COMPARATIVE DISC ELECTROPHORETIC PROTEIN ANALYSES AND SEROLOGICAL RELATIONSHIPS OF SELECTED SPECIES OF Meloidogyne AND SOME HOST PLANTS

By

FRANKLIN HON-CHING CHOW

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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The author expresses his sincere appreciation to the chairman of his supervisory committee, Dr. Vernon G. Perry, for his generous assistance and enthusiastic encouragement throughout this study, and to the cochairman of his supervisory committee, Dr. S. G. Zam, for his advice during the periods of research.

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

COMPARATIVE DISC ELECTROPHORETIC PROTEIN ANALYSES AND SEROLOGICAL RELATIONSHIPS OF SELECTED SPECIES OF Meloidogyne AND SOME HOST PLANTS

By
Franklin Hon-Ching Chow

June, 1977

Chairman: Vernon G. Perry
Cochairman: Stephen G. Zam
Major Department: Entomology and Nematology

Disc electrophoresis and serological techniques were used to distinguish between Meloidogyne arenaria, M. javanica, and two populations of M. incognita. The buffer soluble protein extracts obtained from adult females and eggs of each of the populations were used in this study. Root extracts of selected host plants were serologically tested with the antibodies of the nematodes to determine their relationships to the host range of the nematodes.

Total protein, glycoprotein, mucoprotein, and lipoprotein contents of nematode populations were analyzed by disc electrophoresis. Total protein electrophoresed in 7 percent polyacrylamide gel stained by coomassie blue gave the best protein separation both between and within species. The glycoprotein, mucoprotein, and lipoprotein patterns where observed were identical in all populations studied. One glycoprotein band and one lipoprotein band were observed in extracts of both adult females and eggs; however, no mucoprotein band was
demonstrated in adult female extracts, but two were shown in egg extracts.

The four populations of *Meloidogyne* could be separated both by immunodiffusion and immunoelectrophoresis. Several attempts to obtain purified adult female and egg population-specific antibodies by affinity chromatography were unsuccessful.

Although there were immunoprecipitin bands of root extracts on 'Porto Rico' sweetpotato when applied against nematode antisera, no evidence of the relationship between the nematodes and their host range was shown by serological techniques.
INTRODUCTION

The root-knot nematodes of the genus *Meloidogyne* are damaging parasites of most agricultural crops grown in the world. Some 35 species were recognized by Esser et al. (27). Certain of these species are similar in morphology and host responses so that identification has proven to be difficult even when attempted by experienced nematologists. The characters used for species differentiation are variable even from a population produced on an individual plant. Yet identification is of the utmost importance if integrated pest management procedures are to be successfully employed.

At the present time, identification of the root-knot nematodes is based partly on the morphology of adult males, females, and second stage larvae, and some nematologists use host responses to parasitism as an aid to identification. Many researchers, however, have found that these criteria are often confusing and hence not satisfactory.

Modern technology in cytology (75, 76), enzyme and protein analyses (21, 22, 42), and serology (39, 40, 42, 50, 54) has proved very helpful in identifying certain nematodes. Some of the above authors reported that techniques such as disc electrophoresis, immunodiffusion, and immunoelectrophoresis offer substantial promise for identification of root-knot nematodes.
The objectives of the research reported herein were to further determine if disc electrophoresis and serological techniques could be used as aids in identifying populations of *Meloidogyne*; to determine if purification of species-specific antibodies by affinity chromatograph can be utilized and; to determine if relationships could be demonstrated between host ranges and serological reactions. In order to accomplish these objectives, one population each of *Meloidogyne arenaria* (Neal) and *M. javanica* (Treub), and two populations of *M. incognita* (Kofoid and White) were selected for investigation (Table 1). All populations were derived from single egg mass isolates and were maintained on tomato plants, *Lycopersicon esculentum* Mill 'Rutgers' in a greenhouse.
Table 1. Species and populations of Meloidogyne.

<table>
<thead>
<tr>
<th>Species</th>
<th>Population No.</th>
<th>Place Collected</th>
<th>Original Host</th>
<th>Date Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. arenaria</td>
<td>000</td>
<td>Greece - Supplied by Dr. J.N. Sasser (N.C. State University)</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>M. incognita (S)</td>
<td>118</td>
<td>St. Johns County, Florida</td>
<td>Irish Potato Solanum tuberosum L.</td>
<td>March 1971</td>
</tr>
<tr>
<td>M. incognita (A)</td>
<td>122</td>
<td>Alachua County, Florida</td>
<td>Irish Potato Solanum tuberosum L.</td>
<td>May 1971</td>
</tr>
<tr>
<td>M. javanica</td>
<td>129</td>
<td>Suwannee County, Florida</td>
<td>Tobacco Nicotiana tabacum L.</td>
<td>August 1971</td>
</tr>
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</table>
CHAPTER I
DIFFERENTIAL HOST TEST

Allen (2) in 1952 demonstrated the intraspecific variations of four populations of M. incognita acrita on 12 hosts. Sasser (62, 63) described a method to identify root-knot nematodes by host reaction and identified an unknown population by his methods. He emphasized, however, that some populations may not be identifiable by this method.

During a two-year tobacco rotation research program Sasser and Nusbaum (65) reported the existence of strains or races within M. incognita acrita that differ in their ability to attack crops. Van der Linde (78) identified a root-knot nematode as M. incognita acrita by host responses despite morphological evidence which showed the nematode to be more similar to M. incognita.

Biotypes of root-knot nematodes were further demonstrated by Goplen et al. (33) within M. incognita acrita, M. hapla, and M. javanica in tests with alfalfa varieties.

In my investigations each of the four root-knot nematode populations was identified by examination of 10 female perineal patterns and by morphometrical measurements of 15 larvae. Host differential tests were conducted in an attempt to confirm the identifications and also to seek possible relationships between host ranges and serological reactions.
Materials and Methods

Nine differential host plants were used to observe the host responses to different populations of root-knot nematodes. They were corn, Zea mays L., 'Minnesota A 401'; cotton, Gossypium hirsutum L., 'Deltapine 16'; peanut, Arachis hypogaea L., 'Florunner'; pepper, Capsicum frutescens L., 'California Wonder'; strawberry, Fragaria ananassa Duch., 'Albrition'; sweetpotato, Ipomoea batatas (L.) Lam., 'Allgold' and 'Porto Rico'; tobacco, Nicotiana tabacum L., 'NC-95'; and watermelon, Citrulus vulgaris Schard., 'Charleston Grey.' 'Rutgers' tomato was used as an indicator of inoculum potential.

