Root-Knot Nematodes: A Globa

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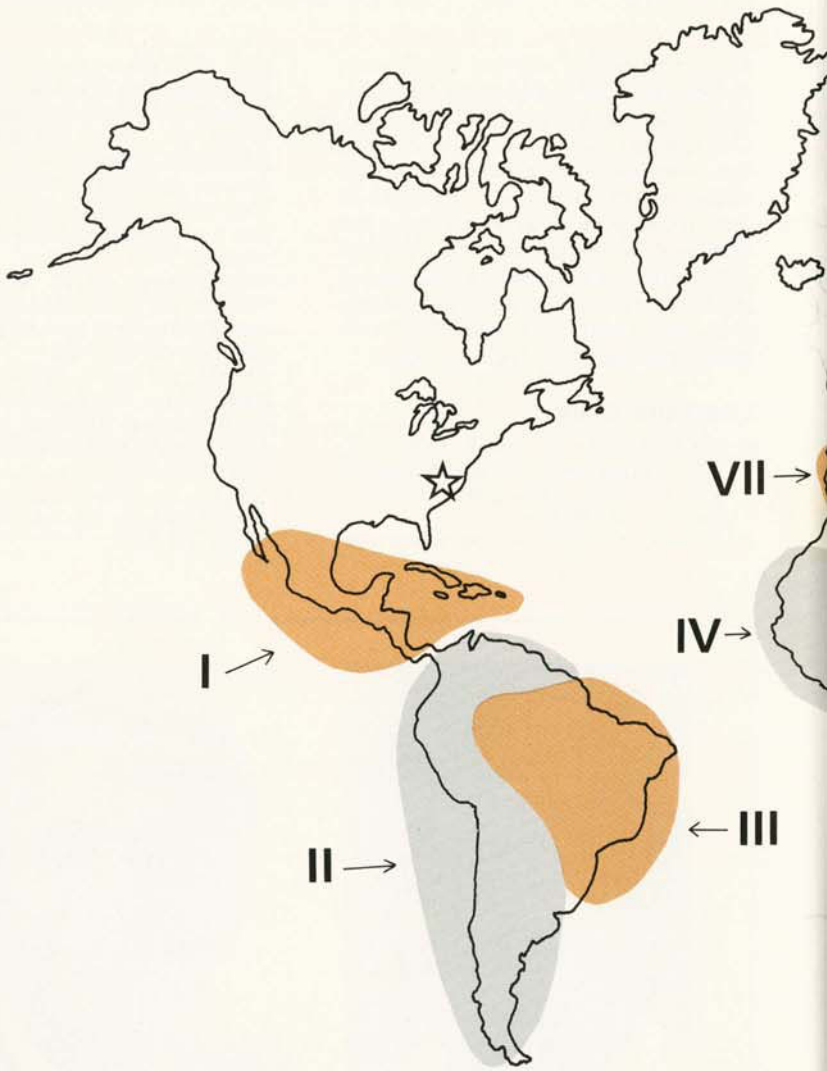

One of the major obstacles to the production of adequate supplies of food in many developing nations is the damage caused by plant-parasitic nematodes, especially the root-knot group, *Meloidogyne* spp. Their worldwide distribution, extensive host ranges, and involvement with fungi, bacteria, and viruses in disease complexes rank them among the top major plant pathogens affecting the world's food supply. Collectively, the various species of *Meloidogyne* attack nearly every crop grown. Not only are yields greatly affected but quality is also reduced, especially for root crops such as potatoes, yams, and peanuts (Fig. 1).

The literature on root-knot nematodes is extensive and contains significant contributions from many parts of the world. The purpose of this paper is not to review the literature but to present the activities and principal research findings of the International *Meloidogyne* Project (IMP). This worldwide view of the genus *Meloidogyne* has been made possible through funding by USAID.

The Beginning of IMP

In addition to being the most economically important group of plant nematodes, root-knot nematodes are the most well known. The characteristic galls produced on the roots of most susceptible plants are easily recognized. Much research has been conducted on the biology of this group of nematodes during the past several decades, leading to significant advances in understanding this complex group of plant pests.

