively from extracts of *M. arenaria*, whereas only three isoenzymes of malate dehydrogenase and one form of a-glycero-

phosphate dehydrogenase were present in extracts of M. incognita. The same number of sites of esterase activity were detected for both species, but distinct characteristic differences did occur with respect to the relative activity at certain sites and electrophoretic migration rates (6).

Variation in nematode enzyme patterns has been shown to occur as a result of influence by the host-plant (2, 6, 7, 9). Some differences were detected in peroxidase patterns from extracts of M. incognita propagated on tomato, tobacco, and bean (6, 7). Patterns of six other enzymes were not influenced by these hosts, indicating that host influence may be limited to certain enzymes.

Differences have also been noted in soluble protein patterns obtained by electrophoretic separation of extracts of Meloidogyne species (1, 6). These differences, however, are not as striking as differences in enzyme patterns.

Serology: Serological techniques have been employed recently in determining differences or similarities among the soluble proteins of Meloidogyne species (5, 6). Because of the specificity of the antigen-antibody reaction, serological techniques are regarded to be very reliable in determining homologies between proteins of different animal species. Serological tests failed to demonstrate significant differences in soluble proteins between M. arenaria and M. incognita. For example, reaction of proteins separated from extracts of M. incognita by polyacrylamide-gel electrophoresis, with homologous antisera and antisera prepared against M. arenaria proteins showed that most of the proteins of these two species are homologous (6). Similarly, gel-diffusion (Ouchterlony) tests showed that one immunoprecipitate was unique with M. incognita, whereas another eight, based on band position and coalescence, were common to both species (5).

## Prospects for the Future

Molecular approaches to taxonomy of Meloidogyne species, although still in its infancy, shows considerable promise as being a useful tool in identification and characterization of this important nematode genus. Future electrophoretic

investigations should consider the use of microtechniques, which would permit analyses of fewer nematodes. Additional enzymes also need to be studied to determine their usefulness in these investigations. For serological investigations, refinement in the methods currently being used is needed. Partial purification of nematode antigens, (for example, using only antigens from the nematode cuticle), would result in simplification of the precipitin patterns and permit an easier interpretation of the interaction of precipitin bands.

The capabilities of these techniques to differentiate and characterize root-knot nematode species makes them useful and ultimately they may facilitate the identification of these nematodes.

## References


1. Dickson, D. W., J. N. Sasser, and D. Huisingh. 1970. Comparative disc-electrophoretic protein analyses of selected Meloidogyne, Ditylenchus, Heterodera, and Aphelenchus spp. J. Nematol. 2:286-293.

2. Dickson, D. W., D. Huisingh, and J. N. Sasser. 1971. Dehydrogenases, acid and alkaline phosphatases, and esterases for chemotaxonomy of selected Meloidogyne, Ditylenchus, Heterodera and Aphelenchus spp. J. Nematol. 3:1-16.

3. Hansen, E. L. and E. J. Buecher. 1970. Biochemical approach to systematic studies with axenic nematodes. J. Nematol. 2:1-6.

4. Hussey, R. S. 1971. A technique for obtaining quantities of living Meloidoygne females. J. Nematol. 3:99-100.

5. Hussey, R. S. 1972. Serological relationship of Meloidoygne incognita and M. arenaria. J. Nematol. 4:101-104.

6. Hussey, R. S., J. N. Sasser and D. Huisingh. 1972. Disc-electrophoretic studies of soluble proteins and enzymes of Meloidoygne incognita and M. arenaria. J. Nematol. 4:183-189.

7. Hussey, R. S. and J. N. Sasser. 1973. Peroxidase from Meloidogyne incognita. Physiol. Plant Pathol. 3:223-229.

8. Hussey, R. S. and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Pl. Dis. Reptr. 57:1025-1028.

9. Ishibashi, N. 1970. Variation of electrophoretic protein patterns of Heteroderidae (Nematodes: Tylenchida) depending on the developmental stages of the nematode and on the growing conditions of the host plant. Appl. Entomol. Zool. 5:23-32.

10. Triantaphyllou, A. C. and R. S. Hussey. 1973. Modern approaches in the study of relationships in the genus Meloidogyne. Europ. Pl. Prot. Bull. 9:61-66.


DISCUSSION:

Sasser - The esterases in M. hapla appear distinctive, are they related to pathogenicity?

Hussey - No, esterases are probably related to lipid metabolism and not involved with pathogenicity.

Starr - Might not the virologists' techniques for isolation and characterization of different RNA fractions be a useful tool, especially radio flurography.

Golden - I would encourage further work in this area, could be very helpful in identification of different races.

Dropkin - What is the relation of this type of work to increased food production?

Sasser - This work should be helpful in species and race identification, a necessary component of an integrated control program.

Dropkin - A very premature and optimistic attitude. I would recommend a de-emphasis of this portion of IMP.

Rickard - Serological techniques for species identification, using eggs as the antigen source, could be a uniform and easy technique that could be used by the regional investigators. Could easily demonstrate the presence of mixed populations.

Mai - NCSU is probably the best prepared institution for this work. One reason for past failures was that the biologists and biochemists did not work closely together. Would not be a problem here at Raleigh.

Van Gundy - After comparing the 30-odd Meloidogyne species will probably find that most are closely related, with only a few distinctive species.

Mai - This would be useful information to help determine if there are, in fact, 30 different species.

Tarte - Rather than develop these techniques for species identification, wouldn't it be more important to determine pathogenic variation?

Sasser - I really agree with Dr. Dropkin, the biochemical work is not the major thrust of IMP. This work is designed not only to separate M. hapla from other species but to help understand the genus in general. With a program of this type (IMP), the biochemical work can be attempted without jeopardizing other aspects.

Barriga - I believe this should be a complementary to help clarify the problem, but not a main part of the program.

Madamba - Is highly specific population identification required? Major efforts should be geared to major crops and root-knot species. I would recommend delaying the biochemical work until the project is well established.

Sasser - A better understanding of the biochemistry of Meloidogyne spp. may make it possible to prevent the "breaking" of resistance. Maybe an emphasis should be placed on applied research for the first two years, but to include more basic research in subsequent years.

Ngundo - Kenya would not be happy with long term research which did not benefit directly food production and in a reasonably short time.

de Moura - I believe the work is very important and needs more emphasis.

Caveness - I am more worried about maintaining current levels of food production than increasing production. We need all the help we can get; crop rotation and resistant cultivars will have to be an important complement of pest management. These methods may be helpful in identifying slight differences between root-knot populations.

Sasser - While this work is not recommended for the regional centers, which often have more pressing problems, it should be started and maintained at the central institute for development of techniques that may be useful in 10-15 years.

Tarte - I am in complete agreement with Dr. Sasser.

## I. POSSIBLE MECHANISMS INVOLVED IN HOST SPECIFICITY - Dr. Jeng-Sheng Huang

### Introduction

The study of host specificity in plant diseases is generally regarded as a part of plant pathology which explains why one pathogen could parasitize a group of plants but not others.

Understanding of the mechanisms involved in host specificity would be helpful to the plant breeder in screening and developing for individuals which are resistant to a specific pathogen.

Since host specificity is expressed only when a host plant interacts with a pathogen, it is essential for a person who studies host specificity to know what capacity a pathogen has to cause disease and what defense reaction a plant has to counteract pathogenic attack.

Today I would like to discuss some of the possible mechanisms involved in pathogenicity of and resistance to root-knot nematodes which I consider to be important in host specificity. My emphasis will be on what we do know and what we do not know on each mechanism.

### Growth Regulators (Diagram 1)

The hypertrophy and hyperplasia induced by root-knot nematodes are indications of changes in plant growth regulatory substances. In fact, there are reports of auxins in extracts of Heloidogyne and increase in levels of growth regulators in nematode-induced root-knot. Several questions however remain to be answered:

First, the recovery of growth-regulatory substances from root-knot nematodes does not indicate that these substances are synthesized by the nematodes. They may simply be the result of the intake of plant growth regulators by the nematodes during feeding or other physiological activities. This question may be answered by examining the IAA synthesis system in nematodes by using

radioactively labeled tryptophan and nematode extracts.

Second, besides the possible contribution of auxins from nematodes, what other mechanisms may account for the increase in growth regulators in root knot?

1) Secretion of glycosidases by nematodes into plant tissue may release IAA from glycocides.

2) Secretion of proteolytic enzymes by nematodes into plants may free protein-bound IAA. In addition, proteolytic enzymes may break down proteins and supply IAA precursor (tryptophan) and IAA systhesis activators (such as phenylalanine).

3) Secretion of IAA oxidase inhibitors which inactivate the enzyme and results in accumulation of IAA.

These three possibilities have been mentioned in several publications. Definite evidence, however, has not been provided.

Cell-Wall Degradation (Diagram 2)

One of the most distinct effects of root-knot nematodes in host cells is the formation of giant cells. Giant cells usually develop from cells directly penetrated by the nematode's stylet and from neighboring cells incorporated by cell wall dissolution. In addition, giant cells may have thickened walls, deposits of extra wall material and wall ingrowth. These indicate that the cell wall metabolism is surely altered in the presence of Heloidogyne. The question I am concerned with is how do these changes happen?

Plant cell wall consists mainly of polysaccharides and proteins. Cell wall polysaccharides may further be divided into pectic substances, celluloses and hemicelluloses.

Cellulases ($C_x$), the enzymes which hydrolyze cellulose, endopolymethyl-galacturonase and endo-pectin methyl-trans-eliminase, the enzymes which attack certain pectic substances, have been found in some root-knot nematodes. The im-

portance of these enzymes in relation to giant cell formation, however, has not been studied. Besides the possible involvement of cell-wall degrading enzymes, root-knot nematodes may secrete certain compounds which interfere with the normal metabolism of cell wall proteins, pectins, celluloses, hemicelluloses and other wall components.

Hypersensitive Reaction (Diagram 3)

Hypersensitive reaction is a form of host response against nematode infection which is characterized by the formation of necrosis immediately surrounding the nematode.

Dropkin and his co-workers reported that only 4% of the larvae of a species of root-knot nematode that entered roots of a resistant tomato variety grew but 88% of them induced necrosis of host cells. In contrast to this, 73% of the larvae entered roots of a susceptible variety grew inside the tissue but none induced necrosis of host cells. The data suggest that necrosis of host cells and growth of larvae in the tissues are mutually exclusive.