All plants were rooted before inoculation. During transplantation into a 15-cm clay pot, each plant was inoculated with 10,000 eggs and then maintained in a greenhouse with a temperature range of 25-30°C. There were 5 replicates per host plant. After 60 days, the plants were removed, the roots washed and examined for galls. Roots were rated according to the following scale modified from the S-76 Technical Committee work plan (3).

- 0 = no gall
- 1 = 1 - 2 galls per plant
- 2 = 3 - 10 galls per plant
- 3 = 11 - 30 galls per plant
- 4 = 31 - 100 galls per plant
- 5 = greater than 100 galls per plant
Results

The results of the differential host tests are shown in Table 2.

Galls were not observed on corn, cotton, pepper, strawberry, or either variety of sweetpotato when inoculated with *M. arenaria*. Both *M. incognita* populations had the same host range, although there were differences in degree of damage; galls were not observed on corn, cotton, peanut, strawberry or tobacco. Following inoculation of *M. javanica*, galls were not observed on corn, cotton, peanut, pepper, strawberry, or 'Porto Rico' sweetpotato.

Discussion

Host responses of these four root-knot nematode populations differ from the standard reaction of *Meloidogyne* spp. compiled by the Southern Regional Nematology Technical Committee (3). The committee reported that corn and pepper were susceptible hosts for *M. arenaria*, that corn was a susceptible host for *M. incognita*, and that 'Porto Rico' sweet potato was a susceptible host for *M. javanica*. Galls were not observed in these combinations during this experiment. Nonetheless, the host differential test confirmed that the populations were identified correctly.
<table>
<thead>
<tr>
<th>Host Plants</th>
<th>M. arenaria</th>
<th>M. incognita (S)</th>
<th>M. incognita (A)</th>
<th>M. javanica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (Minnesota A 401)</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>1,2,1,2,2</td>
</tr>
<tr>
<td>Cotton (Deltapine 16)</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
</tr>
<tr>
<td>Peanut (Florunner)</td>
<td>4,5,4,4,3</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
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<tr>
<td>Pepper (California Wonder)</td>
<td>0,0,0,0,0</td>
<td>2,2,2,2,2</td>
<td>4,4,4,3,3</td>
<td>0,0,0,0,0</td>
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<tr>
<td>Strawberry (Albritton)</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
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<tr>
<td>Sweetpotato (allgold)</td>
<td>0,0,0,0,0</td>
<td>3,4,4,3,4</td>
<td>5,3,4,4,5</td>
<td>4,3,3,3,3</td>
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<tr>
<td>Sweetpotato (Porto Rico)</td>
<td>0,0,0,0,0</td>
<td>3,3,4,4,4</td>
<td>5,4,4,5,4</td>
<td>0,0,0,0,0</td>
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<td>Tobacco (NC-95)</td>
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<td>0,0,0,0,0</td>
<td>0,0,0,0,0</td>
<td>5,5,5,5,5</td>
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<tr>
<td>Watermelon (Charleston Grey)</td>
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<td>5,4,5,5,5</td>
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<td>Tomato (Rutgers)</td>
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<td>5,5,5,5,5</td>
<td>5,4,5,5,5</td>
<td>5,5,5,5,5</td>
</tr>
</tbody>
</table>

*Host ratings
0 = no gall
1 = 1-2 galls per plant
2 = 3-10 galls per plant
3 = 11-30 galls per plant
4 = 31-100 galls per plant
5 = greater than 100 galls per plant
CHAPTER II
DISC ELECTROPHORESIS

Introduction

Disc electrophoresis is a very sensitive analytical method used to separate charged protein molecules. It has proved to be an excellent analytical method for protein characterization of animal or plant tissue. Not only can the gel pore sizes be selected for optimal resolution (59), but also one can optimize 'charge separation' (60). By operating at any pH between 3 and 11, the maximal difference among the net charge of molecules can be obtained (60). Disc electrophoresis can be carried out at 0°C as well as at higher temperatures and is, therefore, applicable to enzymes (29, 56) and other thermolabile molecules (55). Proteins at or below microgram quantities can be detected by polyacrylamide gel electrophoresis (68). Therefore, the technique is ideal for separating protein from micro-organisms.

Electrophoretic separation of water soluble proteins may have applicability in the future in the taxonomy of a variety of micro-organisms, such as bacteria (31, 34, 37, 70), fungi (10, 12, 24, 53, 80), protozoa (45), and nematodes (5, 21, 22, 26, 28, 41, 42, 43, 77).
Literature Review

The enzyme acetylcholinesterase, which hydrolyzes the synaptic nerve impulse transmitter, acetylcholine, was reported in *Trichodorus christiei*, *Pratylenchus penetrans*, *Xiphinema americanum*, *Dorylaimus* sp., and *Helicotylenchus nannus* by Rohde in 1960 (61). Although the enzyme was detected histochemically in his study, it was the first study in which a single protein was detected in plant parasitic nematodes. Esterases were later detected and demonstrated histochemically in free-living nematodes by Lee (46) and in *Meloidogyne* spp. by Bird (7).

During 1966, specific and distinct phosphatases, esterases, and protein patterns were demonstrated by disc electrophoresis in *Ditylenchus triformis* and *Panagrellus redivivus* (4). Protein compositions of *Panagrellus silusiae*, *Aphelenchoides fragariae* and three rhabditid species were investigated by means of agar electrophoresis by Gysels (35). Two glycoproteins and one lipoprotein were detected in *P. silusiae*. Gysels also emphasized the importance of the biochemical information in addition to the traditional systematic methods for species identification.

Chow and Pasternak (11) carried out their research on protein changes during maturation of *Panagrellus silusiae*. Although differences in the intensity of certain bands at various larval stages and the adult were reported, no differences in the number of protein pattern bands in acrylamide gel
Electrophoresis were observed. Specific enzyme activities were different during different larval stages; however, the authors suggested that during maturation a precise regulator program operates to control the sequential appearance of specific enzymatic proteins.

Erkisson and Granbery (26) reported a noticeable variation in staining intensity of protein bands and several bands which differ with regard to occurrence and position within four races of *Ditylenchus dipsaci*. Dickson et al. (21) worked more extensively on four species of *Meloidogyne*, two species of *Ditylenchus*, and one species each of *Heterodera* and *Aphelenchus*. They found characteristic electrophoretic protein patterns for each genus and differences in number of bands within genera.

Ishibashi (43) reported different patterns of esterases and acid phosphatases for *Meloidogyne* spp. isolated from different host plants or host plants growing under different conditions. Evans (28) reported differences in esterase, amylase, and acid phosphatase profiles among seven isolates of *Aphelenchus avenae*. Nevertheless, Dickson et al. (21, 22) found that the enzyme patterns were stable in populations cultured on several different hosts.

