

Antigenic Relationship of *Meloidogyne incognita*, *M. javanica*, and *M. arenaria*

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Supported in part by Cotton Incorporated from funds made available through the Commodity Credit Corporation, USDA.

Journal Series Paper No. 2210 of the Arizona Agricultural Experiment Station.

Accepted for publication 3 December 1973.

ABSTRACT

Serological relationships of the eggs and larvae of *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* were studied. Based on agar-gel double-diffusion tests and immunoelectrophoresis, all three species are closely related

serologically; however, *M. incognita* and *M. javanica* each possess two to three unique serologically active components not shared by the other two species.

Phytopathology 64:698-701.

Additional key words: root-knot nematode, intragel cross-absorption.

Serological tests have been used extensively to identify bacteria, fungi, and viruses, but there are few cases where this important technique has been utilized to characterize plant parasitic nematodes. Webster and Hooper (13) identified two distinct serological groups among six *Heterodera* species and found that three species of *Ditylenchus* were serologically distinct. Scott and Riggs (9) showed that two races of *Heterodera glycines* were serologically unrelated to *Heterodera betulae*. Hussey (5) noted that adult females of *M. incognita* and *M. arenaria* had many precipitin bands in common. However, one precipitin band unique to *M. incognita* was found when antiserum to *M. incognita* was reacted with homologous and heterologous antigens.

We have examined the serological relationships of larvae and eggs of *M. incognita*, *M. javanica*, and *M. arenaria*.

MATERIALS AND METHODS.—*Meloidogyne incognita* (Kofoid and White) Chitwood, was propagated on chili pepper and *Meloidogyne javanica* (Treub) Chitwood, and *Meloidogyne arenaria* (Neal) Chitwood, on tomatoes in the greenhouse. A single isolate of each species was used for all tests. Eggs were obtained by the method of McClure et al. (6). Larvae were collected by placing eggs on a nylon screen (pore size, 20 μ m) over a modified Baermann funnel. Hatched larvae migrated through the screen and were collected in water containing 13 μ g/ml of Aretan (3).

Preparations of larval and egg antigens.—Freshly prepared eggs and larvae, collected within 24 h of hatching, were washed several times with sterile water and were suspended in 2.5 volumes of 0.05 M potassium

phosphate buffer (pH 7.2) containing 0.14 N sodium chloride and 0.001 M magnesium chloride. These suspensions were homogenized by passage through a chilled French pressure cell four times, and used to immunize rabbits. Antigenic preparations were centrifuged at 20,000 g for 30 min and the supernatants were stored at -16 C until used in serological reactions.

Preparation of antisera.—Antisera were produced in New Zealand white doe rabbits by intravenous, intraperitoneal, and intramuscular injections over 14 days (2). Antisera also were produced by foot-pad injections (R. S. Hussey, *personal communication*). Sera were stored frozen at -16 C in small quantities until use.

The homologous titers of antisera were determined by agglutination tests with antigens adsorbed on polystyrene latex particles (Colabtex, Colab, Chicago) (7). For agar-gel diffusion tests Ionagar No. 2 (Colab, Chicago) (0.7%) was dissolved in 0.01 M potassium phosphate buffer (pH 7.2) containing 0.14 N sodium chloride and 0.02% merthiolate. Ten ml of the above agar was added to 100 mm plastic petri dishes. Intragel cross-absorption (11) was used for determination of species-specific precipitin bands.

Immunoelectrophoretic analysis.—Prior to electrophoretic separation, the protein concn of antigenic preparations from the eggs of *M. incognita* was increased about fivefold using a Zeineh Microconcentrator, Model UF-T (RHO Scientific, Inc.). Glass plates (9.5 \times 5.5 cm) were covered with a thin layer of 0.9% Ionagar No. 2, prepared with 0.02 M barbital buffer, pH 8.2. Chambers were filled with the above buffer (0.06 M) and antigenic components from the eggs of *M. incognita* were separated

by applying a potential of 70 V. After electrophoretic separation, one trough was filled with nonabsorbed antiserum for the eggs of *M. incognita* and the other with the same antiserum which had been absorbed with an antigenic preparation from the eggs of *M. javanica*. Cross-absorption for the above test was done according to Wimalajeewa and DeVay (15).