Phenolic compounds, particularly chlorogenic acid, are thought to be the cause of browning and the resistant reaction in certain tobacco-root-knot nematode and tomato-root-knot nematode interactions. Furthermore, there is a correlation between the concentration of phenolic compounds and resistance in tomato cultivars. The concentration of phenolics in resistant and immune cultivars is 2-3 times the amount found in susceptible cultivars.

Most phenolics in plant tissues are in bound form as glycosides which have low physiological and biochemical activity and low toxicity to both host cells and pathogens. The glycosides may be hydrolyzed by B-glycosidases to form physiologically and biochemically active aglycones or free phenolic compounds which are toxic.

In addition to the possible toxic effect of the free phenolics on nematodes free phenolics may interfere with host metabolism in several ways. The compounds

may stimulate IAA oxidase synthesis and therefore decrease auxin level. This will result in no gall formation. Again, some questions remain to be answered. Do root-knot nematodes produce glycosidases? Is the concentration of the free phenolic high enough to damage host cells and the nematodes?

## Conclusions

Each higher plant has a complicated biochemical and physiological system. So does each nematode. You probably can imagine the complexity of the problem when host and pathogen are brought together and studied at one time.

Some plant pathologists recognize the complexity of the problem and have sought to study parts of the host-parasite interaction one at a time until a point is reached when we put the pieces together to form a whole picture. Tissue cultures have been used as working systems in fungal, viral and bacterial diseases and have been found to be suitable for study of host-parasite interactions in certain cases. There are several advantages in this system. First, host specificity and disease physiology can be studied on certain tissues and the complexity of whole plant can be avoided. Today we can induce root, stem, and leaf formation on several callus tissues. Second, the interactions between tissue cultures and pathogens can be studied without the interference of contaminating organisms. Third, the host materials can be grown under defined and repeatable growth conditions. Fourth, resistance and susceptibility of tissue cultures to pathogens may be varied by modification of growth regulators or other growth conditions.

In fact, Peacock in 1959 reported that root tips of a resistant tomato species (*Lycopersicon peruvianum*) remained resistant to the nematode larvae after 27 passes in culture solution. Similarly, root tips of a susceptible species (*L. esculentum*) remained susceptible to the nematode after 33 passages. I am sure that nematologists are taking advantage of the tissue culture system now and I believe with the help of advanced technology we will be in a much better position to discuss host-root-knot nematode interactions in the next few years.

Diagram 1: Growth Regulators

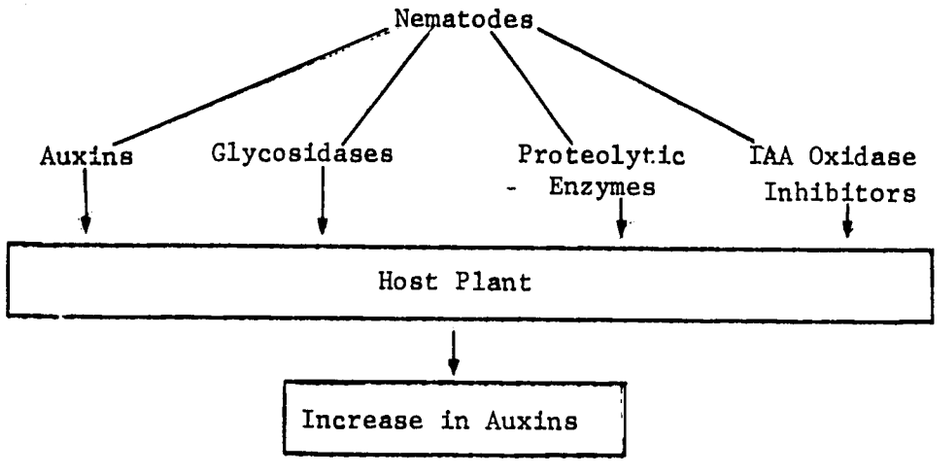



Diagram 2: Cell-wall Degrading Enzymes

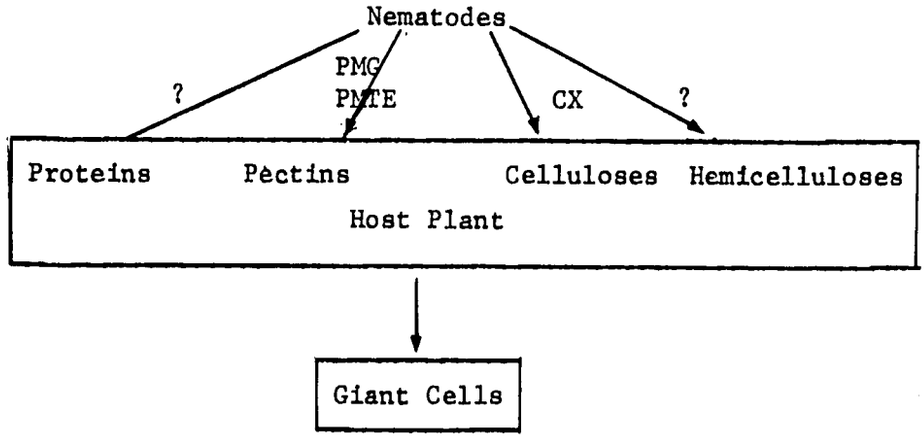
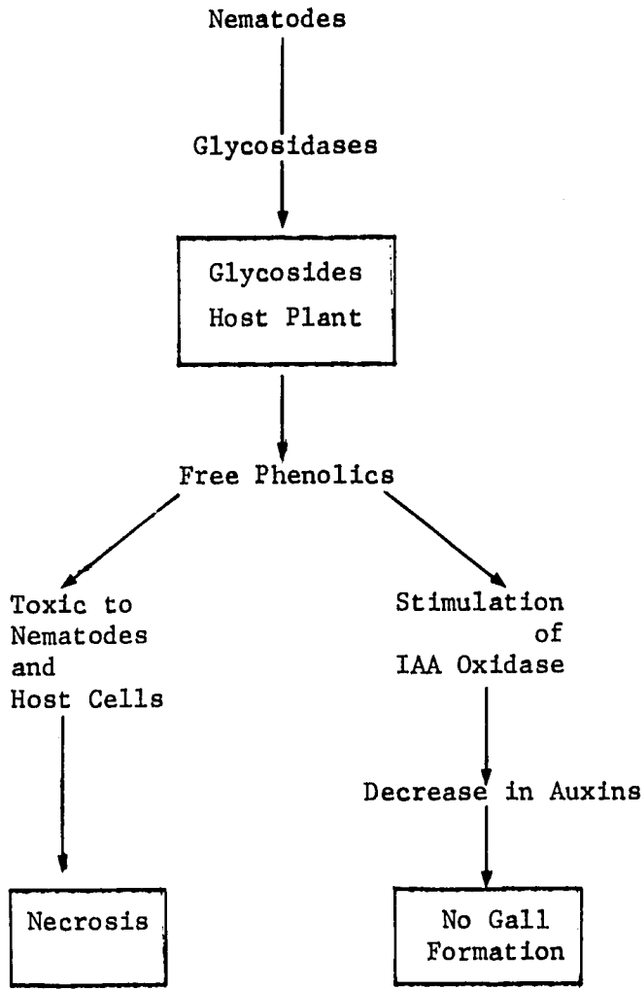


Diagram 3: Hypersensitive Reaction and Phenolic Compounds



## REFERENCES

Pi, C. L. and R. A. Rohde. 1967. Phenolic compounds and host reaction in tomato to injury caused by root-knot and lesion nematodes. Phytopathol. 57:344 (Abstr.)

Giebel, J. 1974. Biochemical mechanisms of plant resistance to nematodes. A review. J. Nematology 6:175-184.

Peacock, F. C. 1959. The development of a technique for studying the host-parasite relationships of the root-knot nematode Meloidogyne incognita under controlled conditions. Nematologica 4:43-55.

Dropkin, V. H., J. P. Helgeson, and C. D. Upper. 1969. The hypersensitivity reaction of tomatoes resistant to Meloidogyne incognita. Reversal by cytokinins. J. Nematology 1:55-61.

Dropkin, V. H. 1969. Cellular responses of plants to nematode infection. Ann. Rev. Phytopathol. 7:101-122.

Webster, J. M. 1969. The host-parasite relationships of plant-pathogenic nematodes. In Advances in parasitology, 7:1-40, edited by B. Dawes, Academic Press, London.

Viglierchio, D. R. 1971. Nematodes and other pathogens in auxin-related plant growth disorders. The Bot. Rev. 37:1-21.

Milne, D. L., D. N. Bechaff, and P. W. W. Buchan. 1965. The nature of resistance of Nicotiana repanda to the root-knot nematode, Meloidogyne javanica. S. Afr. J. Agri. Sci. 8:557-567.

Singh, B. and B. Choudhury. 1973. The chemical characteristics of tomato cultivars resistant to root-knot nematodes (Meloidogyne spp.) Nematologica 19:443-448.

## DISCUSSION:

Hussey - We are not really certain about the role of auxins in root-knot nematode-host relationships, since studies which suggest auxins are involved are based on paper chromatography which, alone, is not acceptable evidence. Recent work suggests that products of IAA oxidases may have more auxin activity than the precursor molecule.

Huang - There are many different auxins and not all have been characterized.

## J. ECOLOGY OF ROOT-KNOT NEMATODES - Dr. S. D. Van Gundy

Agricultural land represents a specialized environment subject to the whims of the farmer; ranging at times from a dry barren waste to a lush jungle of plant growth and moisture. There are relatively few places in the world where conditions allow nematodes to grow and reproduce throughout the year, the least of which is cultivated land. In addition to the plant-parasitic nematodes' ability to reproduce is its ability to survive from one crop season to another, to endure long periods of cold and dry and to survive bathing in a variety of chemical additives.

The important environmental factors affecting nematode populations can generally be categorized as climatic influences, soil influences, food influences, influence of other organisms and intra-specific influences. Since food influences will be covered by other participants, the ecological relationships between nematodes and their external environment will be approached in this discussion as key elements of a pest management program.

### Climate

Climate generally has a determining influence on the position of the border of the range of any species, animal or plant. The main direct climatic factors are temperature, moisture, light and cyclic influences. The main indirect factor is the affect of climate on food, i.e. crop plants grown in a particular region or time of year. Temperature probably is the most important nonbiological factor which affects all aspects of the environment, direct and indirect and for this reason covered in detail in this section.