Early studies on host ranges of the common species occurring in the southeastern United States revealed that individual species differed in their host preferences (5). This observation gave encouragement to the possibility that species could be separated on the basis of host response (4). The North Carolina differential host test has been in the process of development for many years. After it became somewhat dependable for identification of many of the populations from the United States, arrangements were made to assemble populations from other parts of the world at North Carolina State University for testing under uniform conditions. It was recognized that the problem of root-knot nematodes could not be solved by studying a few populations from a small geographical area. A better picture of the biological status of this group of nematodes could be acquired only from intensive study of many populations from various parts of the world. Thus, the idea for the International *Meloidogyne* Project was conceived.

Justification for the project was based primarily on the need to minimize the damage caused by these pests to impor-

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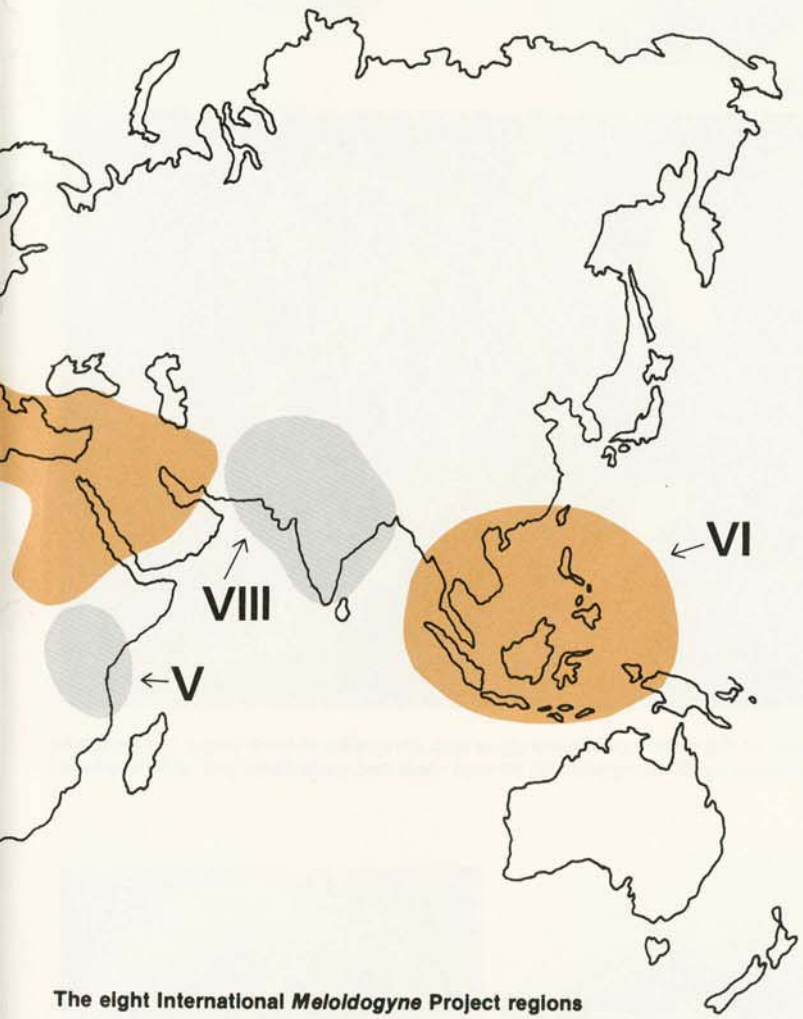
Menace to Crop Production



The eight International *Meloidogyne* Project regions

tant food and fiber crops in developing countries. Because these same nematode species are also important agricultural pests in the United States, especially in North Carolina and other southern states, several faculty from the School of Agriculture were already devoting a considerable portion of their time to research on control.

Phase I in implementing the International *Meloidogyne* Project began with organization of the Research Center at North Carolina State University and establishment initially of six overseas regional centers; two regions were added later. A "regional investigator" was selected to coordinate the program of research for 10–12 cooperators within each region.