RESULTS.—A close serological relationship between *M. incognita*, *M. javanica* and *M. arenaria* was shown by the presence of a number of common precipitin bands

(Fig. 1). However, some species-specific antigens also were demonstrated. Their presence was further confirmed by cross-absorption tests (Fig. 2). *M. incognita* possessed one specific precipitin band which was not present in *M. javanica* or *M. arenaria* (Fig. 2-A, B) and at least two precipitin bands unique to *M. javanica* which were not associated with either *M. incognita* or *M. arenaria* (Fig. 2-C, D). The single specific precipitin band of *M. incognita* was resolved into three components by cross-absorption and immunoelectrophoresis (Fig. 3). No

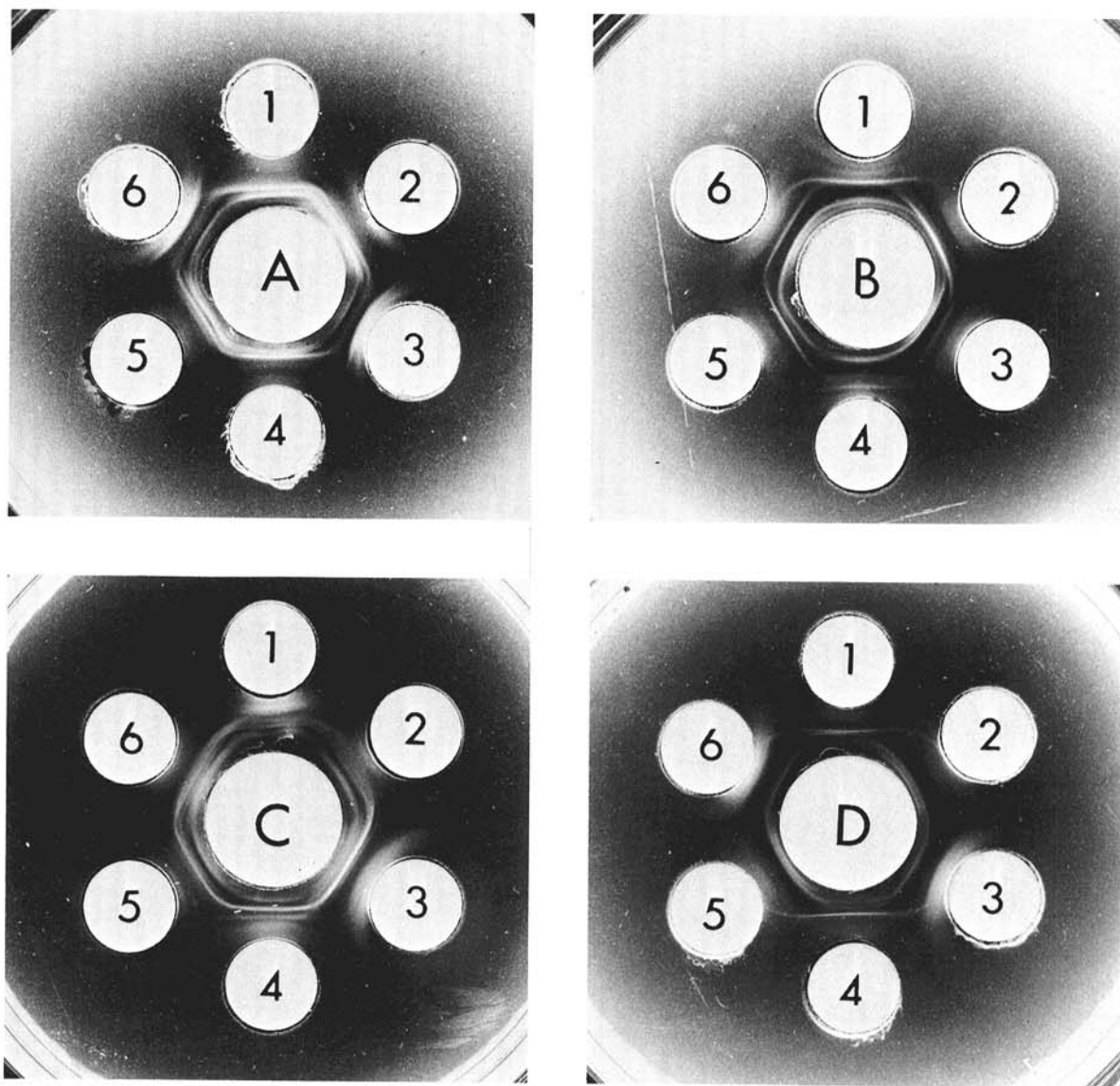


Fig. 1—(A to D). Precipitin patterns in an agar double diffusion test showing the antigenic relationships of *Meloidogyne* species. **A**) Antiserum for the eggs of *Meloidogyne incognita* (center well) reacted with antigen preparations from the eggs of *M. javanica* (1 and 4), *M. arenaria* (2 and 5), and *M. incognita* (3 and 6). **B**) Antiserum for the eggs of *M. javanica* (center well) reacted with antigen preparations from the eggs of *M. javanica* (1 and 4), *M. incognita* (2 and 5), and *M. arenaria* (3 and 6). **C**) Antiserum for the larvae of *M. incognita* (center well) reacted with antigen preparations from the larvae of *M. incognita* (1 and 4), *M. arenaria* (2 and 5), and *M. javanica* (3 and 6). **D**) Antiserum for the larvae of *M. javanica* (center well) reacted with antigen preparations from the larvae of *M. javanica* (1 and 4), *M. arenaria* (2 and 5), and *M. incognita* (3 and 6).

precipitin bands were formed in similar tests where antiserum for *M. incognita* was absorbed with its homologous antigen.

Antisera produced by foot-pad injection had better titers (12,800-25,600) than those produced by a combination of intravenous, intraperitoneal, and intramuscular injections (6,400-12,800).

DISCUSSION.—Identification of species of *Meloidogyne* is based upon the perineal pattern of adult

females, morphometrics of eggs and larvae and differential host tests. However, considerable intraspecific variation of the perineal pattern occurs (1) and usually many individuals must be examined for a positive determination (14). Furthermore this method is time-consuming and requires the skills of a trained nematologist. Mixed populations, which occur on a single host and which show pronounced intraspecific variation, can present serious difficulties (14). Host range