Root-knot nematodes (*Meloidogyne* spp.) are world-wide in distribution but some species are limited to specific geographic areas by soil temperature and soil types. It appears that *M. hapla* (Sayre, 1964) and possibly *M. naasi* (Franklin *et al.*

1971) are normally temperate region species (Cryophils) since they are able to survive soil temperatures below 0° C. On the other hand M. incognita, M. javanica, M. arenaria (Thomason and Lear, 1961), and possibly M. exigua are normally subtropical or tropical region species (Thermophils) since they do not appear to have any extended survival in soils below 10° C. Since these nematodes are continuously moved about by man their current distribution ranges overlap, particularly the occurence of temperate species in many of the subtropical and tropical areas (Siddiqui et al., 1973; Whitehead, 1969). The susceptibility of larvae to succumb to temperatures from 0-10° C is related to the existence of temperature induced phase transitions in the lipid position of the biological membranes in the nematode. Meloidogyne javanica exhibits a phase transition at 10° while M. hapla does not and probably accounts for the apparent differences in geographical distribution (Lyons, et al., 1975). There are also temperature differences between populations of the same species which have been termed "thermotypes." Comparisons of a Venezuelan and a Netherlands population of M. incognita indicated that the minimum temperature for the Netherlands population to start infestation and reproduction was about 5°C lower than the Venezuelan (Dao, 1970). Still another species, M. naasi, has chilling requirement for hatching. Storage of soil for 1 week at 0° or 10° C followed by an incubation at 20° C produced 3 times as many emerging larvae as unchilled soil (Franklin et al., 1971).

The distribution of Meloidogyne spp. in agricultural soils in California (Siddiqui et al., 1973) and East Africa (Whitehead, 1969) have been surveyed in some detail. In California M. hapla is distributed throughout the agricultural areas but has a tendency to be more common in the cooler coastal areas. Meloidogyne incognita and M. javanica are more commonly found in the hot interior valleys, while M. naasi and M. thamesi are found in cooler coastal and northern areas of California primarily restricted to barley and potatoes, respectively. In Africa, M. hapla was found in many areas of East Africa; it only flourished at high alti-

tudes (6000 ft. and above). Meloidogyne javanica and M. incognita were restricted to areas below 6500 ft. This general pattern is typical of numerous other observations on the world-wide distribution of root-knot nematodes.

There are numerous reports on the effects of temperature on the embryology, hatching, movement, infection, penetration, growth, development and survival of the various root-knot species. Bird and Wallace and co-workers have probably made the most comprehensive studies in this area and a summary is presented in Table 1.

The optimum temperature for embryogenesis in M. javanica is between 25 and 30° C (Bird, 1972), 30° C for hatching, 25° C for mobility, 25-30° C for growth (Bird or Wallace, 1965) and 32-34° for egg production (Thomason and Lear, 1961). The optimums for M. hapla are about 5° C less than for M. javanica. Since optimum temperatures for root-knot do not occur throughout the crop growing season it is often better to forecast generation time by means of a heat factor (e.g. °C x hours). In the case of M. javanica it takes approximately 9,300 centigrade hours to complete the life cycle from second stage larva to egg on tobacco (Milne and DuPlessis, 1964). This is an area which will need more attention as nematode pest management programs are developed for specific nematodes and specific crops.

Temperature has a strong influence on the survival of Meloidogyne eggs and larvae in soil. For example, the larvae of M. javanica begin their life in soil with a third of their body weight in lipids as a storage energy reserve. It is used at a rate dependent upon rate of metabolism which is in turn influenced by temperature, moisture, aeration, etc. (Van Gundy et al., 1967; Wallace, 1966 a). The more food reserves the nematode has when the host root enters its environment the greater are its chances of getting to the infection site and entering the root. Extended survival and infectivity in soil must then be related to conservation of food reserves and physiological condition of aged larvae rather than chronological age. In general the optimum temperatures for survival of eggs and larvae is from 10-15° C (Bergeson, 1959; Thomason et al., 1964).

Table 1. Minimum, optimum, and maximum temperatures recorded for root-knot nematode species

| Nematode species | Activity or process | Temperature in °C min. | opt. | max. |
|---|---|---|---|---|
| *Meloidogyne hapla* | Hatch | | 25 | |
| | Mobility | | 20 | |
| | Invasion | 5 | 15-20 | 35 |
| | Growth | 15 | 20-25 | 30 |
| | Reproduction | 20 | 25 | |
| | Survival | ±0 | | |
| *Meloidogyne javanica* | Hatch | 10 | 30 | 35 |
| | Mobility | | 25 | |
| | Invasion | | 20-30 | |
| | Growth | | 25-30 | |
| | Reproduction | | 24-28 | |
| | Survival | ±10 | | |

Soil Environment

The soil environment is a constantly changing habitat of physical and chemical factors. Since they are also interrelated it is difficult to relate nematode distribution, population levels, pathogenicity, etc., to any one factor. In addition to the general effects of temperature, moisture, aeration, soil texture, and soil solution are important factors which must be considered in the ecology of nematodes (Stolzy and Van Gundy, 1968).

Simply stated, soil can be thought of as a three-phase model: solid, liquid and gas. The solids may be inorganic or organic. The liquid usually contains a variety of ions and gases. The gas phase is inversely proportional to the liquid phase. The solid inorganic phase is made up of sand, silt and clay particles and these may be augmented in varying degrees. Most studies in the soil have been to isolate various segments of the system and study them separately. The pest management approach for the future must approach this environment from a holistic point of view.

Soil moisture is an extremely important factor because nematodes are dependent upon water for most of their activities. In moist soils (40-60% of field capacity) nematodes are active and move through the soil cavities in water films. In dry soils nematodes become inactive and may eventually die due to desiccation. In extremely wet soils nematodes often become inactive and may die because of lack of oxygen. Therefore, it is extremely difficult to separate soil texture from soil moisture and soil aeration.

There are numerous observations and reports suggesting an association between soil type and distribution of root-knot species or the severity of disease expression caused by various root-knot species. Sasser (1954) has reported that infestations of *M. incognita*, *M. incognita acrita* and *M. hapla* are more severe in sandy loam soils than in the heavy clay soils of Eastern Maryland. In California the root-knot nematodes are a major problem on the coarse textured

soils of warm interior Imperial, Coachella, and San Joaquin Valleys and a relatively minor problem in the nonirrigated coastal fine-textured soils. In Arizona the heaviest infestations of M. incognita acrita occur on the coarse texture soils (O'Bannon and Reynolds, 1961). On the other hand, Whitehead (1969) found the relative frequencies of M. javanica and M. incognita in East Africa were not correlated with soil texture. Even though there are variable and conflicting observations it generally appears that the various species of root-knot nematodes occur in a wide-range of soil types but that the damage to crop plants is often accentuated on sandy soils or sandy patches within a given field. Damage particularly in the field situation may in part be related to moisture stress experienced by infested plants on sandy soils. Therefore, distribution of a species and damage by a species as related to soil texture may be quite different. In detailed laboratory studies (Wallace, 1969) particle size had a significant effect on the numbers of larvae hatching from egg sacs; highest numbers in fine sand (150-250 u) and lowest numbers in coarse sand (500-1000 u). Reproduction rate was highest in the coarse sand and invasion of roots was highest in fine sand. The moisture distribution in pore spaces is also important and closely related to soil texture. In general, maximum hatch of M. javanica occurs when most of the pores are emptied of water and corresponds to a stage of the moisture characteristic which is sometimes called field capacity (Wallace, 1966b, 1968b). The effects of moisture are also closely associated with aeration. Wallace (1968) found a linear relationship between rate of hatch of M. javanica and oxygen concentration over a range of 5% $O_2$ to 20% $O_2$. No hatch occurred in the absence of oxygen.

Exposures of embryonated eggs to anaerobic conditions for short periods (1 week) appear to be lethal presumably from buildup of lactic acid within egg shell. At low oxygen levels metabolic and movement activity of larvae is reduced and the subsequent infection rate is reduced. Continued low oxygen levels around larvae and females within plant roots caused correspondingly reduced growth and

reproduction (Van Gundy and Stolzy, 1961;Van Gundy, et al., 1967). The effect of $O_2$ on invasion, movement and hatch of M. hapla in organic soil appears to be modified by the level of $CO_2$ present. Wong and Mai (1973) found the activity was not adversely affected at 10% $CO_2$ but was at 30% $CO_2$. High suction potentials directly or indirectly because of increased aeration induce high metabolic activity in M. javanica larvae causing them to be using up body reserves faster and thereby reduce their survival time in soil (Van Gundy, et al., 1967).

Soil Water

In addition to the direct effects of water and aeration on root-knot nematode activity, there are a variety of chemicals to be found in the soil water which also affect nematode activity directly or indirectly. Some of the important ones are dissolved salts, hydrogen ion concentration, root exudates, fertilizers, organic compounds, and various pesticides.

Differences in osmotic potential between the nematodes' body fluids and the soil solution occur with changes in concentration of dissolved salts in the soil water. In general, hatch of plant parasitic nematodes stops at osmotic pressures of 15-16 atmospheres. Osmotic pressures in agricultural soils seldom exceed 2 atmospheres. Wallace (1966b) observed that there was no significant difference between the hatch of M. javanica eggs in deionized distilled water and any of several solutions up to 2.5 atmospheres. As osmotic potential increased further, hatch decreased up to 12.5 atmospheres. Embryos and first stage larvae were more resistant to water loss than unhatched second stage larvae because of changes in the egg membrane after the first molt in the egg. The egg sac appeared to give more protection to water loss from the unhatched second stage larvae than the egg shell. In drying environments such as soil the egg sac tends to maintain a high moisture level. Since the nematode cuticle allows passage of water rapidly back and forth, solutions of high osmotic potential cause second stage larvae

to shrink in size and sometimes become inactive or quiescent. In all cases, root-knot nematode larvae are able to osmoregulate and become active when returned to more favorable osmotic pressures. Very little is known about osmoregulatory mechanisms, however, studies on Aphelenchus avenae indicate that the average residence time of a water molecule in a nematode is approximately 0.9 sec, thus water movement in and out of nematodes is a very dynamic process (Marks, et al., 1968).

The root-knot nematodes appear to survive, hatch, and reproduce over a wide pH range, 4.0-8.0. Wallace (1966a) did find that hatch of M. javanica was inhibited below pH 5.2 and reach its optimum between 6.4 and 7.0.