Phase II involved bringing the regional investigators to North Carolina State University for a 1-wk planning conference, during which the scientific aspects of the project were discussed in detail. In addition to the regional investigators and Research Center scientists, six internationally known authorities on root-knot nematodes attended the conference. Up-to-date reviews were given on such important aspects of the genus as: 1) taxonomy and morphology, 2) ecology, 3) cytogenetics, 4) breeding for resistance, 5) disease complexes involving root-knot nematodes and fungal pathogens, and 6) influence of different agricultural systems on control strategies. Other topics, as well as details of discussions, are recorded in the proceedings of the conference (1).

Phase III consisted of holding research planning conferences in each of the eight regions (see map), with 10–12 "country cooperators" participating in each conference. Participants summarized the state of knowledge concerning the root-knot nematodes in their country and outlined research they could do in keeping with the goals and objectives of the International *Meloidogyne* Project. Special consideration was given to research methodology relating to the genus *Meloidogyne*, including collecting and maintaining populations, identifying species, inoculation techniques, conducting differential host tests, evaluating resistance, and interpreting results.

Research began in 1976. Scientists at the project headquarters at North Carolina State University focused on the following areas: 1) morphological and taxonomic studies, with emphasis on finding new and more reliable characters for use in species identification; 2) cytogenetic studies involving comparisons of populations with regard to chromosome numbers, mode of reproduction, sexuality, and genetic basis of ability to reproduce on resistant crops; 3) differential host tests to detect pathogenic variation; 4) biochemical investigations of general protein pattern and specific enzyme systems; and 5) correlation studies of environmental factors on distribution, survival, and development of new pathotypes and/or species.

Scientists in cooperating countries within each region emphasized the following areas: 1) collection of nematode populations from diverse habitats, hosts, and types of agriculture; 2) identification

of species in field collections; 3) determination of the resistance and/or susceptibility of economic crops in the region to the root-knot nematode species present; 4) differential host tests to detect pathogenic variation; 5) search for new sources of resistant germ plasm in crop varieties, plant introductions, and breeding lines; 6) participation in studies to determine the relationships of ecological factors to survival and pathogenicity of *Meloidogyne* spp.; 7) use of crop response information in developing effective rotation schemes for control of root-knot nematodes; and 8) evaluation of nematicides and appraisal of their role in an integrated crop protection system.

Improving Control Methods

Project personnel decided at the beginning of the program that efforts should be directed toward conventional methods of control, namely, cultural practices, use of nematode-free planting stock, crop rotation, resistant cultivars, and, under certain circumstances, use of nematicides. Project goals were to make each of these methods more effective and more economical through better understanding of the pathogen. Methods of control likely to be used in developing countries should be adaptable to the small farmer with minimum financial resources. To accomplish our goals, we have concentrated our research efforts in the following areas: 1) developing a rapid, reliable method of identifying the species and races; 2) extending information on distribution of species and races; 3) assessing variability in the pathogen; 4) determining host susceptibility and/or resistance of local crops; 5) discovering sources of resistance; 6) identifying ecological factors affecting survival and pathogenicity; and 7) evaluating cropping systems within each country, utilizing information gained through the project and other important pest management principles.