Trudgill and Carpenter (77) used disc electrophoresis to identify protein patterns of six *Heterodera* spp. which correlated with the shape of the cyst either rounded or lemon shaped. The authors separated electrophoretically two groups of *H. rostochiensis* pathotypes and suggested that they comprised two species (77). Later, Stone (72) described a second species,
**H. pallida**, from what had been considered *H. rostochiensis*.

Distinct electrophoretic differences in enzymes and water soluble proteins of *Ditylenchus dipsaci* and *D. triformis* were demonstrated by Hussey and Krusberg (41). Hussey et al. (42) later reported differences of \( \alpha \)-glycerophosphate dehydrogenase patterns detected when *Meloidogyne* spp. were propagated on bean rather than on tobacco or tomato.

Berge and Dalmasso (5) reported considerable intraspecific and interspecific heterogeneity among various populations of *Meloidogyne* spp.; 19 hydrolytic enzymes were studied in his work.

**Materials and Methods**

Large quantities of nematodes were obtained by rearing on 'Rutgers' tomato after inoculating seedlings approximately 15 cm tall with 15-20 egg masses. The seedlings were grown in 15-cm diameter clay pots filled with Arredondo fine sand previously fumigated with methyl bromide at the rate of 3 kg/m\(^3\). Plants were maintained for 45 days in a greenhouse at 25-30°C.

All plants used in the experiments were fertilized weekly with 250 ml of 2 percent Nutri-Sol solution (12-10-20) (Nutri-Sol Chemical Company, Inc., Tampa, Florida 33614).

Adult females were recovered from infected roots by a modification of the methods reported by Dropkin et al. (23), Dickson et al. (21), and Hussey (38). Roots from 40 heavily infected tomato plants were washed free of soil and chopped into 2-3 cm sections. Approximately 150 grams of chopped roots
were softened in a 1000 ml Erlenmeyer flask by 250 ml 1:1 (v:v) water: pectinol 59-L (Rhine and Haas Co., Philadelphia, Pennsylvania) during 8 hours of agitation at 280 oscillations per minute on a Burrell wrist-action shaker at room temperature. Then the softened roots were placed on nested 30-and 60-mesh sieves and adult females and egg masses of *Meloidogyne* were dislodged from the roots by spraying with a high pressure stream of water. Debris on the 30-mesh sieve was discarded. Females, egg masses, and root tissue debris collected on the 60-mesh sieve were poured into beakers.

A 60-mesh sieve was placed in a pan filled with water and 50 ml of the above mixture poured into the sieve. The sieve was raised to the surface of the water to allow all material to float. Then the sieve was submerged slowly and most of the females, egg masses, and root tissue debris floated. By blowing gently on the water surface, most of the egg masses and root tissue debris fell to the bottom while the females remained floating. The females and other floating matter were skimmed from the surface and were collected and emptied into beakers. Repetition of this process cleaned the females from other debris. Using this technique, 10 g of adult females from 40 heavily infected plants could be obtained within 2 hours after the roots were softened.

Relatively large quantities of nematode eggs were obtained by the method of McClure et al. (51). Egg masses were treated with one percent sodium hypochlorite, and eggs were collected on a 450-mesh sieve. In most cases, approximately 6-8 g of
eggs could be obtained from 40 heavily infected tomato plants. Eggs and females of *Meloidogyne* spp. were washed three times in deionized water and suspended (1:4, V/V for eggs; 1:1.5, v/v for females) in iced 0.05 M potassium phosphate (pH 7.4) buffer containing 0.15 M sodium chloride and 0.001 M magnesium chloride (42). Eggs and females were homogenized using a Sonifier cell disruptor Model W185 (Branson Sonic Power Company, Danbury, Connecticut) for one minute, set at maximum speed and equipped with a standard tip. Homogenized tissues were extracted at 4°C for 8 hours and then centrifuged at 60,000 x g (rotor type 40, at 30k rpm, Beckman Model L-2) at 4°C for one hour. The clear supernatants were retained. Supernate protein concentrations were determined fluorometrically with 4-phenylspiro (furan-2(3H), 1'-phthalan)-3,3'-dione (fluorescamine) with bovine serum albumin as a standard (8). Buffer solution was added to the protein samples to adjust the concentration to 2 mg/ml.

Aliquots (100 mg) of adjusted samples were electrophoresed immediately. Buffer soluble protein extracts that were used as immunogens were stored at -75°C for future use.

Disc electrophoresis and selected staining techniques were used to determine the various protein classes of females and eggs of various *Meloidogyne* spp. Standard 7 percent polyacrylamide gel was used to analyze the electrophoretic profiles of total protein, glycoprotein, and mucoprotein (19). The total concentration (T%) of amide and the proportion of bis-acrylamide (C%) were T% = 7.2 and C% = 2.4. Gels were prepared
in 5.5 mm i.d. glass tubes, with 6 cm separating gel and 1.5 cm of stacking gel. Fifteen to one hundred µl (100-200 µg) of prepared sample mixed with 40 percent sucrose solution (3:1, v/v) were overlaid onto the top of the gel. Electrophoresis was conducted at 4°C and a constant current of 3 ma/tube applied until the tracking dye (bromophenol blue) migrated to within one cm of the anodic end of the gel. The pH of the stacking gel was 6.6-6.8, separating gel 8.8-9.0, Tris-glycine electrode buffer 8.2-8.4, and the running pH was 9.5. For the demonstration of the total protein profile, a minimum of ten gels were removed from the glass tubes, fixed and stained in 0.2 percent coomassie blue, R-250, in a mixture of methyl alcohol: deionized water: acetic acid (5:5:1) for 2 hours and then destained by constant washing in the dye solvent. Glycoprotein was stained by alcian blue (79), and mucoprotein was stained by toluidine blue (58).

A minimum of five buffer soluble protein extracts from Meloidogyne spp. were prestained by Sudan Black B in ethyl acetate-propylene glycol and electrophoresed in 5.5 percent gel (T% = 5.7, C% = 3.2) without stacking gel to obtain the lipoprotein patterns (52). Photographs of lipoprotein patterns were taken immediately after separation because the bands will fade on standing.

All stained gels were scanned on Beckman spectrophotometer ACTA C11, Beckman scanner 2 at 280 nm and recorded on a Beckman 10 inch recorder.
The relative mobility \((E_f)\) values of each protein band was calculated as the distance of band migration divided by the distance of dye migration obtained by measurements on the densitometer tracings of stained gels.