There are a variety of chemicals such as fertilizers, organic acids, and pesticides in the soil solution which may affect root-knot. Wallace (1969) found that high nutrient levels (200 mg/pot) stimulated hatching of M. javanica from egg sacs. Hatching of M. javanica is also stimulated by low concentrations (0.48 ug/ml) of aldicarb in soil solution (Hough & Thomason, 1975). Other organic chemicals in the soil may be toxic, such as decomposition products of rye residues which were directly responsible for immobilizing M. incognita larvae in three hours and killing in 24 hours (Patrich, et al., 1965). Unfortunately, little more is known about the effects of these chemicals on the activities of root-knot nematodes.

One of the major factors affecting behavior and distribution of plant-parasitic nematodes in soil are roots and their emanations (Jones, 1960). The spacial distribution of Meloidogyne spp. is closely associated with root distribution in grape vineyards (Ferris and McKenry, 1974). Grape roots and galls have been found as deep as 510 cm (17 ft) below the surface (Raski and Lear, 1962). Although this is largely a response to food source there are other factors that influence nematode behavior. Viglierchio and Lownsbery (1960) have shown a significant increase in hatch of M. hapla, M. incognita acrita, and M. javanica due to root emanations. Wallace (1966) found that hatching of M. javanica was inhibited in nonsterile soil. Inhibition

disappeared when the soil was either sterilized or planted to a tomato seedling. It was further observed that larvae stored in a porous cup imbedded in the soil near a tomato seedling used their body reserves much faster than those not associated with a tomato seedling (Van Gundy, et al., 1967).

The relationship between numbers of Meloidogyne in the soil, rate of reproduction and growth of host plant is a complex ecological situation. Environmental factors may influence both plant and nematode. They may influence the nematode in the soil, soil water, and in the plant. A good understanding of these interactions are needed for the development of nematode pest management program.

## LITERATURE CITED


Bergeson, G. B. 1959. The influence of temperature on the survival of some species of the genus Meloidogyne, in the absence of a host. Nematologica 4:344-354.

Bird, A. F. and H. R. Wallace. 1965. The influence of temperature on Meloidogyne hapla and M. javanica. Nematologica 11:581-589.

Bird, A. F. 1972. Influence of temperature on embryogenesis in Meloidogyne javanica. J. Nematology 4:206-213.

Dao, D. F. 1970. Climatic influence on the distribution pattern of plant parasitic and soil inhabiting nematodes. Meded. Landbouwhogeschool Wageningen 70-2, 181 pp.

Ferris, H. and M. V. McKenry. 1974. Seasonal fluctuations in the spatial distribution of nematode populations in a California vineyard. J. Nematol. 6:203-210.

Franklin, M. T., S. A. Clark and J. A. Course. 1971. Population changes and development of Meloidogyne naasi in the field. Nematologica 17:575-590.

Hough, A. and I. J. Thomason. 1975. Effects of Aldicarb on the behavior of Heterodera schachtii and Meloidogyne javanica. J. Nematol. 7:221-228.

Jones, F. G. W. 1960. Some observations and reflections on host finding by plant nematodes. Deel XXV, No. 3-4:1009-1024.

Lyons, J. M., A. D. Keith, and I. J. Thomason. 1975. Temperature-induced phase transitions in nematode lipids and their influence on respiration. J. Nematol. 7:98-104.

Marks, C. F., I. J. Thomason and C. E. Castro. 1968. Dynamics of the permeation of nematodes by water, nematocides and other substances. Exp. Parsitol. 22: 321-337.

Milne, D. L. and D. P. De Plessis. 1964. Development of Meloidogyne javanica (Treub.) Chit. on tobacco under fluctuating soil temperatures. S. African J. Agric. Sci. 7:673-680.

Patrick, Z. A., R. M. Sayre, and H. J. Thorpe. 1965. Nematicidal substances selective for plant-parasitic nematodes in extracts of decomposing rye. Phytopathology 55:702-704.

Raski, D. J. and B. Lear. 1962. Influence of rotation and fumigation on root-knot nematode populations on grape replants. Nematologica 8:143-151.

O'Bannon, J. H. and H. W. Reynolds. 1961. Root-knot nematode damage and cotton yields in relation to certain soil properties. Soil Sci. 92:384-386.

Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne sp.) Univ. Md. Agric. Exp. St. Bull. A-77.

Sayre, R. M. 1964. Cold-hardiness of nematodes. I. Effects of rapid freezing on the eggs and larvae of Meloidogyne incognita and M. hapla. Nematologica 10:168-179.

Siddiqui, I. A., S. A. Sher and A. M. French. 1973. Distribution of plant parasitic nematodes in California. Dept. Food and Agric., Sacramento, Calif., 324 pp.

Stolzy, L. H. and S. D. Van Gundy. 1968. The soil as an environment for microflora and microfauna. Phytopathology 58:889-899.

Thomason. I. J. and B. Lear. 1961. Rate of reproduction of Meloidogyne spp. as influenced by soil temperature. Phytopathology 51:520-524.

Thomason, I. J., S. D. Van Gundy, and J. D. Kirkpatrick. 1964. Motility and infectivity of Meloidogyne javanica as affected by storage time and temperature in water. Phytopathology 54:192-195.

Van Gundy, S. D., A. F. Bird and H. R. Wallace. 1967. Aging and starvation in larvae of Meloidogyne javanica and Tylenchulus semipenetrans. Phytopath. 57: 559-571.

Van Gundy, S. D. and L. H. Stolzy. 1961. Influence of soil oxygen concentrations on the development of Meloidogyne javanica. Science 134:665-666.

Viglierchio, D. R. and B. F. Lownsbery. 1960. The hatching response of Meloidogyne species to the emanations from the roots of germinating tomatoes. Nematologica 5:153-157.

Wallace, H. R. 1966a. Factors influencing the infectivity of plant parasitic nematodes. Proc. Royal Soc., B, 164:592-614.

Wallace, H. R. 1966b. The influence of moisture stress on the development, hatch and survival of eggs of Meloidogyne javanica. Nematologica 12:57-69.

Wallace, H. R. 1968a. The influence of aeration on survival and hatch of Meloidoygne javanica. Nematologica 14:223-230.

Wallace, H. R. 1968b. The influence of soil moisture on survival and hatch of Meloidogyne javanica. Nematologica 14:231-242.

Wallace, H. R. 1969. The influence of nematode numbers and of soil particle size, nutrients and temperature on the reproduction of Meloidogyne javanica. Nematologica 15:55-64.

Whitehead, A. G. 1969. The distribution of root-knot nematodes (Meloidogyne spp.) in tropical Africa. Nematologica 15:315-333.

Wong, T. K. and W. F. Mai. 1973. Meloidogyne hapla in organic soil: effects of environment on hatch, movement, and root invasion. J. Nematol. 5:130-138.

K. DISEASE COMPLEXES IN TOBACCO INVOLVING NEMATODE AND FUNGAL PATHOGENS - Dr. N. T. Powell

Flue-cured tobacco of the genus Nicotiana grown in North Carolina, as well as in other southeastern states in the U.S.A., is constantly subjected to a number of important soil-borne diseases. Among the pathogens which cause these diseases are highly destructive fungi, bacteria and nematodes. These pathogens, for the most part, are perfectly capable of causing damage to plants without assistance from other organisms.

However, it has been known for some time that when certain pathogens exist together, the destruction is often much greater than when either organism occurs alone. This is especially true when one of the pathogens is the root-knot nematode (Meloidogyne incognita). For example, damage from black shank (caused by Phytophthora parasitica var. nicotianae), bacterial wilt (caused by Pseudomonas solanacearum) or Fusarium wilt (caused by Fusarium oxysporium f. nicotianae) is greatly magnified if root-knot nematodes are present along with the causal agents of these other diseases. The nematodes are capable of predisposing plants to infection by other organisms. The knowledge that root-knot nematodes have a profound influence on the expression of other diseases raises the possibility that many subtle interactions could exist involving, along with nematodes, fungi which have never been suspected before. It is well known that root knot-infected roots "break down" or decay in the field much quicker than do non-infected roots. It is unlikely that the nematodes alone inflict this damage. Could not disease complexes contribute to this breakdown? This possibility was tested in experiments using M. incognita along with several cosmopolitan soil fungi. These fungi included Rhizoctonia solani and Pythium ultimum, both of which are capable of causing seedling damping-off in tobacco soon after transplanting. R. solani inflicts a very sporadic and unimportant stalk disease known as "soreshin" under certain conditions. However, neither of these fungi has been considered as an important tobacco pathogen, and certainly not a root pathogen

after plants have progressed beyond juvenile stages.

Five additional soil-borne fungus genera, none of which are known to attack tobacco, were tested in combination with M. incognita to determine whether the nematode rendered tobacco roots susceptible to invasion by such microorganisms. The fungi included were Curvularia sp. and Botrytis sp. - pathogens of certain crops other than tobacco under some conditions; Aspergillus sp. and Penicillium sp. - pathogenic on certain crops, but rarely regarded as important pathogens of growing plants; and Trichoderma sp. - a very common soil inhabitant, but not regarded as pathogenic on plants.

This report includes a series of different experiments using the various fungi in combination with M. incognita. Methodology differed slightly with certain fungi and these differences are noted appropriately. The overall objective, however, was to resolve whether certain soil inhabiting fungi, which are seemingly unimportant on tobacco can become parts of complexes with root-knot nematodes and contribute to economic damage.

M. incognita and R. solani: Root necrosis did not differ among any of the varieties when they were inoculated with the fungus alone, indicating that these varieties do not differ in reaction to R. solani. Also, no appreciable differences in root necrosis were noted following exposure to the fungus after artificial wounding. M. incognita-resistant "N. C. 95" developed less necrosis than nematode susceptible varieties when the nematodes were applied alone.

In treatments wherein M. incognita and R. solani were combined, the roots of nematode-susceptible varieties were consistently more necrotic than resistant "N. C. 95". Decay was especially severe in nematode-susceptible plants inoculated with R. solani 3 weeks after inoculation with M. incognita. Little damage occurred in roots of "N. C. 95" with this treatment.

In general, roots of susceptible varieties were more necrotic than the resistant variety in treatments wherein nematodes preceded the fungus by 10 days. Differences were less pronounced with simultaneous inoculation with the nematode

and fungus, although nematode-susceptible roots were more necrotic than those of "N. C. 95".