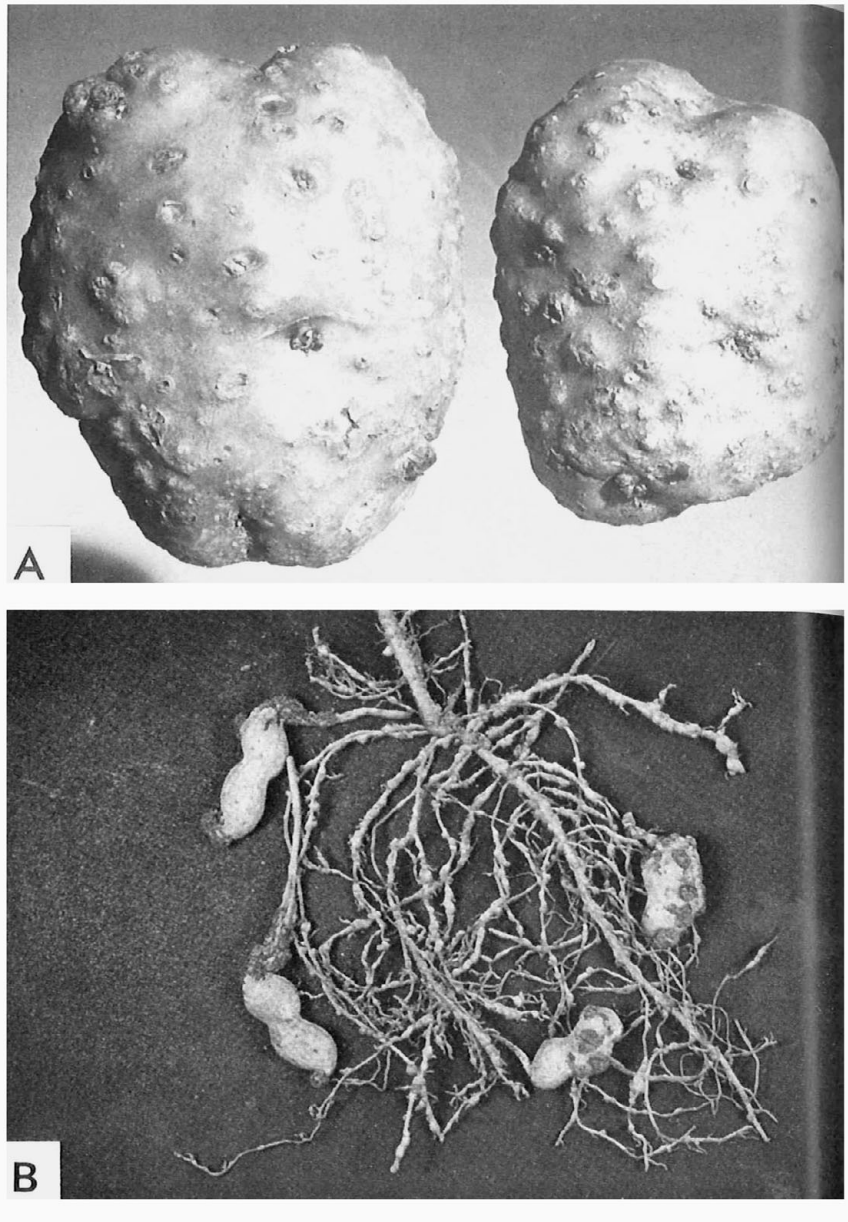


Fig. 1. Examples of the effects of *Meloidogyne* spp. on quality of food crops. (*A*) Potatoes severely parasitized by *M. incognita*. (*B*) Peanut roots and pods damaged by *M. arenaria*.

Identifying Species and Races

An all-inclusive approach to identification has been undertaken. The conventional morphological characters, such as perineal pattern of adult females, and various morphometric characters of larvae, females, and males are used with efforts to understand their variability. Supplemental parameters include response to a standard set of host differentials, cytological data (chromosome number and mode of reproduction), and, in some instances, biochemical data.

Recent morphological studies by project personnel using the scanning electron microscope (SEM) have detected significant differences in the morphology of larval, female, and male heads that are proving useful in distinguishing species. Details of structure observed with the SEM cannot be seen with the conventional light microscope. Results of this

