A PROGRESS REPORT OF RESEARCH COMPLETED AT THE RESEARCH CENTER OF THE INTERNATIONAL *MELOIDOGYNE* PROJECT

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Introduction

Research on several aspects of the biology, behavior, and biochemistry of *Meloidogyne* spp. is being carried out at North Carolina State University in connection with the International *Meloidogyne* Project. This report will cover only a portion of the work; namely, the frequency of occurrence of the major *Meloidogyne* species, pathogenic variation within these species, results of cytogenetic studies on many of the populations sent to the Research Center, and results from some of the biochemical studies.

Identification of and Pathogenic Variation Within Field Populations.

Five hundred and seven field populations have been sent to Raleigh by the local country cooperators. Of these populations, 399 have been studied for pathogenic variation and species identification. *Meloidogyne incognita* and *M. javanica* together make up nearly 80 % of these populations, followed by *M. hapla* and *M. arenaria* (Table 1). Other species *(M. exigua, M. microtyla, M. graminicola, M. graminis* and *M. naasi)* are only rarely encountered. Data on the distribution and frequency of these species is biased by the location and type of crops sampled; the majority of the samples being from vegetable and field crops in tropical regions. Species such as *M. hapla* and *M. naasi* would be expected to be found in higher frequencies in the temperate climates. Other species such as *M. brevicauda* and *M. mali* have very limited host ranges (tea and *Malus* spp., respectively) and would not be expected to be found unless these crops were sampled. For these reasons, those species with wide host ranges and tropical to subtropical habitats would be expected to predominate. Nevertheless, it is noteworthy that *M. incognita* and *M. javanica* are the most frequently encountered species.

During the identification of the field populations it was noted that a mixture of species was occasionally encountered. Six per cent of the field populations contained more than one species. Mixtures of *M. incognita* and *M. javanica* occurred with the highest frequency. Mixtures of other species occurred with lower frequencies.

A major portion of the work on the *Meloidogyne* populations sent to Raleigh has involved the study of pathogenic variation with each species. This involves tests on the ability of each population to infect and reproduce on a standardized set of differential hosts. Previous studies have demonstrated that populations of *M. incognita, M. javanica, M. hapla* and *M.arenaria* respond to the host differentials in a characteristic or "usual" way (Table 2) (2). Deviations from the "usual" host responses therefore, are an indication of pathogenic variation. The results of these studies are not intended to be used in developing crop rotation systems because of the limited number of host differentials used. Nor can it be inferred that the results of the studies completely characterize the pathogenic variation within a species. The limited number of host differentials used in these studies are only intended to give some indication of the pathogenic variation that exists among populations of *Meloidogyne* species from widely separated geographic regions of the world.

Based on the ability to parasitize cotton (cv'Delta Pine 16') and tobacco (cv 'NC 95'), four host races of *Meloidogyne incognita* have been identified (Table 3). Based on the ability to parasitize peanut (cv'Florrunner'), two host races of *M. arenaria* have been identified. There are no morphological differences among the host races of *M. incognita* or *M. arenaria.* The host races of these species are apparently worldwide in distribution, and for *M. incognita* do not appear to have developed through selection pressures. With *M. arenaria,* some selection pressure may be involved in the development of the two host races; Race 1, which attacks peanuts, has generally been found in regions where peanuts are frequently cultivated. Race 2 of *M. arenaria,* which does not attack peanuts, is generally from

regions where peanuts are not grown. No host races have been detected among the different populations of *M. javanica* and *M. hapla.*

Approximately 200 of the populations received have been examined cytogenetically (Table 4). These studies have revealed that more than one chromosomal form can be detected in the different populations of *M. incognita, M. hapla,* and *M. arenaria.* All populations of *M. javanica* examined to date have a single chromosome form with a somatic chromosome number of 43 to 48. Cytological information, including chromosome numbers, morphology, and behavior, can assist identification of most common species, especially when identification of a population on a morphological or host specificity characteristic is not possible or is doubtful. A feature of great value for species identification is the peculiar behavior of the prophase chromosomes during maturation of the oocytes of *M. incognita.* It involves a characteristic clumping of the prometaphase chromosomes which prolongs considerably the duration of this stage. This feature easily differentiates *M. incognita* from all other species of *Meloidogyne* studied thus far.

Biochemical Studies.

The biochemical studies have concentrated on the study of nematode-produced-enzymes that may play a role in the host-parasite relations of *Meloidogyne* species. An attempt is being made to characterize these enzymes in order that any role they might have in the parasitic mechanism can be assessed. Because of the difficulty in collection of a sufficient amount of nematodes to work with, a "micro" polyacrylamide gel electrophoresis system has been developed (3). Using the "micro" system the isozyme composition of different enzyme systems can be evaluated using small samples.

Peroxidase activity in *M. incognita* females was first reported by Hussey and Sasser (1). The present work (4) confirms this initial report of two peroxidase isozymes detectable in *M. incognita* females; two isozymes with identical electrophoretic mobilities have been found also in *M. arenaria, M. hapla,* and *M. javanica* females which were reared on tomato. However, no significant peroxidase activity could be detected in extracts of eggs or preparasitic larvae of any of these species. Electrophoretic analysis of extracts from *M. hapla* and *M. incognita* adult males also failed to reveal any peroxidase activity.