Histological observations revealed extensive fungal colonization of roots in treatments in which nematodes were allowed to become well established before fungal inoculation. Hyphal strands were not restricted posteriorly or anteriorly in relation to the nematodes. Vigorous hyphae encircled the nematode and were concentrated within the locus of nematode infection. No evidence of fungal invasion was observed in M. incognita-resistant "N. C. 95" in any treatment.

M. incognita and Pythium or Trichoderma: Plants were inoculated with these organisms and observed for disease symptoms, both below- and above-ground, throughout the course of the experiments. Non-inoculated control plants remained healthy. Also, no symptoms of above-ground plant parts were observed in plants inoculated with either Pythium or Trichoderma alone. Leaves of plants receiving M. incognita remained green, although these plants were slightly stunted.

Plants inoculated with either Pythium or Trichoderma after root-knot nematodes developed symptoms above-ground four weeks after fungus inoculation. Plants were stunted and became chlorotic; leaves and internodes were reduced in size when compared to non-inoculated plants. These symptoms were followed by permanent wilting of the leaves. Those plants exposed to Trichoderma after nematodes developed more severe symptoms than those receiving Pythium. In fact, some plants inoculated with Trichoderma were almost dead when the tests terminated.

No evidence of root damage was observed among control plants or among plants inoculated with either fungus alone. Roots which received only M. incognita were galled and developed slight necrosis as the experiment neared termination.

The first necrotic symptoms were seen in roots of plants jointly inoculated with both M. incognita and Pythium twenty-four days after inoculation with the fungus. However, roots inoculated with M. incognita and Trichoderma decayed faster than those exposed to nematodes and Pythium. Among plants receiving the former treatment, roots were severely decayed eighteen days after the first necrotic symptom was detected.

Root disease indices assigned when experiments were terminated confirmed observations made during the course of the investigations. The greatest necrosis was recorded in roots inoculated with M. incognita and Trichoderma, but those plants receiving the nematodes and Pythium were also severely decayed. No necrosis developed in roots inoculated with either fungus alone or in noninoculated control plants.

M. incognita and Curvularia, Botrytis, Aspergillus or Penicillium: Some of the plants receiving only M. incognita developed very slight necrosis. Plants inoculated with either Botrytis or Penicillium following M. incognita developed chlorotic symptoms above-ground two weeks after fungus inoculation. In addition, these plants were stunted and leaves wilted as the tests approached termination. Plants receiving Curvularia or Aspergillus after root-knot nematodes became chlorotic, but suffered little reduction in growth.

Root symptoms of all plants jointly inoculated with M. incognita and either of the fungi tested were severely necrotic when experiments were terminated. Roots receiving Botrytis and Penicillium were damaged more than those exposed to other treatments. Inoculation with Curvularia and Aspergillus following M. incognita also resulted in significant root necrosis.

Field trials have substantiated these findings, with combination inoculation treatments reducing income per acre by up to $800.00.

It is becoming increasingly more apparent that much damage to plants, previously ascribed to the activity of plant parasitic nematodes is due to disease complexes. This may be particularly applicable in situations involving root-knot nematodes in tobacco. This impression does not tend to lessen the economic significance of plant parasitic nematodes as plant pathogens; rather, it strengthens and emphasizes their importance. It does appear, however, that root-knot nematode damage in tobacco reaches its zenith when these pathogens are components of complexes.

One of the most meaningful aspects of the work contained in this report is the evidence that root-knot nematode infection is capable of elevating normally minor pathogens on a particular host to major status. For example, neither Pythium nor

Rhizoctonia are generally regarded as important pathogens of tobacco roots after plants have progressed beyond juvenile stages, although they are recognized as causal agents of damping-off of young seedlings. If, however, plants have been previously infected by root-knot nematodes (three or four weeks in these tests), the roots become very susceptible to invasion and decay by these fungi. On the other hand, they appear virtually harmless on these tobacco roots unless they have been predisposed by prior nematode invasion. Tests including M. incognita along with fungi which are not regarded as pathogens on tobacco further substantiate the importance of nematodes in disease complexes. Curvularia, Botrytis, Aspergillus, and Penicillium are not "tobacco pathogens" in the typical sense of this term. Furthermore, Trichoderma has virtually no plant pathogenic reputation at all. However, if a tobacco root system has been previously colonized by root-knot nematodes, each of these fungi can invade this altered root system and induce extensive necrosis. In fact, damage by Trichoderma is as great under these conditions as that by a recognized pathogenic fungus, such as Pythium.

These observations lead to the conclusion that root-knot nematode infections on certain hosts effectively predispose these roots to subsequent infection by whatever other microorganisms might be present in the rhizosphere. These results suggest that this is true regardless of whether the subsequent invader is capable of attacking the host under other conditions.

Questions are provoked about established host range descriptions when one realizes the possibilities in complex diseases. Presumably, host ranges for most pathogens have been based on inoculations or observations under monopathogenic conditions and fail to consider possible predisposition factors. The successful colonization of tobacco roots altered by root-knot nematode infection by microorganisms which, ostensibly, do not attack that host stresses the validity of these questions.

Accurate disease diagnosis is obviously complicated when interactions are present. A tendency to ascribe a particular disease syndrome to a single pathogen must be avoided. It is possible that failure of disease control practices in certain

situations could be due to incomplete diagnosis of disease complexes, resulting in incomplete or inappropriate control treatment.

The nature of biopredisposition by root-knot nematodes is not defined in this work. However, it is obvious that the most pronounced changes are physiological rather than mechanical. The observation that roots are maximally predisposed to fungus invasion after nematodes have been present for a period of time substantiates this. The extensive decay of galls by soil microorganisms also indicates that these areas have been physiologically altered.

Disease complexes deserve the careful attention of plant pathologists. After all, the complex situation must be the normal case in nature. It would seem naive to give primary emphasis to plants influenced by a single pathogen, when, under natural growing conditions, this is indeed rare.

## L. PROGRESS, PROBLEMS AND PERSPECTIVES IN BREEDING FOOD CROPS FOR ROOT KNOT RESISTANCE - Dr. George Fassuliotis

Root-knot nematodes are a major depressive factor in food production. The high cost of control with soil fumigants makes breeding and selection a practical, economical and environmentally acceptable approach to combat this problem. Many resistant crops are being grown in infested fields because of the progress made over the past 50 years. To date there are available over 152 cultivars or breeding lines of vegetable and field crops with resistance to one or more species of nematodes.

The USDA and many state experiment stations are giving much support to breeding programs to develop nematode resistant crops. Many of these programs were in effect even before the controversial book "Silent Spring" was ever conceived.

Most nematologists eventually become involved, directly or indirectly, in a plant breeding program. Breeding for root-knot nematode resistance involves the same basic principles as in breeding for resistance to other diseases. In developing a breeding program for nematode resistance, it is advisable that the nematologist work closely with the breeder so that each could appreciate the other worker's problems. The role of the nematologist in such a program may be characterized as follows:

a) define and assess the root-knot nematode problems

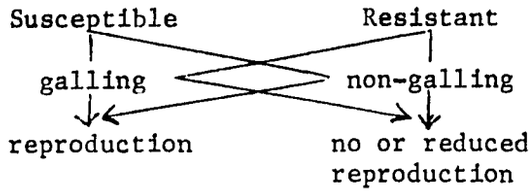
b) identify the species of root-knot nematodes involved in crop decline

c) establish and propagate pure populations of the species for use as inoculum

d) establish reliable screening techniques to eliminate chances of escapes

e) screen cultivars, accessions, related wild species, and unrelated species for sources of resistance

f) define the nature of resistance. Reactions to infection may be diagrammed thus:




Progress in breeding vegetables for root knot resistance has been extremely encouraging as we can see by the number of cultivars listed in Table 1. Cultivars of beans, lima beans, pepper, southernpeas, soybeans, and sweetpotato are currently available. Most are resistant to M. incognita, but resistance to M. arenaria and M. javanica is also found in some of these cultivars.

Progress in breeding for resistance cannot be discussed without reference to some of the problems that have confronted breeders and nematologists. Root-knot nematodes have a very wide host range making the search for resistance difficult. One of the major problems hampering development of resistant cultivars in more food crops is the apparent absence of resistant germplasm from which selections can be made or that can be crossed readily with the susceptible commercial cultivar. The following account will demonstrate the type of problems encountered in the tomato improvement program. The resistant genome in Lycopersicon peruvianum could not be used until the incompatibility barriers to hybridization with L. esculentum were overcome. When pollen from L. peruvianum was placed on the stigma of L. esculentum, well-shaped fruit could be produced on the tomato plant; however, the ovules aborted and failed to produce viable seeds. An interspecific cross was obtained after the sterility barriers were broken with embryo culture. Backcrossing to an L. esculentum parent also presented problems because the $F_1$ was practically self-sterile. After a backcross was eventually achieved, tomatoes containing the Mi gene for resistance were developed. So far as known, all resistant tomato cultivars derive their resistance from a cross "Michigan State Forcing" x L. peruvianum, PI 128657.

Resistance in beans, southernpeas, peppers, soybeans and sweet potato is readily achieved since resistant gene(s) are available within the crop species and can be crossed with commercial cultivars. There is a noticeable lack of resistance in the Cucurbita, however, several wild species of Cucumis (C. anguria, C. longipes,

C. ficifolius, C. heptadactylus, and C. metuliferus) have been reported as resistant to M. incognita. Attempts to cross these species with cantaloupe or cucumber have been unsuccessful. Fruit can be occasionally produced on the wild species when pollinated with C. melo but the seeds are not viable. We are currently exploring the use of embryo culture to break the sterility barriers.

No adequate levels of resistance to root-knot nematodes has been found in eggplant, Solanum melongena; it is available in the wild species, S. sisymbriifolium. Incompatibility barriers prevent hybridization of the latter species with eggplant. To break these barriers we are attempting to produce a somatic hybrid by fusion of eggplant with S. sisymbriifolium protoplasts. This approach for overcoming natural barriers to sexual hybridization between the wild species and commercial types is still in its infancy and many technical details remain to be worked out. However, current research indicates that this technique is feasible for crop improvement. Somatic hybridization involves (a) determination of cultural requirements for callus growth and differentiation of the plant species, (b) isolation of protoplasts from either callus or plant tissues, (c) fusion of protoplasts, (d) regeneration of fused protoplasts into a callus, and (e) induction of organogenesis of the hybrid callus to produce a whole plant. In our preliminary work with eggplant and the resistant wild species we have thus far been successful in regenerating whole plants from callus and have been able to obtain fusion of protoplasts. There seems to be no standard method for the isolation and culture of protoplasts and regeneration of plants from callus. Each tissue and cell-type poses special problems which must be overcome by empirically adjusting procedures.