A protein band was considered similar among the various populations if their \(E_f\) values were within a \(\pm 0.008\) range and showed similarity in staining intensity. To compare total number of similar protein bands among the various populations, the percent similarity was calculated as shown below (81).

\[
\% \text{ similarity} = \frac{\text{no. of pairs of similar bands}}{\text{no. of different bands} + \text{no. of pairs of similar bands}} \times 100
\]

In this study, the term 'distinctive band' is used to refer only to darkly stained well defined protein bands having optic densities above 0.2. Those having an optical density below 0.2 were not employed in calculating the percent similarity. This does not imply that weakly stained protein bands are not important in taxonomic relationships. The reasons weakly stained protein bands were not employed for percent similarity calculations were the inconsistent migration, diffuse appearance and weak staining of these bands among the populations.
Results

Total protein

Adult females

All four populations studied had similar protein patterns (Figure 1).

Among the four populations of *Meloidogyne* nematodes, 15-21 protein bands were demonstrated by disc electrophoresis, in which *M. arenaria* had 15, *M. incognita* (S) 21, *M. incognita* (A) 19, and *M. javanica* 17. There were 8 distinctive bands in *M. arenaria* and *M. javanica* and 9 distinctive bands in both *M. incognita* populations. The $E_f$ values (distance of protein migration/distance of dye migration) of all the bands in adult females are shown in Table 3.

Bands with $E_f$ values of 0.481-0.490, 0.662-0.668, 0.681-0.683, and 0.717-0.725 were common in all populations and were considered to be distinctive bands. Bands with $E_f$ value of 0.470-0.473 and 0.644-0.647 were common to both populations of *M. incognita*. The percentage similarity of adult females among species was calculated as described previously.

The percentage similarity of adult females among species was as follows:

1. between *M. arenaria* and *M. incognita* (S)
   
   (% similarity = $\frac{5}{7} + \frac{5}{5} \times 100 = 41.7\%$)

2. between *M. arenaria* and *M. incognita* (A)

   (% similarity = $\frac{5}{7 + 5} \times 100 = 41.7\%$)
Figure 1. Total protein patterns of coomassie blue stained gels and corresponding densitometer tracings of stained gels of adult females of *Meloidogyne* spp.
Table 3. Disc electrophoretic analyses of buffer soluble protein extracts of adult females of *Meloidogyne* spp.

<table>
<thead>
<tr>
<th>Protein</th>
<th>M. arenaria</th>
<th>M. incognita (S)</th>
<th>M. incognita (A)</th>
<th>M. javanica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-----</td>
<td>0.072</td>
<td>0.073</td>
<td>0.075</td>
</tr>
<tr>
<td>2</td>
<td>0.080</td>
<td>0.082</td>
<td>0.086</td>
<td>0.081</td>
</tr>
<tr>
<td>3</td>
<td>-----</td>
<td>0.105</td>
<td>0.107</td>
<td>0.108</td>
</tr>
<tr>
<td>4</td>
<td>-----</td>
<td>0.134**</td>
<td>0.136**</td>
<td>0.136**</td>
</tr>
<tr>
<td>5</td>
<td>0.148**</td>
<td>-----</td>
<td>-----</td>
<td>0.151**</td>
</tr>
<tr>
<td>6</td>
<td>-----</td>
<td>0.167</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>7</td>
<td>-----</td>
<td>0.199**</td>
<td>0.202**</td>
<td>0.214**</td>
</tr>
<tr>
<td>8</td>
<td>0.270**</td>
<td>0.260</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>9</td>
<td>0.285**</td>
<td>-----</td>
<td>0.288**</td>
<td>0.285**</td>
</tr>
<tr>
<td>10</td>
<td>0.299*</td>
<td>0.305**</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>11</td>
<td>-----</td>
<td>0.347</td>
<td>0.346</td>
<td>0.378</td>
</tr>
<tr>
<td>12</td>
<td>0.379</td>
<td>0.380</td>
<td>0.377</td>
<td>0.393</td>
</tr>
<tr>
<td>13</td>
<td>0.407</td>
<td>-----</td>
<td>-----</td>
<td>0.431</td>
</tr>
<tr>
<td>14</td>
<td>0.434</td>
<td>0.425</td>
<td>0.445</td>
<td>0.481**</td>
</tr>
<tr>
<td>15</td>
<td>0.459</td>
<td>0.452</td>
<td>0.470**</td>
<td>0.522</td>
</tr>
<tr>
<td>16</td>
<td>-----</td>
<td>0.473**</td>
<td>-----</td>
<td>0.559</td>
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<tr>
<td>17</td>
<td>0.490**</td>
<td>0.490**</td>
<td>0.487**</td>
<td>0.607</td>
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<tr>
<td>18</td>
<td>-----</td>
<td>0.523</td>
<td>0.517</td>
<td>0.663**</td>
</tr>
<tr>
<td>19</td>
<td>0.555</td>
<td>0.555</td>
<td>0.555</td>
<td>0.662**</td>
</tr>
<tr>
<td>20</td>
<td>0.601</td>
<td>0.604</td>
<td>0.605</td>
<td>0.681**</td>
</tr>
<tr>
<td>21</td>
<td>-----</td>
<td>0.647**</td>
<td>0.644**</td>
<td>0.717**</td>
</tr>
<tr>
<td>22</td>
<td>0.668**</td>
<td>0.663**</td>
<td>0.662**</td>
<td>0.720**</td>
</tr>
<tr>
<td>23</td>
<td>0.683**</td>
<td>0.681**</td>
<td>0.681**</td>
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<tr>
<td>24</td>
<td>0.725**</td>
<td>0.719**</td>
<td>0.717**</td>
<td>-----</td>
</tr>
</tbody>
</table>

| Total Bands | 15 | 21 | 19 | 17 |
| Total Distinctive Bands | 8 | 9 | 9 | 8 |

* Average of 2 replicates
** Distinctive Bands
3. between *M. arenaria* and *M. javanica*

\[
\text{\% similarity} = \frac{4}{4 + 6} \times 100 = 60% 
\]

4. between *M. incognita* (S) and *M. javanica*

\[
\text{\% similarity} = \frac{5}{5 + 6} \times 100 = 45.5% 
\]

5. between *M. incognita* (A) and *M. javanica*

\[
\text{\% similarity} = \frac{3}{3 + 7} \times 100 = 30% 
\]

The percentage similarity of adult females within *M. incognita* was:

\[
\text{\% similarity} = \frac{8}{2 + 8} \times 100 = 80% 
\]

**Eggs**

The protein profiles of eggs obtained from each nematode population were more similar than those from the adult females (Figure 2). Among the four populations of *Meloidogyne* nematodes, 17 or 18 protein bands were demonstrated by disc electrophoresis, in which *M. javanica* had 17, and other populations had 18. *M. incognita* populations had 10 distinctive bands, *M. arenaria* and *M. javanica* had 9. The *E_f* values of all bands in eggs are shown in Table 4.