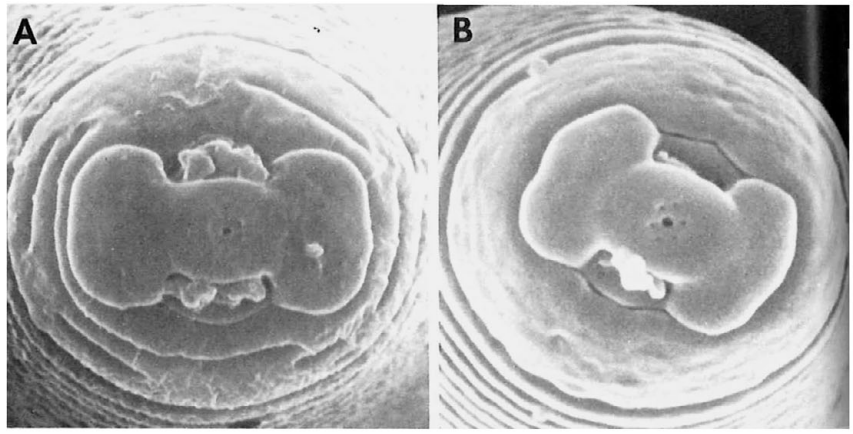


Fig. 2. Head structures of larvae of two *Meloidogyne* spp. as observed with the scanning electron microscope: (*A*) *M. incognita* with distinct annulation in the head region. (*B*) *M. hapla* with no annulation forward of the beginning of the body annules. (Photographs provided by J. D. Eisenback.)

promising research have been published in detail (2,3). Figure 2, adapted from the publication of Eisenback and Hirschmann (2), illustrates the differences in larval head structures between two well-known species when observed with the scanning electron microscope.

Through the network of approximately 100 scientists representing 80 countries, root-knot nematode populations are obtainable from a major portion of the earth's land surface. Furthermore, because of interest in ecological factors affecting *Meloidogyne* survival and pathogenicity, some effort has been made to collect populations from diverse habitats and hosts. These field populations, consisting of 20–30 egg masses of the nematode, are forwarded to project headquarters in 1% saline solution for increase on appropriate hosts. In most cases, Rutgers tomato serves as a good host. Populations from rice or coffee, however, are usually cultured initially on these crops to reduce the risk of losing a valuable culture.

Once the inoculum has been increased, various studies are made to aid identification and determine extent of variability. Previous studies have shown that the more common species, *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria*, differ in ability to infect and reproduce on certain crop plants. These differences may be with reference to a single crop or may involve two or more crops. The usual response of populations of the more common and widespread species is shown in Table 1. This pattern of host response is based on hundreds of tests on populations of the indicated species, primarily from the United States. Testing a population on these differentials indicates its probable identity. For example, a population that attacks the resistant tobacco NC 95, watermelon, and tomato but not cotton, pepper, and peanut would probably be *M. javanica*. A population that reproduces well on resistant tobacco, pepper, peanut, and tomato but fails to reproduce on cotton and watermelon would most likely be *M. hapla*.

Final determination is always based on additional data, especially morphological and cytological. The primary purpose of subjecting all populations to the differential host test is to detect significant pathogenic variation. For example, will a population of *M. hapla* from Kenya, Korea, or Costa Rica react in the same way as a population from North Carolina, New Jersey, or Ohio? Or, do all populations of *M. javanica* behave alike when used to inoculate the same differential host and maintained under nearly identical conditions of soil type, temperature, and nutrition? Because the extent of variability among populations of the same species is of primary concern to the plant breeder, considerable attention has been given to assessing the extent of variation.

The Important Species

Our team of scientists have now studied more than 500 live populations of *Meloidogyne* species sent by overseas cooperators. We have been able to identify most of the various populations to species. The frequency of species identified thus far is shown in Table 2.

The finding that about 99% of the root-knot nematode populations studied comprise only five species is encouraging and gives hope that crop plants can be bred for resistance to these few species. Crop plants with multiple resistance to *M. incognita* and *M. javanica* would provide resistance to 84% of the root-knot nematode populations encountered in the tropical and subtropical regions of the world. These percentages, especially those of *M. hapla* and *M. naasi*, are influenced by the fact that most collections were made from warm climates. Furthermore, a large percentage of collections came from cultivated fields that apparently are inhabited primarily by *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria*. *M. exigua* has been collected only from coffee in Central and South America.

Although approximately 40 species of *Meloidogyne* have been described, only five or six are important agricultural species. The remaining species appear to be highly host-specific, and apart from the original description, little additional information is available concerning their biology or distribution. It is possible, but not likely, that some of these species could become economically important in the future.