Some of the other more common problems familiar to nematologists have been presented in several excellent reviews covering the subject of host resistance. These are:

a) Variability of pathogenicity among populations of a species

b) Development of new pathotypes after successive cropping with a resistant crop

c) Instability of resistance factors to high soil temperatures

d) Extreme hypersensitivity reactions by resistant plants to infection which may stunt or kill the plant

e) Increase in susceptibility to other diseases and nematodes

f) Non-acceptance of new cultivars because of traditional culinary customs

g) Linkage of undesirable horticultural traits to resistance

PERSPECTIVES: An important element in breeding for nematode resistance is the availability of resistant genes. There is enormous potentiality for development of resistant vegetable crops through a combination of conventional breeding methods and new breeding technology. With an increase in the number of nematologists throughout the world concerned with increasing food production, more effort is being expended into seeking sources of resistance.

There is at present a surge of research activity in the field of developmental botany. Plant tissue and cell culture techniques will eventually play a key role as an adjunct to conventional breeding techniques for crop improvement. The nematologist and plant breeder must be aware of these new developments and utilize this knowledge for incorporating root knot resistance into food crops. It will be essential to utilize these techniques especially where resistance is found only in wild species that cannot be crossed with commercial types.

Advances are being made in the hybridization of protoplasts of widely divergent species. It is not inconceivable that we may be able to transfer whole genomes from a resistant plant to a susceptible plant to acquire a hybrid that conventional breeding methods fail to achieve. The potentials of protoplast hybridization are enormous, and we as nematologists interested in crop improvement must aggressively seek out new methods to achieve our goals.

## Selected References

1. Bingefors, S. 1971. Resistance to nematodes and the possible value of induced mutations. In Mutation breeding for disease resistance, Interntl. Atomic Energy Agency 209-235.

2. Good, J. M. 1972. Management of plant parasitic nematode populations. Proc. Tall Timers Conf. on Ecological Animal Control by Habitat Management, Tallahassee, Florida, Feb. 1972, 109-127.

3. Kehr, A. E. 1975. New developments in plant cell and tissue culture. HortScience 10:4-5.

4. Malo, S. E. 1964. A review of plant breeding for nematode resistance. Proc. Soil and Crop Science of Florida 24:354-365.

5. Sasser, J. N. 1972. Managing nematodes by plant breeding. Proc. Tall Timbers Conf. on Ecological Animal Control by Habitat Management, Tallahassee, Florida, Feb. 1972, 65-80.

6. Singh, B., D. S. Bhatti and K. Singh. 1974. Pest Articles and News Summaries 20:58-67. Haryana Agricultural Univ., Hessar, India.

TABLE 1 Vegetable Cultivars Resistant to *Meloidogyne* spp. (root-knot nematodes)

| Crop and Cultivar | Species | | | | References |
|---|---|---|---|---|---|
| | aren | hap | inc | jav | |
| BEANS (COMMON) | | | | | |
| Alabama #1 | | | x | | 1 |
| Alabama #18 | | | x | | 1 |
| Alabama #19 | | | x | | 1 |
| Coffee Wonder | | | X | | 1 |
| Isbell's Nematode Resistant | | | x | | 1 |
| Manoa Wonder | | | x | | 2 |
| Springwater Half Runner | | | x | | 1 |
| Wingard Wonder | | | x | | 1 |
| BEANS (LIMA) | | | | | |
| Hopi 5989 | | | x | | 3 |
| Nemagreen | | | x | | 4 |
| Western | | | x | | 3 |
| White Ventura N | | | x | | - |
| PEPPER | | | | | |
| Burlington | x | | | | 6 |
| California Wonder Special | x | | | x | 6 |
| Early California Wonder | | | | x | 6 |
| Nemaheart | x | | x | x | - |
| Oakview Wonder | x | | | x | 5 |
| Red Chile | x | | x | x | 5 |
| Ruby King | x | | | | 6 |
| Santanka x S | x | | x | x | 6 |
| SOYBEANS (EDIBLE) | | | | | |
| Mokapu Summer | | | x | | 7 |
| Kailua | | | x | | 7 |

| Crop and cultivar | Species | | | | References |
|---|---|---|---|---|---|
| | aren | hap | inc | jav | |
| SOYBEANS (Continued) | | | | | |
| Kaikoo | | | x | | 7 |
| Kahala | | | x | | 7 |
| SOUTHERNPEAS (COWPEAS) | | | | | |
| Blackeye 5 | | | x | | 11 |
| Blackeye 7 | | | x | | 11 |
| Brabham Victor | | | x | | VL 1/ |
| Browneye 7 | | | x | | 11 |
| Browneye 9 | | | x | | 11 |
| Brown Seeded Local | x | | x | x | 8 |
| Calif. Blackeye No. 5 | | | x | | VL |
| Chinese Red | | | x | | 11 |
| Chino 2 | | | x | | 11 |
| Clay | | | x | | VL |
| Colossus | | | x | | VL |
| Early Red | | | x | | 11 |
| Early Sugar Crowder | | | x | | 11 |
| Floricream | | | x | | VL |
| Groit | | | x | | 11 |
| Iron | x | x | x | x | 10 |
| Iron 3-5 | | | x | | 11 |
| Iron 9-1 | | | x | | 11 |
| Iron 9-10 | | | x | | 11 |
| Magnolia Blackeye | | | x | | VL |
| Miss. Crowder | x | | x | x | 8 |
| Miss. Purple | | | x | | VL |
| Miss. Silver | | | x | | VL |

| Crop and cultivar | Species | | | | References |
|---|---|---|---|---|---|
| | aren | hap | inc | jav | |
| SOUTHERNPEAS (Continued) | | | | | |
| New Era | | | x | | 11 |
| Purple Hull Pink Eye | x | | x | x | 8 |
| Red Ripper | | | x | | 11 |
| Rice | | | x | | 11 |
| Suwanne | | | x | | 11 |
| Victor | | | x | | 11 |
| Zipper Cream | | | x | | VL |
| SWEET POTATOES | | | | | |
| Acadian | x | | | | 16 |
| Apache | | | x | | 18 |
| Allgold | x | | | | 16 |
| Buster Haynes Red | | | x | | VL |
| Centennial | x | | | | 16 |
| Goldrush | x | | | | 16 |
| Heartogold | x | x | x | x | 12, 14, 15 |
| Hopi | | | x | | 18 |
| Jasper | | | x | | VL |
| Jewel | | | x | | 16 |
| Kandee | | | x | | VL |
| Keyline White | | | x | | VL |
| Maryland Golden | x | | | x | 19 |
| Nemagold | | | x | | 12 |
| Nugget | | | x | | 18 |
| Orlis | | | x | | 13 |
| Porto Rico | x | | | | 16 |

| Crop and cultivar | Species | | | | References |
|---|---|---|---|---|---|
| | aren | hap | inc | jav | |
| SWEET POTATOES (Continued) | | | | | |
| Red Jewel | | | x | | VL |
| Sunnyside | | | x | | 18 |
| Tinian (PI 153655) | | | x | x | 15 |
| Whitestar | | | x | | 18 |
| White Bunch | | | x | | VL |
| White triumph | | | x | | VL |
| TOMATOES | | | | | |
| Anahu | | | x | x | 34 |
| Anahu R | | | x | | 23 |
| Atkinson | | | x | x | 24, 31 |
| Beefeater | | | x | | 26 |
| Beefmaster | | | x | | 26 |
| Better Boy | | | x | | 33 |
| Big Seven | | | x | | 33 |
| Bigset (H) | | | x | | VL |
| Bonus (H) | | | x | | 33 |
| BWN-21-F1 | | | x | | VL |
| Calmart | | | x | | VL |
| Coldset | | | x | | 30 |
| Florida-Hawaii Cross | | | x | | 34 |
| Gawaher (Giza-1) | | | x | x | 28 |
| Gilestar | | | x | | 34 |
| Hawaii-55 | | | x | | 34 |
| Healani | | | x | x | VL, 31 |
| Kalohi | | | x | x | 34, 31 |

| Crop and cultivar | Species | | | | References |
|---|---|---|---|---|---|
| | aren | hap | inc | jav | |
| TOMATOES (Continued) | | | | | |
| Kewalo | | | x | | VL |
| Kolea | | | x | | VL |
| Merbein Canner | | | x | | 34 |
| Merbein Early | | | x | | 34 |
| Merbein Mid-Season | | | x | | 34 |
| Merbein Monarch | | | x | | 34 |
| Monte Carlo | | | x | | 34 |
| Nematex | x | | x | x | 20 |
| N-52 (H) | | | x | | VL |
| Nemared | | | x | | 22 |
| Pelican | | | x | | 25 |
| Peto 662 VFN | | | x | | 33 |
| Puunui | | | x | | VL |
| Red Glow (H) | | | x | | 33 |
| Ronita | | | x | | VL |
| Roodeplaat Albesto | | | x | | 27 |
| Rossol | | | x | | VL |
| Small Fry | | | x | | 30 |
| Sunburst | | | x | | 26 |
| Terrific (H) | | | x | | 33 |
| Tuckcross K | | | x | | VL |
| Vine Ripe | | | x | | 26 |
| VFN Bush | | | x | | VL |
| VFN-8 | x | | x | x | 32 |
| VFN 368 | | | x | | VL |
| PI 270435 L. peruvianum | | x | | | 21 |

# REFERENCES


## Beans

1. Blazey, D. A. et al. 1964. Nematode resistance in the common bean. J. Heredity 55:20-22.

2. Hartmann, R. W. 1968. Manoa Wonder, a new root-knot nematode resistant pole bean. Hawaii Agr. Exp. Sta., Univ. of Hawaii Cir. 67, 12pp.

3. Allard, R. W. 1954. Sources of root-knot nematode resistance in lima beans. Phytopathology 44(1):1-4.

4. Wester, R. E., H. B. Cordner, and P. H. Massey, Jr. 1958. Nemagreen-- a new lima. Amer. Veg. Grower, May.

## Pepper

5. Hare, W. W. 1956. Resistance in pepper to Meloidogyne incognita acrita. Phytopathology 46:98-104.

6. Hare, W. W. 1957. Inheritance of resistance to root-knot nematodes in pepper. Phytopathology 47:455-459.

## Soybean

7. Gilbert, J. C., J. T. Chinn, and J. S. Tanaka. 1970. Four new tropical vegetable-type soybeans with root-knot nematode resistance. Hawaii Agr. Exp. Sta., Univ. of Hawaii, Res. Report 178, 7 pp.