The distinctive bands with the *E_f* values of 0.110-0.121, 0.220-0.228, 0.238-0.246, 0.369-0.375, 0.383-0.388, 0.490-0.495, 0.658-0.666, 0.686-0.691, and 0.731-0.742 were common in all
Figure 2. Total protein patterns of coomassie blue stained gels and corresponding densitometer tracings of stained gels of eggs of *Meloidogyne* spp.
<table>
<thead>
<tr>
<th>Protein Bands</th>
<th>M. arenaria</th>
<th>M. incognita (S)</th>
<th>M. incognita (A)</th>
<th>M. javancia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.121**</td>
<td>0.119**</td>
<td>0.113**</td>
<td>0.110**</td>
</tr>
<tr>
<td>2</td>
<td>0.159**</td>
<td>0.220**</td>
<td>0.225**</td>
<td>0.222**</td>
</tr>
<tr>
<td>3</td>
<td>0.228**</td>
<td>0.243**</td>
<td>0.238**</td>
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</tr>
<tr>
<td>4</td>
<td>0.246**</td>
<td>0.229**</td>
<td>0.225**</td>
<td>0.253**</td>
</tr>
<tr>
<td>5</td>
<td>0.292</td>
<td>0.346</td>
<td>0.349</td>
<td>0.375**</td>
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<td>0.379</td>
<td>0.369**</td>
<td>0.385**</td>
</tr>
<tr>
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<td>0.379</td>
<td>0.392</td>
<td>0.383**</td>
<td>0.388**</td>
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<tr>
<td>8</td>
<td>0.383**</td>
<td>0.417</td>
<td>0.409**</td>
<td>0.419**</td>
</tr>
<tr>
<td>9</td>
<td>0.417</td>
<td>0.492**</td>
<td>0.490**</td>
<td>0.491**</td>
</tr>
<tr>
<td>10</td>
<td>0.492**</td>
<td>0.529</td>
<td>0.532**</td>
<td>0.533**</td>
</tr>
<tr>
<td>11</td>
<td>0.529</td>
<td>0.567</td>
<td>0.564**</td>
<td>0.564**</td>
</tr>
<tr>
<td>12</td>
<td>0.567</td>
<td>0.601</td>
<td>0.601</td>
<td>0.601**</td>
</tr>
<tr>
<td>13</td>
<td>0.601</td>
<td>0.597</td>
<td>0.597</td>
<td>0.597**</td>
</tr>
<tr>
<td>14</td>
<td>0.597</td>
<td>0.651**</td>
<td>0.662**</td>
<td>0.658**</td>
</tr>
<tr>
<td>15</td>
<td>0.651**</td>
<td>0.664**</td>
<td>0.664**</td>
<td>0.685**</td>
</tr>
<tr>
<td>16</td>
<td>0.664**</td>
<td>0.691**</td>
<td>0.691**</td>
<td>0.689**</td>
</tr>
<tr>
<td>17</td>
<td>0.691**</td>
<td>0.734**</td>
<td>0.734**</td>
<td>0.734**</td>
</tr>
<tr>
<td>18</td>
<td>0.734**</td>
<td>0.742**</td>
<td>0.742**</td>
<td>0.742**</td>
</tr>
<tr>
<td>19</td>
<td>0.742**</td>
<td>0.685**</td>
<td>0.685**</td>
<td>0.685**</td>
</tr>
</tbody>
</table>

Total Bands: 18
Total Distinctive Bands: 9

* Average of 2 replicates
** Distinctive Bands
four populations; the band 0.646-0.651 was the only different
distinctive band between the *M. incognita* populations and other
species.

The percentage similarity within *M. incognita* populations
and between *M. arenaria* and *M. javanica* was:

\[
\% \text{ similarity} = \frac{9}{0 + 9} \times 100 = 100\%
\]

This signified no differences.

The percentage similarity between *M. arenaria* and *M.
incognita* (S), *M. arenaria* and *M. incognita* (A), *M. incognita*
populations and *M. javanica* was:

\[
\% \text{ similarity} = \frac{9}{1 + 9} \times 100 = 90\%
\]

**Adult females and eggs**

The four common bands in adult females were also common
in eggs. The band 0.644-0.647 in *M. incognita* females was
also demonstrated in eggs (0.646-0.651). The percentage
similarity between adult females and their eggs are shown
below:

*M. arenaria*

\[
\% \text{ similarity} = \frac{4}{8 + 4} \times 100 = 33.3\% *
\]

*Since the distinctive band 0.285-0.288 in adult females over-
lapped the indistinctive band 0.288-0.294 in the eggs, this
band was ignored in calculation.*
M. incognita (S)

\[ \% \text{ similarity} = \frac{5}{9 + 5} \times 100 = 35.1\% \]

M. incognita (A)

\[ \% \text{ similarity} = \frac{5}{8 + 5} \times 100 = 38.5\% \]

M. javanica

\[ \% \text{ similarity} = \frac{4}{8 + 4} \times 100 = 33.3\% \]

**Glycoprotein**

One glycoprotein band was observed in extracts of adult females and eggs (Figure 3). In extracts of adult females the \( E_f \) value of the band was 0.083-0.086 and of eggs 0.854-0.860.

**Mucoprotein**

There was no detectable mucoprotein bands in adult female extracts, but there were two mucoprotein bands in the egg extracts (Figure 4), with \( E_f \) values of 0.325-0.356 and 0.961-0.969.

**Lipoprotein**

Extracts of adult females and of eggs had one lipoprotein band, a widely diffuse band located at a similar position in all populations studied (Figure 5).

The \( E_f \) values of glycoprotein, mucoprotein, and lipoprotein are shown in Table 5.
Figure 3. Diagrammatic representation of glycoprotein patterns of Meloidogyne spp. adult females (above) and eggs (below) stained with alcian blue. (a = M. arenaria, iS = M. incognita (S), iA = M. incognita (A), and j = M. javanica.)
Figure 4. Diagrammatic representation of mucoprotein patterns of eggs of Meloidogyne spp. stained with toluidine blue. (a = M. arenaria, iS = M. incognita (S), iA = M. incognita (A), j = M. javanica).
Figure 5. Diagrammatic representation of lipoprotein patterns of adult females (above) and eggs (below) of Meloidogyne spp. stained with Sudan Black B. (a = M. arenaria, iS = M. incognita (S), iA = M. incognita (A), j = M. javanica).
Table 5. Disc electrophoretic analyses of glycoprotein, mucoprotein and lipoprotein from buffer soluble protein extracts of *Meloidogyne* spp.