The most important species on a world basis is unquestionably *M. incognita*. Scientists associated with the International *Meloidogyne* Project have long recognized that there is physiological variation within the *M. incognita* group. For example, some populations attack cotton or the resistant tobacco NC 95, while others do not. The differential host tests of about 300 populations of this species revealed a pattern of response to cotton and tobacco indicating at least four host races (Table 3). These appear to be biologically distinct races, since the gall and egg mass ratings on the differential hosts indicated either susceptibility or resistance; intermediate reactions were rare. The consistency of host response for the four races; their widespread occurrence, especially races 1, 2, and 3; and the number of populations involved indicate that these are stable taxons. The four races are as yet morphologically indistinguishable and have no apparent cytological differences. All have 41–46 chromosomes, the approximate number determined for this species by Triantaphyllou (7).

There are two host races of *M. arenaria*. One severely infects and reproduces on peanut, causing serious crop losses in Virginia, North Carolina, Georgia, Florida, Alabama, and Texas. This race not only attacks the fibrous roots but also causes galls and other malformations on the pegs and pods, greatly reducing quality (Fig. 1B). This race is responsible for the common name of *M. arenaria*, the "peanut root-knot nematode."

In our collections, however, more populations of *M. arenaria* fail to infect and reproduce on peanut than do. No morphological or cytological differences between the two populations have been observed. For the present, we have designated them races 1 and 2, with race 1 being pathogenic on peanut.

We have not found distinct host races of *M. javanica* or *M. hapla*. A few populations of *M. javanica* parasitize California Wonder pepper, but most do not. Other species have not been studied sufficiently to determine the existence of races, although some variability has been reported. Differential host responses to the various *Meloidogyne* species and races are summarized in Table 4.

Ecological Factors

An important objective of the International *Meloidogyne* Project is to gain some insight concerning ecological factors that influence survival and pathogenicity of root-knot nematodes. Although significant contributions have been made (8), a clearer understanding is needed of the effects of biotic and abiotic factors closely associated with various species of the genus, particularly on their survival, population increases, and potential damage to the host. The established network of cooperators provides an ideal mechanism for obtaining detailed information on each population sent to us for study. Using forms we provide, the cooperators record host location, elevation, severity of damage, other organisms associated with the disease, cropping history for 24 mo preceding sample collection, agronomic practices used, weather data, and other information. In addition, 300 g of soil is taken from the rhizosphere of the plant where the nematode was collected and forwarded to project headquarters for physical and chemical analyses. Data on the identity of the nematode, host response on the standard differential hosts, and cytological information (chromosome number and mode of reproduction) are determined for each nematode population.

These data are currently being analyzed to elucidate any significant correlations that might exist between ecological factors associated with the nematode population and nematode behavior. We hope to gain information on the influences of soil factors (texture, pH, organic matter), climatic factors (temperature, rainfall), and cultural practices (cropping history, fertilizer and pesticide use) on root-knot nematode survival and pathogenicity.

Prospects for Control

Although we have gained a better perspective of the root-knot nematode than we had at the beginning of our project, it has become increasingly evident that we are dealing with a complex group of plant pathogens. Many more years of concentrated effort will be needed to develop effective and low-cost methods of control. The most practical approach for the small farmer is to use resistant cultivars. Progress in the development of resistant cultivars has been substantial, and much of the information on their existence and availability has been compiled in a recent IMP publication (6). Development of resistant cultivars will be greatly enhanced as we learn more about the genetics of resistance and the mechanisms that enable the nematode to change or adapt to new stresses of the environment, including the host plant.

For the present, however, we should take advantage of what we have learned and utilize available information to develop a plan of attack. Although eradication of the nematode from the soil is difficult if not impossible, the population density can be lowered to a level where damage to the crop is negligible.

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

Meloidogyne spp.	Differential hosts[a]					
	Tobacco	Cotton	Pepper	Watermelon	Peanut	Tomato
M. incognita	⊟[b] (+)[c]	⊞(−)	+	+	−	+
M. javanica	+	−	⊟	+	−	+
M. hapla	+	−	+	⊟	⊞	+
M. arenaria	+	−	+	+	⊞(−)	+

[a]Plant cultivars: tobacco, NC 95; cotton, Deltapine 16; pepper, California Wonder; watermelon, Charleston Grey; peanut, Florrunner; tomato, Rutgers.
[b]Key differential hosts for that species.
[c]Some populations attack differential host but others do not.