When plants other than tomato were used to culture the nematodes, it was found that the peroxidase isozyme profile of the females changed with the host. Females of *M. hapla, M. javanica,* and *M. incognita* had a single peroxidase isozyme when cultured on bean or eggplant as compared with two isozymes when cultured on tomato. The electrophoretic mobility of each peroxidase isozyme was different for each host. When the nematodes were cultured on tobacco, no peroxidase activity was detectable following anionic electrophoresis. Activity was detected in total activity assays. In another series it was found that the peroxidase isozymes from nematodes reared on tomato were electrophoretically identical to some of the peroxidase isozymes from tomato root tissues. The host root tissues included nongalled roots, *Meloidogyne* root-galls, and isolated syncytial complexes.

These data show that i) only adult females of *Meloidogyne* spp., which have fed on the host, have significant levels of peroxidase activity; ii) the host has significant influence on the peroxidase isozyme profiles of *Meloidogyne* spp. females; and iii) tomato roots have the same peroxidase isozymes as detected in females reared on tomato. Collectively, these observations suggest that the peroxidase activity associated with *Meloidogyne* spp. females is not of nematode origin. Rather, it appears to be of host origin and was ingested by the nematode during feeding.

Another enzyme system that is currently being investigated is the glycosidase system. Both β-glucosidase and β-galactosidase activities have been reported for second stage larvae of *Heterodera rostochiensis* (5). The present work involves characterization of different glycosidase activities in *M. javanica* and *M. incognita* (preparasitic larvae and females) and *H. glycines* females.

Extracts of *M. javanica* and *M. incognita* females and larvae are able to release the p-nitrophenol aglycone from β-glucosides, β-galactosidase, and β-fucosides. Generally, the β-galactosidase activity is greater than the β-glucosidase or β-fucosidase activities. Immature, white *H. glycines* females also contain β-glucosidase and β-galactosidase activities; β-fucosidase activity has not been tested. With *H. glycines,* however, the β-glucosidase activity is higher than the β-galactosidase activity. Work is currently in progress to determine the isozyme composition of each of these enzyme systems.

Based on the present information, no con-

clusions can be drawn as to the significance of glycosidase activity in the host-parasite relations of these nematodes.

Table 1. *Meloidogyne* species identified from field population sent to the IMP Research Center (Raleigh, NC) by local country cooperators.

Species	% of 399 populations studied
M. incognita	55.6
M. javanica	24.3
M. hapla	6.5
M. arenaria	4.5
Unknown	7.0
Others[a]	2.1

[a] Includes populations of *M. exigua, M. microtyla, M. graminicola, M. graminis* and *M. naasi.*

Table 2. Usual response of host differentials to attach by the common *Meloidogyne* species.

Species	Differential hosts[a]					
	Tobacco	Cotton	Pepper	Watermelon	Peanut	Tomato
M. incognita	±	±	+	+	-	+
M. javanica	+	-	-	+	-	+
M. hapla	+	-	+	-	+	+
M. arenaria	+	-	+	+	±	+
M. exigua	-	-	+	+	-	-

[a] Plant varieties include: Tobacco, NC95; cotton, Delta Pine 16; pepper, California Wonder; watermelon, Charleston Grey; peanut, Florrunner; tomato, Rutgers.

Table 3. Host races of *Meloidogyne incognita.*

Race	Host reaction		% of 222 populations studied
	Tobacco	Cotton	
1	-	-	62.6
2	+	-	22.0
3	-	+	10.0
4	+	+	5.4

Table 4. Major chromosomal forms detected in different populations of *Meloidogyne incognita, M. javanica, M. hapla* and *M. arenaria.*

Species	Number of populations studied	Chromosome number
M. incognita	91	2N = 40-45
	5	2N = 32-36
M. javanica	63	2N = 43-48
M. hapla	3	N = 15
	7	N = 16
	11	N = 17
	2	2N = 45
	2	2N = 48
M. arenaria	2	2N = 36
	13	2N = 50-54

Table 5. Peroxidase activity in different developmental stages of *Meloidogyne arenaria, M. hapla, M. javanica,* and *M. incognita.*

Species	Stage of development	Peroxidase specific activity[a]	No. of peroxidase isozymes detected
M. arenaria	adult females	1.43	2
	eggs	0.007	0
	preparasitic L_2	0.004	0
M. hapla	adult females	1.08	2
	eggs	0.02	0
	adult males		0
M. javanica	adult females	1.44	2
	eggs	0.014	0
M. incognita	adult females	1.54	2
	preparasitic L_2	0.024	0
	adult males		0

[a] Specific activity is the change in absorbance at 460 nm. per min per mg. protein using H_2O_2 and o-dianisidine as cosubstrates.

Table 6. β-glycosidase activities in *Meloidogyne javanica, M. incognita* and *Heterodera glycines.*

	μM p-nitrophenol released/min/mg. protein from		
	β-glucoside	β-galactoside	β-fucoside
M. javanica			
females	1.10	9.20	1.05
preparasitic L_2	1.25	5.75	1.10
M. incognita			
females	2.66	5.04	3.49
preparasitic L_2	4.77	8.27	3.38
H. glycines			
females	13.50	4.15	—

Literature Cited

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Discussion

Razak: 1. How did you obtain the peroxidase from the nematode? 2. What method did you employ to remove the gel from the capillary tube?

Starr: 1. A crude enzyme preparation was made by macerating the nematodes in phosphate buffer (pH 7.4) and centrifuging the suspension at 10,000 g for 20 min. The resulting supernatant was used for all enzyme analyses. 2. gels were removed from the glass capillary tubes by gently breaking the glass.

Inagaki: How about the applicability of electrophoretic technique in identifying species of root-knot nematodes?

Starr: With certain enzyme systems there is a good possibility that isoenzyme analysis can be used to aid in the identification of certain populations and species.