<table>
<thead>
<tr>
<th></th>
<th>M. arenaria</th>
<th>M. incognita (S)</th>
<th>M. incognita (A)</th>
<th>M. javanica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult females</td>
<td>0.085</td>
<td>0.083</td>
<td>0.084</td>
<td>0.086</td>
</tr>
<tr>
<td>eggs</td>
<td>0.854</td>
<td>0.859</td>
<td>0.854</td>
<td>0.860</td>
</tr>
<tr>
<td>Mucoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult females</td>
<td>no bands observed</td>
<td>no bands observed</td>
<td>no bands observed</td>
<td>no bands observed</td>
</tr>
<tr>
<td>eggs</td>
<td>0.325</td>
<td>0.336</td>
<td>0.355</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>0.963</td>
<td>0.961</td>
<td>0.968</td>
<td>0.969</td>
</tr>
<tr>
<td>Lipoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult females</td>
<td>0.446</td>
<td>0.527</td>
<td>0.509</td>
<td>0.450</td>
</tr>
<tr>
<td>eggs</td>
<td>0.442</td>
<td>0.469</td>
<td>0.470</td>
<td>0.455</td>
</tr>
</tbody>
</table>

*Average of 2 replicates*
Discussion

Differences among the protein patterns of adult females of the four populations studied show the potential of protein analyses as a complement to other methods presently used to identify species of *Meloidogyne*. There were protein bands which existed in only one population, bands that existed only within species, and bands common in all populations studied. As pointed out by Gysels (35), Trudgill and Carpenter (77), Dickson *et al.* (21, 22), and Hussey (41, 42) the differences in protein patterns may give useful information on nematode systematics.

The protein patterns of eggs did not show as many differences as those of the adult females, but some characteristic distinctive bands were demonstrated in both adult females and eggs.

One specific band was present only in *M. incognita*. Its $E_f$ value was 0.644-0.647 for adult females and 0.646-0.651 for eggs. This band may prove to be unique to *M. incognita*.

Unlike Chow and Pasternak's (11) report of no differences in number of bands in *Panagrellus silusiae* during maturation, there were differences between adult females and eggs of *Meloidogyne* spp. The protein patterns obtained from adult females were specific for the species, whereas eggs of the species did not show consistent differences.

Depending upon the host plants on which they have fed, nematodes have different total protein patterns (21, 22, 27,
41, 42). Since a stable protein profile of a certain population is important for reliable use in identification, more work on the differences of protein patterns due to the host plants is necessary.

The glycoprotein and lipoprotein patterns show little promise for purposes of identification. Although different $E_f$ values of lipoproteins were obtained the difference may have been due to experimental error since lipoprotein patterns were represented by a widespread band in the middle of the gel, and the $E_f$ values were taken at the points with the highest density.

The percentage similarity served as an index for comparing the different protein patterns. In this study the reasons for counting only the distinctive band were to improve the reproducibility of protein patterns.

Due to the low concentration of certain proteins or variability of protein preparation, some bands were not demonstrated in all replicates. Therefore, only those bands which were darkly stained and consistently present were used in order to give reproducible patterns of every population.

It is possible that some lightly stained bands may play an important role in the protein profile characteristics or even be related to the host plant. However, before an acceptable and workable system of classification based on protein patterns is established, researchers should focus on the accessible problems; important information on these lightly stained bands may add to the system later.
CHAPTER III

SEROLOGY

Introduction

Serological techniques have been used not only in diagnostic clinics but also in identifying bacteria, fungi, viruses, protozoa, and other microorganisms. Earlier serological work on nematodes was concentrated on animal parasitic nematodes (44, 48, 73, 74).

Molecular mimicries of metazoan parasites to their warm blooded hosts have been known and studied for many years. In the comprehensive review by Damian (18) the phenomenon of hosts and parasites sharing common antigens was discussed. Studies of common antigens between bacterial and fungal parasites and their plant hosts have confirmed the relationship of pathotypes and serotypes (20, 66, 82). However, the serological similarity in plant-animal relationships is still unknown.

Literature Review

Bird (6) in 1964 injected living larvae of M. javanica into rabbits and demonstrated that antibodies were induced and reacted with antigenic materials exuded from the nematode's excretory pore and buccal cavity. The gelatinous matrix and stylet tips of adult females were also antigenic. It must be
noted that all precipitation reactions took place on the surface of the nematodes; the sources of the antigenic materials within the nematodes were not studied. Lee (47) attempted to use immunodiffusion for species identification of *Meloidogyne* spp. by injecting freshly ground females into rabbits. This was the first incidence of using homogenized tissues to induce antibody formation to plant parasitic nematode antigens. Immunodiffusion was used in this study to demonstrate the antigen-antibody precipitation patterns.

Gibbins (30) reported that both qualitative and quantitative differences were found when antigens of various races of *Ditylenchus dipsaci* were used against antisera of another race. Cross-reaction was also obtained against *Aphelenchoides ritzemabosi*. Therefore, the authors suggested that serology may eventually enable biological races of *D. dipsaci* to be distinguished and may lend itself to intergeneric studies.

Taxonomically related nematodes *Panagrellus redivivus* and *Diplogaster* spp. were found serologically common and did not have any specificity. No similar immunoprecipitin bands were formed by either of the two against an unrelated nematode *Aphelenchus avenae* (25). Three species of *Ditylenchus* with five races of *D. dipsaci* and six species of *Heterodera* with two pathotypes of *H. rostrochiensis* were studied serologically by Webster and Hooper (80). They reported that the precipitation response divided the *Heterodera* spp. tested into two groups,
one containing *H. schachtii*, *H. trifolii* and *H. rostochiensis* and the other group containing *H. cruciferae*, *H. carotae* and *H. goettingiana*. Apparently no antigens are common to both groups. The three species of *Ditylenchus* tested were serologically distinct. No significant intraspecific differences were found among races or pathotypes of *Ditylenchus* and *Heterodera*.

Scott and Riggs (67) reported that two races of the soybean cyst nematode were serologically identical and were unrelated to the birch cyst nematode. The authors also reported a greater number of precipitin bands separated by immunoelectrophoresis than by immunodiffusion. Hussey (39, 40) studied two populations each of *M. incognita* and *M. arenaria*. Based on position and coalescence, he found most of the precipitin bands common to both species.

The two populations of *M. incognita* were serologically identical. The two populations of *M. arenaria* differed from each other with respect to one weak band. Later, Hussey et al. (42) reported thirteen immunoprecipitates developed with *M. incognita* antiserum and twelve formed with *M. arenaria* antiserum in an immunodisc electrophoresis test. Most of the immunoprecipitates were common.