## Southernpeas

8. Choudhury, B., R. Rajendran, B. Singh, and T. S. Verma. 1969. Breeding tomato, brinjal and cowpea resistant to root-knot nematodes (Meloidogyne spp.). All India Nematology Symp., New Delhi, India 46-47 (abstract).

9. Hare, W. W. 1959. Resistance to root-knot nematodes in cowpea. Phytopathology 49:318. (Abstract).

10. James, M. 1969. Nine varieties of southern peas resistant to the soybean cyst nematode. Plant Dis. Reptr. 53(4):245.

11. Thomason, I. J. and H. E. McKinney. 1960. Reaction of cowpeas, Vigna sinensis, to root-knot nematodes, Meloidogyne spp. Plant Dis. Reptr. 44(1):51-53.

## Sweet potatoes

12. Cordner, H. B., F. B. Struble, and L. S. Morrison. 1954. Breeding sweet potatoes for resistance to the root-knot nematode. Plant Dis. Reptr. Supplement 227:92-93.

13. Elmer, O. H. 1950. Sweet potatoes in Kansas. Kan. Agr. Exp. Sta. Bull. 341, p. 10-11.

14. Elmer, O. H. 1958. The sweet potato variety Kandee. Kan. Agr. Exp. Sta. Rept. 9-14.

15. Gentile, A. G., K. A. Kimble, and G. C. Hanna. 1962. Reactions of sweetpotato breeding lines to Meloidogyne spp. when inoculated by an improved method. Phytopathology 52:1225-1226.

16. Giamalva, M. J., W. J. Martin and T. P. Hernandez. 1960. Reaction of 8 sweet potato selections to 5 species of root-knot nematodes. Phytopathology 50:575.

17. Giamalva, M. J., W. J. Martin, and T. P. Hernandez. 1963. Sweetpotato varietal reaction to species and races of root-knot nematodes (Meloidoygne). Phytopathology 53:1187-1189.

18. Martin, W. J., L. W. Nielsen, and L. S. Morrison. 1970. Diseases. In Thirty Years of Cooperative Sweet Potato Research 1939-1969. Southern Coop Series Bull. No. 159, pp. 46-48.

19. Sasser, J. N. 1954. Identification and host-parasitic relationships of certain root-knot nematodes (Meloidogyne spp.). Md. Agr. Expt. Sta. Bull. A-77, 30 pp.

Tomatoes

20. Dropkin, V. H. 1969. The necrotic reaction of tomatoes and other hosts resistant to Meloidoygne: Reversal by temperature. Phytopathology 59: 1632-1637.

21. Dropkin, V. H., D. W. Davis, and R. E. Webb. 1967. Resistance of tomato to Meloidogyne incognita acrita and to M. hapla (root-knot nematodes) as determined by a new technique. Proc. Amer. Soc. Hort. Sci. 90:316-323.

22. Fassuliotis, G. and E. L. Corley, Jr. 1967. Use of seed growth pouches for root-knot nematode resistance tests. Plant Dis. Reptr. 51:482-486.

23. Gilbert, J. C., J. L. Brewbaker, J. S. Tanaka, J. T. Chinn, R. W. Hartmann, J. A. Crozier, Jr., and P. J. Ito. 1969. Vegetable improvement at the Hawaii Exp. Sta. Research Report 175, 16 pp.

24. Greenleaf, W. H. 1967. Atkinson--a root-knot and fusarium wilt resistant tomato variety of Rutgers class. HortScience 2:60.

25. Hernandez, T. P., A. Miller, A. J. Adams, R. T. Brown, W. Etzel. 1972. Pelican: a new root knot resistant tomato variety. Louisiana Agriculture 15:3, 16.

26. Jenkins, Jr., S. F. Disease resistance in vegetable varieties. Plant Pathology Information Note 188, North Carolina State University Mimeo publication, no date.

27. Joubert, T. G. and C. E. Rappard. 1971. Roodeplaat Albestoeelworm resistant tomato cultivar. Helminthological Abstracts Series B:33.

28. Moh, A. S., S. H. Nasar, and M. S. Attia. 1972. Gawaher (Giza-1), a tomato variety with resistance to the root-knot nematode. Agricultural Research Review, Arab Republic of Egypt 50:39-45. Fr. Plant Breeding Abstracts 43:7426.

29. Rebois, R. V., B. J. Eldridge, J. M. Good and A. K. Stoner. 1973. Tomato resistance and susceptibility to the reniform nematode. Plant Dis. Reptr. 57:169-172.

30. Sidhu, Gurmel and John M. Webster. 1973. Genetic control of resistance in tomato. I. Identification of genes for host resistance to Meloidogyne incognita. Nematologica 19(4):546-550.

31. Sikora, R. A., Sitaramaiah, K. and R. S. Singh. 1973. Reaction of root-knot nematode-resistant tomato cultivars to Meloidogyne javanica in India. Plant Disease Reptr. 57(2):141-143.

32. Singh, B. and C. Choudhury. 1973. The chemical characteristics of tomato cultivars resistant to root-knot nematode (Meloidogyne spp.). Nematologica 19(4):443-448.

33. Southards, C. J. 1973. A field evaluation of nematode resistant tomato varieties for vine-ripe tomato production. Tenn. Farm and Home Science 85:18-20.

34. Winstead, N. N. and R. D. Riggs. 1963. Stability of pathogenicity of B biotypes of the root-knot nematode Meloidogyne incognita on tomato. Plant Dis. Reptr. 47:870-871.

1/ VL = Tested at the Vegetable Research Laboratory, Charleston, S.C. No other reference located.

## M. INFLUENCE OF DIFFERENT AGRICULTURAL SYSTEMS ON CONTROL STRATEGIES FOR ROOT KNOT - Dr. W. F. Mai

I would like to emphasize the importance of root-knot nematodes in tropical areas by mentioning two personal experiences rather than by showing pictures or citing per cent loss figures.

When I was in Northeastern Brazil, I observed a tomato planting near Afogados in which the top symptoms resembled those caused by extremely poor root symptoms. Upon closer examination I found that the plants had essentially no roots and the scattered roots and the lower stems were heavily infected by root-knot nematodes. The plants were supported by stakes and in this very hot dry climate, the grower irrigated three times each day to supply sufficient water to the stems and scattered roots. The grower continued to grow tomatoes despite these very low yields because tomatoes are a favorite vegetable and thus sell for a high price and most of the local supply was grown 150-200 miles away and transported over unpaved roads. The most surprising finding was that the cause of this problem was not recognized by the grower, peace corps workers, or soil scientists from the U.S.A. In fact, a soil scientist had suggested some plant nutrition amendments as a possible remedy. By this extreme example, I would like to emphasize that losses caused by root-knot nematodes are even more grossly under-estimated because often even severe damage is unrecognized.

A second incident occurred in Jamaica where a retired Cornell horticulturist was trying to test the suitability of a number of white potato cultivars to the environmental conditions in Jamaica. Over a several-year period he had found that the second time he conducted a test on the same location the root-knot damage was so severe that very few marketable tubers were harvested from any cultivar. This occurred on land previously planted to trees and brush or land previously planted to a plantation crop such as sugar cane. This experience illustrates another

way that root-knot nematodes cause damage; the inability to grow food crops needed by the people of a country is an important loss caused by root-knot nematodes.

Today, I will discuss different nematode control practices such as: prevention of spread, land management, and cultural practices, biological control, plant resistance, control by physical factors, and control by chemicals. I will not discuss each practice singly but instead will attempt to indicate the role of each practice in actual or potential integrated control programs. The role of these control practices in pest management will also be emphasized. I use the term "integrated control" to indicate that more than one control practice is used to control a nematode disease. Knowingly or unknowingly in most successful control programs this has been done for a long time. By pest management, I mean that nematode populations are maintained at lower than damaging or economic threshold levels.

Because control strategies differ markedly for small and large growers, whenever possible, I will discuss these differences as well as the resulting differences in research needs. By a small grower, I am referring to one with 1 or 2 acres or less of land and growing crops chiefly for food. On the other hand a large grower uses more land and most or all of the food or fiber grown is sold.

The use of land management and particularly crop rotation to reduce nematode populations is one of the most effective and most widely used control practices. Most cultural procedures reduce a root-knot nematode population over a period of weeks, months or years as opposed to rapid kill obtained by nematicides or heat. Usually a satisfactory economic control cannot be obtained by a single cultural or land management practice but by a combination of them as well as by one or more additional control practices.

Controlling a single nematode by crop rotation is complicated by factors such as economic value of crops, soil type, need to consider the effect of crop rotation on control of other soil-borne diseases and insects, and manpower and equipment needs for growing specific rotation crops. Another problem is that a

crop rotation that reduces populations of a species of root-knot nematodes may increase populations of another nematode species. Despite these problems crop rotations are widely used by small and large growers.

Both the small grower and the large grower have valid reasons for not rotating. The small grower often owns or controls insufficient land to provide food, fiber and a little cash for his family. Not growing a favored food or cash crop for one or more years would result in economic hardship to the family. On the other hand the large grower must use high priced equipment and specialized manpower as effectively as possible and thus he tends to grow the most profitable crop as often as possible on the best land. As a result both small and large growers all over the world have severe root-knot nematode problems and as population pressures increase, the problems will become more severe.

Because of a lack of experimental data it is difficult to plan a crop rotation to control a single root-knot nematode species even though effects of the rotation other than nematode control are not considered. Information is needed on the rate of build-up of root-knot species on most crop plants, the influence of environmental and cultural practices on survival rate, and economic threshold levels for different crops. Because of the uneven distribution of root-knot nematodes in field soils and the difficulties in approximating field conditions and measuring yields in greenhouses or growth chambers perhaps the use of microplots in experiments would help answer some of these problems. This information is urgently needed for cash crops primarily grown by large growers and particularly for food crops primarily grown by small growers.

The use of root-knot nematode-free planting stock and other sanitation procedures are sure and effective means of nematode control and should be a part of any integrated control program. The cost of such practices is relatively low but both small and large growers continue to use nematode-infected transplants and other vegetative plant materials and thus spread root-knot nematodes into

uninfested fields and increase inoculum in infested fields.