Table 2. Frequency of *Meloidogyne* spp. identified from 558 populations received from cooperators

Meloidogyne spp.	Number studied	Percent of total
M. incognita	298	54
M. javanica	167	30
M. hapla	40	7
M. arenaria	40	7
M. exigua	7	1
Others[a]	6	1

[a]*M. megatyla, M. microtyla, M. naasi, M. graminicola, M. graminis, M. oryzae.*

Table 3. Host races of *Meloidogyne incognita*

Race designation	Differential hosts[a]		Percent of 298 populations studied
	Cotton	Tobacco	
1	−	−	67
2	−	+	18
3	+	−	11
4	+	+	4

[a]Cotton, Deltapine 16; tobacco, NC 95.

Summary

Useful information gained through research done by scientists cooperating in the International *Meloidogyne* Project can be summarized as follows:

1. Although approximately 40 species of *Meloidogyne* have been described, most (perhaps more than 95%) of the damage to agricultural crops is caused by six species. Of these, only four, *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria*, are widely distributed throughout the world in agricultural soils. Two species, *M. naasi* and *M. exigua*, are important in certain regions. *M. naasi* is a parasite of cereal crops in Europe and has been reported from the cooler parts of the United States; *M. exigua* is a parasite of coffee in Central and South America but has not been reported from other coffee-growing regions. About six other species that attack crop plants have been reported, but these have only limited distribution. The approximately 28 remaining species apparently also are limited in distribution and host range and are of little importance in world agriculture.

2. Considerable progress has been made in improving the reliability of identification. Morphology, host response, cytogenetics, and, in some cases, biochemistry have provided additional information to confirm identification.

3. The frequency and relative importance of the major species have been determined. *M. incognita* and *M. javanica* constitute a large proportion of the populations in cultivated fields. Resistance in crops to these two species alone would help alleviate the damage caused

Table 4. Summary of differential host responses to various *Meloidogyne* spp. and races

Meloidogyne spp.	Number studied	Number of countries	Number of pathogenic populations					
			Cotton	Tobacco	Peanut	Pepper	Watermelon	Tomato
M. incognita								
Race 1	199	39	0	0	0	197	199	199
Race 2	54	25	0	54	0	49	54	54
Race 3	35	14	35	0	0	34	35	35
Race 4	10	7	10	10	0	10	10	10
M. javanica	167	48	0	167	0	10	167	167
M. hapla	40	10	0	38	40	36	0	40
M. arenaria								
Race 1	8	3	0	8	8	8	8	8
Race 2	32	14	0	32	0	14[a]	32	32

[a]Variable on pepper.

by root-knot in the tropical and subtropical regions of the world.

4. Distinct host races occur in at least two important species, *M. incognita* and *M. arenaria*. The question of races has to be considered in any control program, including crop rotation and use of resistant cultivars. The occurrence of races raises questions about the validity of reported resistance to *M. incognita* and *M. arenaria*. Is the reported resistance effective against all races of a species? If not, which ones is the cultivar resistant to?

5. Host response data from more than 500 populations studied indicate that populations of a given species from widely separated geographical regions behave similarly on the differential hosts used. For example, *M. hapla*, regardless of origin, previous host, or other influences, reacts almost identically when tested on the six crop cultivars used as differential hosts. The same is true for *M. javanica*. Populations from many countries react similarly. Even the host races are consistent in their reaction on standard differential hosts. In other words, variation in pathogenicity within populations of species tested is not very great. This enhances the likelihood that a resistant variety can be used over a large geographical area. It also suggests that host range determinations for a certain species or race can be done at a single location with the expectation that results will be applicable in other parts of the world where this species or race occurs. The same can be expected for the development of resistant cultivars. Such studies, using a few populations of each of the major species, could be done under standard conditions at minimum cost, compared to testing all crops against all populations in all countries.

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