Misaghi and McClure (54) studied the serological relationships of the eggs and larvae of *M. incognita*, *M. javanica*, and *M. arenaria*. Close serological relationships among those three species were found. However, some species-specific antigens were also demonstrated and confirmed by cross-
absorption tests. The authors suggested that fluorescently labeled antisera may provide a rapid identification of a single specimen of a female root-knot nematode.

Only one report was found dealing with the serology of plant-nematode relationships. McClure et al. (50) reported cross reactions between *M. incognita* and two selected hosts, cotton and soybeans. No evidence of the existence of a common antigen was found.

**Materials and Methods**

**Preparation of antisera**

Prepared samples (0.5 ml, containing one mg protein) were emulsified with an equal volume of Freud's complete adjuvant. The immunogen was injected intramuscularly into female New Zealand white rabbits (2-3 kg) at weekly intervals for 4-5 weeks. Blood samples were obtained by cardiac puncture after 7 weeks. Antibody titer was measured by serial dilution of antigen. If antibody titer was low, one ml of the prepared sample was injected directly into the rabbit's muscle until the titer of the antisera was high, which usually took five days. High antibody titer was determined when a precipitin band was formed by diluting the antigen 1 to 2 with phosphate buffer saline (0.05 M, pH 7.4). Antisera were stored in small quantities at -75°C until needed.

**Preparation of root extracts**

The host plants used in the differential host test were
planted in 15-cm clay pots under a temperature range of 25-30°C. After 40 days, plant roots were taken down, washed clean and rinsed in ice-cold buffer. The buffer contained 0.05 M potassium phosphate, 0.05 M sodium ascorbate, 0.001 M magnesium chloride, pH 7.2. Roots were blended in ice-cold buffer (1 g/3 ml) for two minutes at maximum speed and extracted for 12 hours at 4°C. The extracted root homogenate was filtered through No. 1 Whatman filter paper. Filtrates containing extracted soluble root proteins were stored at -75°C until needed.

**Immunodiffusion and immunoelectrophoresis**

Immunodiffusion was carried out in 0.5 percent agar gel in 0.02 M sodium phosphate buffered saline, pH 7.2 (49). The plates were developed at room temperature for 48 hours, washed in one percent NaCl, dried and stained with Amidoblue Black B and destained in a solution of acetic acid:water:methyl alcohol (1:5:5) (49).

A one percent agarose gel in 0.02 M sodium barbital, HCl buffer, pH 8.6, was used for immunoelectrophoresis (49). A constant current of 3 ma/slide frame was applied for 80 minutes. The incubation, staining and destaining were the same as for immunodiffusion.

**Results**

**Immunodiffusion**

**Adult females**

Based on the position and coalescence, some of the pre-
cipitin bands were common in all populations studied. There were differences in precipitin bands both between and within species (Figure 6). The antisera of each population demonstrated about the same precipitin patterns against antigens of other populations. In Figure 6A, a specific band of identity was demonstrated between M. arenaria antigen and M. arenaria antiserum. No cross reactions of this antigen was observed with any other population antisera. This is an antibody unique to M. arenaria only, and is immunologically able to separate M. arenaria from M. incognita and M. javanica in this study. Differences between species may also be observed by comparing the precipitin patterns of Figures 6B, C, and D. The differences within species are easily observed by comparing the bands of identity in Figures 6B and C. M. incognita (A) has one more distinctive band whereas no precipitin bands occur for M. incognita (S). In Figure 6C, as pointed out by the arrow, M. incognita (A) has one more precipitin band than did M. incognita (S).

Eggs

The buffer soluble protein extracts of eggs did not induce as many antibodies as did that of adult females (Figure 7). However, there were immunoprecipitate bands occurring in the egg precipitin pattern which were not exhibited in adult females. When comparing the identity between M. incognita (A) and M. incognita (S) in Figure 7B and between M. incognita (A)
Figure 6. Immunodiffusion patterns of different populations of adult females of Meloidogyne spp. Arrows in figure 6A: differences between species of Meloidogyne spp. in 6B, C differences within species of *M. incognita*. 
Figure 7. Immunodiffusion patterns of different populations of eggs of Meloidogyne spp. Arrows in figure 7A, upper 7B, 7C, 7D: Differences between species of Meloidogyne spp., in lower 7B: differences within species of M. incognita.
Adult females and eggs

There were significant serological differences between adult female antigens and egg antigens of each population against the antisera of adult females and eggs (Figures 8 and 9). The non-identity serological differences between adult females and eggs within populations are also demonstrated in Figures 8 and 9.

Immunological relationships of Meloidogyne species to host plants

The root extracted protein antigens of ten host plants against the antisera of all nematode populations showed no serological relationship except for 'Porto Rico' sweetpotato. This variety formed two immunoprecipitin bands (Figure 10).

The precipitin between 'Porto Rico' sweetpotato and antisera occurred with adult females and with eggs of all populations studied. It shows no relationship to the host range of nematode populations, since only the two populations of *M. incognita* reproduced on this host.

Immunoelectrophoresis

Adult females

Significant differences in immunoelectrophoresis patterns existed both between and within species (Figure 11). In Figure 11A five distinctive precipitin bands were separated on *M. arenaria* compared with four on *M. incognita* (S);
Figure 8. Immunodiffusion patterns of antigens of adult female of *Meloidogyne* spp. against their own antisera and against antisera of their own eggs.
Figure 9. Immunodiffusion patterns of antigens of eggs of *Meloidogyne* spp. against their own antisera and against antisera of their own adult female.
Figure 10. Immunodiffusion pattern of antiserum of adult female *M. arenaria* against plant root extracts.
Figure 11. Diagrammatic representation of immunoelectrophoretic patterns of different populations of adult females of *Meloidogyne* spp.
similar results are shown in Figures 11B, C, E, and F. In Figure 11D, a positive charged antibody induced by M. incognita (S) was separated by the electric field and moved toward the cathode of the microslide, thereby separating the two populations of this species.

Eggs

There were too few significant precipitin patterns in eggs to separate species or populations within species (Figure 12). In this study only M. arenaria and M. incognita populations showed slight differences (Figures 12A and B).

Adult females and eggs

Significant immunoelectrophoretic differences between the adult females and eggs are shown in Figure 13. In this study, 4-5 precipitin bands were separated in adult females whereas only 2-3 precipitin bands were separated in eggs.