Nematode-free materials are obtained by growing them under nematode-free conditions and by treating infected materials by heat or nematicides. Disinfecting plant materials is difficult or impossible for many of the small growers in developing countries. The most important problem is that in most cases he does not recognize the causal agent or control principle. If he recognizes the need for heat treatment he rarely has fuel to generate the heat and does not have equipment to maintain a constant temperature. There is usually a small difference between the temperature required to kill root-knot nematodes and the temperature that damages host tissue. Also most small growers do not have either sufficient cash or credit to purchase chemicals or knowledge to use them effectively. In fact, many small growers cannot read the labels and thus may use chemicals incorrectly so that personal injury occurs to members of the family. Developing procedures for obtaining nematode-free planting material is an area in which a government or international agency could give meaningful help to small growers. It is realized that, because of factors such as the large number of growers, their educational background, and general lack of transportation to inaccessible locations make the extension aspects of such a program formidable to say the least.

In large farming operations the owners or supervisors are often well trained in economics but are poorly trained or have no training in entomology, nematology, and plant pathology. As a result even inexpensive and uncomplicated control procedures are often not utilized.

The disposal of root-knot-infected roots after harvest is another relatively uncomplicated control procedure that should be used more extensively. It is particularly important in growing crops such as tobacco where the plant stays alive several weeks after harvest. One or two generations of root-knot nematodes may develop between harvest and such natural occurrences as frost. To prevent this buildup commercial tobacco growers in North Carolina and other areas cut the stalks

soon after harvest and the root system of the plant is then turned out and exposed. Thus the numbers of root-knot nematodes are reduced through the drying action of wind and sun.

In Afganistan many of the small growers use both top and roots of crops as fuel. It is frequently stated that because of this practice these growers have few or no root-knot nematode problems. Experimental data are needed to substantiate this broad statement. I was told that there was not a nematode problem on sugar beets in Afganistan. Within a month, however, report of a survey team from Great Britain stated that unless the sugar-beet cyst nematode was controlled the sugar-beet industry would essentially be lost. The survey was requested because sugar yields had been declining.

Obviously field experiments to test the effect of this procedure and modifications of it are needed in Afganistan and probably many other countries. Data from experiments on the effect of various ways of disposing of infected roots after harvest are urgently needed. Jim Starr and I in trying to obtain such information in connection with the northern root-knot nematode as a pathogen of lettuce have found that these kinds of data are not easy to obtain. A major difficulty is the unevenness of root-knot nematode populations under field conditions. Because of this factor and difficulties inherent to sampling populations of any organism in the soil large differences often are not statistically significant.

More large and small growers should use this control procedure. Although the small growers do not have machinery they often have considerable labor to carry out such a practice. In addition, the small grower often can use the debris for fuel. Research is needed to determine which procedures are effective in reducing root-knot populations and the most effective ways for both small and large growers to carry out these procedures.

Weeds and "volunteers" during the winter or dry season are important in maintaining and even increasing root-knot nematode populations. When all plants

are removed from an area, i.e. bare fallow is practiced, soil erosion is likely to occur during rainy seasons and blowing of top soil during dry seasons.

Recently valuable data have been obtained on the susceptibility of weeds in Nigeria and Georgia in the USA. More research is urgently needed in this area. Before results from such research can be applicable to wide geographic areas the root-knot species and the pathotype or race used in the tests must be known.

A better soil conservation measure than bare fallow is to grow a cover crop during the winter. An increase in populations of root-knot may occur if a susceptible crop is grown and a reduction if the cover crop is resistant. Low winter temperatures may limit nematode reproduction so that populations do not increase even though the cover crop is susceptible. With some cover crops the "trap-cropping" principle may be operative. In trap cropping the larvae enter the roots, and many develop into an immobile stage but fail to develop into adults; thus they are trapped within the roots.

The addition of organic matter resulting from the plowing under of cover crops increases the numbers and predacious activity of nematode-trapping fungi, predacious nematodes, and internal parasites of nematodes. Also, nematicidal substances, such as butyric acid, form during the decomposition of cover crops such as rye and timothy. No practical control of nematodes by predacious fungi, other biological agents, or toxic substances resulting from decomposition of organic residues is known.

Growers on Mainland China use a great deal of organic fertilizer. US scientists who have been to China reported that root-knot nematode problems were not evident. Perhaps a group of US nematologists should visit China to assess root-knot nematode problems and the relationship between the use of organic fertilizer and root-knot nematode damage. Also a group of Chinese nematologists would profit from a trip to the USA.

Factors responsible for the survival or mortality of root-knot nematodes in soil are not understood.

Cover crops resistant to root-knot nematodes are needed by both small and large growers. Because of the few available resistant cover crops research designed to develop new ones by plant breeding should be emphasized. For most areas a cover crop should be suitable for animal food.

Planting Date

Root-knot nematodes are inactive during the winter months because low temperatures inhibit their activities. By planting crops when the soil temperature is favorable for crop growth and unfavorable for nematode development it is often possible to obtain relatively high yields despite the presence of nematodes. Although other factors often determine planting time, whenever possible, this inexpensive control measure should be practiced by both small and large growers.

Potentially, the most effective method of controlling nematodes is the use of nematode-resistant or tolerant cultivars. At present, largely because of limited research in this area and difficulties in breeding for resistance to soil-borne organisms, relatively few nematode-resistant cultivars are available to either small or large growers.

An example of the neglect of research to obtain crops resistant to nematodes is the research program at the International Vegetable Research Institute in Taiwan. According to the most recent annual report no nematologist is on the staff and little or no research in nematology is being conducted at this Institute. Because considerable plant breeding is being conducted it would be economical to include a research program to develop nematode-resistant cultivars. As has happened at other locations in the past it is quite likely that a cultivar will be developed which will not be suitable for many locations because of its susceptibility to one or more species of root-knot nematode.

Resistance-breaking biotypes have been reported for some but not all root-knot nematodes. Usually resistance caused by more than one gene (horizontal resistance) is not as likely to "break down" as is resistance caused by one gene (vertical resistance). Thus resistance caused by more than one gene and tolerance (cultivars that yield well despite the presence of nematodes) should be emphasized in a breeding program.

In plant-breeding programs to control diseases caused by root-knot nematodes and microorganisms such as fungi or bacteria, it is important to breed for resistance to all organisms but particularly for resistance to nematodes. In such complex diseases there is usually more growth response from controlling nematodes than from controlling the secondary organisms in the disease complex.

It is frequently stated that nematicides are too expensive for use by small growers in developing countries. Nematicides are widely used by large growers in tropical areas to control nematodes attacking plantation crops such as cotton. There is no question that the price of nematicides is often higher in a tropical country than in industrial nations in which the nematicides are manufactured and small growers have little cash or credit. It appears, however, that to control at least some nematode diseases a small quantity of nematicide must be used in addition to other control measures, such as land management and cultural practices and plant resistance or tolerance.

In order to use a small quantity of nematicides wisely more information is needed concerning methods and timing of applications. As an aid in selecting application methods more information is needed concerning kinds of nematode damage that actually cause substantial yield losses in contrast to symptoms caused by nematodes. It is generally considered that damage to roots of young seedlings results in higher yield reductions than damage to roots of older seedlings. Thus it might be economical for a small grower to buy a nematicide to control nematodes attacking roots of young seedlings but not to control nematodes attacking roots

of older seedlings. A nematicide applied to obtain a high per cent kill of nematodes attacking roots of young seedlings would have the greatest possibility of being practical. In other words there is a need for "cost analysis" of nematode control procedures. In some countries such as West Pakistan, to increase food production, the government buys pesticides and in some cases applies them for growers. As far as I know nematicides were not involved in this program.

Relatively nonvolatile nematicides applied as granular formulations which can be safely used by workers without complicated machinery are urgently needed by small growers. Also needed are systemic nematicides which can be safely applied as sprays by small growers to kill root-knot nematodes. New, safe, effective, and easy methods to apply nematicides must be developed for these small growers. Although new nematicides should be field tested, such research must not become a substantial per cent of an investigator's research effort. This situation is likely to occur when support from chemical companies constitutes a major part of the total research support. Funds and encouragement are urgently needed for high quality field research on ways to make small dosages of nematicide more effective and on nonchemical control measures.

For most small growers, in order to make the use of nematicides economical, other control measures must be used in an integrated control program to reduce root-knot nematode populations. These additional control procedures should include as many as possible of the following:

1) rotation of resistant and susceptible food crops
2) use of root-knot resistant cover crops
3) use of root-knot free vegetative planting material
4) use of planting date least suitable for root-knot nematode reproduction and pathogenicity
5) use of most resistant and/or tolerant variety
6) removal and destruction of infected plant parts

If root-knot nematode populations are too high at the time of nematicide application, particularly with low dosages, the treatment is likely to be totally ineffective. For field treatments an 80-85% kill is better than average. If pre-treatment populations are so high that even after 85% kill they are at or above the damaging level yield losses will be high.

In conclusion, I have spent far more time discussing problems of small growers than large growers in developing countries. This was done because I am convinced that there is a more urgent need to solve problems of small growers and they are far more difficult to solve. I consider this to be the greatest challenge to nematologists of the world. Before we have any chance to solve these problems we need results from high-level field research and new extension techniques. Such research must be supported but just as important nematology students must be trained to do this difficult research and to consider it important and basic.

DISCUSSION:

Sasser - Biological control, trap crops, and other control methods have been reported, but aren't many of them really impractical in and by themselves?

Mai - Generally, but you must define what's practical for North Carolina as compared to what's practical in the Philippines or Kenya.

Sasser - Isn't there a need for nematode-free planting stock; there are many examples of problems arising from nematode-infested planting stock. This is a practical, solvable problem, but probably requires governmental support.

Mai - Yes.

Starr - With the development of effective crop rotation schemes, isn't it possible that it may be necessary to introduce new crops into a region. This could be a difficult problem, i.e., attempting to change local customs.

Ngundo - With the small land holdings of most people in Kenya crop rotation may be difficult. Growers can't afford to have unproductive land.

Van Gundy - Many of these programs will require an effective advisory service, these are generally not available. A problem that limits the use of resistant cultivars is that they are so quickly overcome by the pathogens.

Madamba - Many root-knot nematode problems of the tropics and subtropics may be due to the long growing seasons, and, thus, continuous croppings. More work needs to be done in developing rotation programs which utilize tropical crops. There are also many barriers to the use of chemicals; there is often government opposition to importing foreign-made chemicals, which may be increased in cost by ten times.

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