Host relation

An obscure immunoprecipitin band was observed when the root extract antigen of 'Porto Rico' sweetpotato was applied against antisera of all adult females and eggs studied (Figure 14). This suggests that 'Porto Rico' sweetpotato has structural proteins similar to those of the antigens of nematodes.
Figure 12. Diagrammatic representation of the immunoelectrophoretic patterns of different populations of eggs of *Meloidogyne* spp.
Figure 13. Diagrammatic representation of the immunoelectrophoretic patterns of adult females compared to eggs of Meloidogyne spp.
Figure 14. Diagrammatic representation of the immuno-electrophoretic patterns of antisera of adult females of Meloidogyne spp. against root extracts of 'Porto Rico' sweetpotato.
Discussion

Serological tests showed that all the populations are closely related. However, there were more identity bands within species than between species both in immunodiffusion and immunoelectrophoresis. Serologically, the adult females demonstrated more non-identity precipitin bands than did the eggs; by means of these patterns all populations studied can be distinguished. The intraspecific differences of two *M. incognita* populations are distinct as are the interspecific differences among *M. arenaria*, *M. incognita*, and *M. javanica*. There is no serological evidence on the relationship of nematode populations to their host range. The precipitin bands on 'Porto Rico' sweetpotato give no significant information in this study.

As pointed out before, identification of *Meloidogyne* spp. by morphology and host range is still confusing and time consuming, and serological techniques may facilitate this identification by providing the necessary information. Modern techniques such as fluorescently labeling antisera, purification of antibodies, and antibodies induced by purified species-specific antigens will make the serological information easier to obtain and more likely to play an important role in the identification of *Meloidogyne* spp.
CHAPTER IV

AFFINITY CHROMATOGRAPHY

Introduction

Research on practical methods and procedures for the isolation and purification of antibodies from antisera have been attempted for twenty years (1, 9, 13, 32, 36, 57, 69, 71, 82, 83). The approach was to produce an insoluble protein antigen matrix which would combine specifically with antibodies to give a complex that could be dissociated into soluble antibodies and insoluble antigens (9).

Different methods for insolubilization of biologically active proteins have been reported. However, covalent binding of the protein to a suitable water insoluble carrier exhibits the greatest flexibility (13, 17, 71). Agarose or sepharose immunoadsorbents activated by cyanogen bromide have been used successfully to purify antibodies (32, 36, 57, 83). Antibodies have also been coupled to agarose or sepharose to purify antigens (1, 13). The use of affinity chromatography for purification of antigens and antibodies shows promise as a useful isolation procedure (14, 15, 16).
Materials and Methods

Affi-gel 10, a N-hydroxysuccinimide ester of succinylated aminoalkyl agarose, (Bio Rad Laboratories, 32nd and Griffin Avenue, Richmond, California 94804) was used in this study for the purpose of covalent binding of the antigen.

Antigens were diluted in 0.1 M sodium bicarbonate buffer, pH 7.4 (2 mg/10 ml) and coupled with 0.33 g affi-gel 10 for 12 hours at 4°C with slight agitation. The affi-gel 10-sodium bicarbonate suspensions then were packed in columns (8mm x 10 cm). Columns were washed with 0.1 M potassium phosphate buffer (pH 6.8) until no detectable proteins were observed passing from the columns.

The protein contents of the antigens were determined fluororometrically before coupling. All the sodium bicarbonate buffer after coupling and potassium phosphate buffer washes were retained for protein determination in order to calculate the percentage of proteins coupled with the affi-gel 10.

Antisera were diluted with one percent NaCl solution (1:1, v/v) and applied into the column coupled with their own antigens with a flow rate of 0.2ml/min. The columns then were washed with 0.1 M potassium phosphate buffer pH = 6.8, and eluted with 1 M acetic acid or 3 M sodium thiocyanates (NaCNS) in 0.1 M potassium phosphate buffer pH 6.8. Those solutions eluted with acetic acid were brought back to pH 7.0 by 0.1 M NaOH before freeze drying and those eluted with 3 M sodium
thiocyanates were dialyzed against the potassium phosphate buffer. Both non-absorbed antisera and the desorbed elutions were freeze dried and then dissolved in 0.1 M potassium phosphate buffer pH 6.8 to increase the concentration of the antibodies. These then were tested in immunodiffusion plates.

Results

The coupling of the antigens to the affi-gel 10 was high. The coupling ratios of the proteins in the samples to the affi-gel 10 were 85.8-88.5 percent.

The results of the immunodiffusion showed that the antibodies did not bind to their own antigens. All the precipitin bands formed in the antisera passed through the column directly. Neither acetic acid nor sodium thiocyanate elutions formed any precipitin bands.

Discussion

The purpose for using affinity chromatography was to obtain purified population-specific antibodies. For unknown reasons, it did not work. However, this technique can be refined and when larger amounts of antigens and antisera are available, purified population-specific or species-specific antibodies may be obtained by further investigations.
CHAPTER V

CONCLUSIONS

The total protein patterns analyzed by disc electrophoresis in this study show promise as biochemical techniques to provide information for identification of nematodes.

The total protein patterns of adult females of the four populations proved sufficiently distinctive for identification; those of eggs proved to be much less distinctive.

The protein patterns of glycoprotein, mucoprotein, and lipoprotein did not provide information for identification purposes.

The results of these studies showed that the antibodies induced by the buffer soluble protein extracts could be used to separate all the populations studied. Population-specific antibodies were shown. Antibodies induced by buffer soluble proteins of eggs could not be used to separate populations, although some differences were demonstrated.

Within the four populations of Meloidogyne spp., total protein patterns obtained by disc electrophoresis and serological reactions showed differences between the buffer soluble protein extracts of adult females and eggs.

There were two precipitin bands in immunodiffusion between 'Porto Rico' sweetpotato antigens and the antisera of
the buffer soluble protein extracts of all populations. This does not indicate a serological relationship between the *Meloidogyne* spp. populations and their host range, since it does not correlate to the host response to the nematode populations.

Techniques for obtaining quantities of living *Meloidogyne* females were further refined in this study. Approximately 10gm of clean *Meloidogyne* adult females could be obtained within 2 hours after the infected roots were soften by Pectinol 59-L.


BIOGRAPHICAL SKETCH

Franklin Hon-ching Chow was born January 20, 1945, at Chen-do, China, mainland. He moved to Taiwan in 1949 and attended public school and senior high school in Chia-I. After graduation he attended National Taiwan University from 1964 to 1968 and received a Bachelor of Science Degree with a major in Entomology.

After one year of military service, he worked as a research assistant in the Department of Plant Pathology and Entomology, National Taiwan University for one year.

From 1970 to 1972, he obtained his Master's Degree in Nematology in the Department of Entomology and Nematology, University of Florida, Gainesville, Florida. Since then he has been continuing his study in the Department of Entomology and Nematology, University of Florida, for his Ph.D. degree.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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June, 1977

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