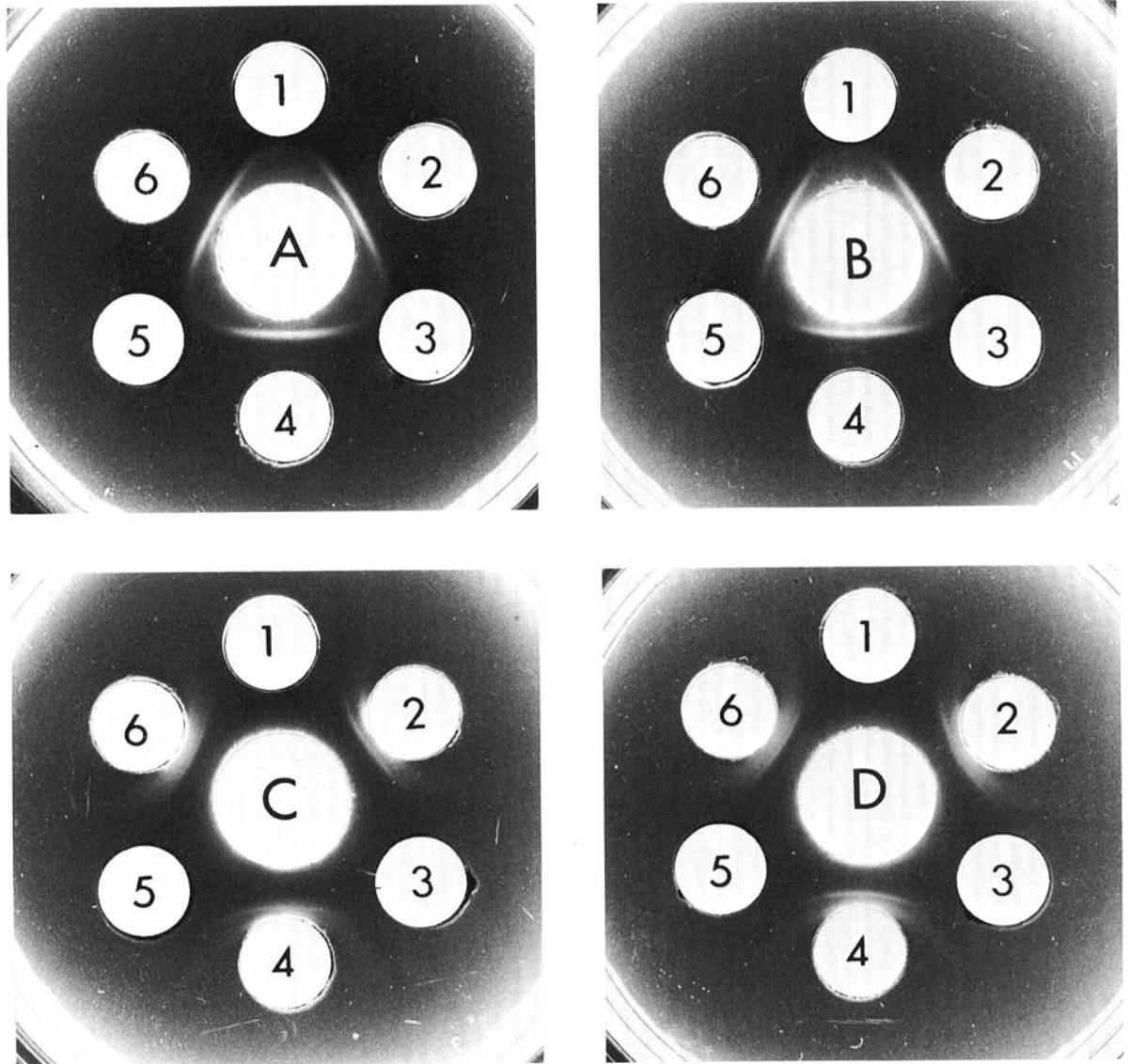


Fig. 2-(A to D). Precipitin patterns in agar double diffusion tests showing the results of cross absorption tests. A) Antiserum for the eggs of *Meloidogyne incognita* absorbed with antigen from the eggs of *M. javanica* (center well) and then reacted with antigenic preparations from the eggs of *M. javanica* (1, 3, and 5), and *M. incognita* (2, 4, and 6). B) Antiserum for the eggs of *M. incognita* absorbed with antigen from the eggs of *M. arenaria* (center well) and then reacted with antigenic preparations from the eggs of *M. arenaria* (1, 3, and 5) and *M. incognita* (2, 4, and 6). C) Antiserum for the eggs of *M. javanica* absorbed with antigen from the eggs of *M. incognita* (center well) and then reacted with antigenic preparations from the eggs of *M. incognita* (1, 3, and 5), and *M. javanica* (2, 4, and 6). D) Antiserum for the eggs of *M. javanica* absorbed with antigen from the eggs of *M. arenaria* (center well) and then reacted with antigenic preparations from the eggs of *M. arenaria* (1, 3, and 5), and *M. javanica* (2, 4, and 6).

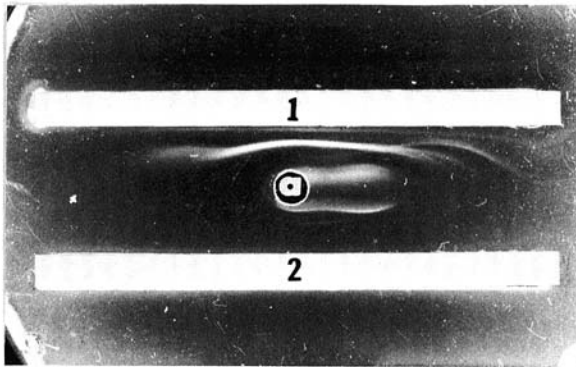


Fig. 3. Precipitin pattern in an immunoelectrophoretic test. Antisera for the eggs of *Meloidogyne incognita*, unabsorbed (1) and absorbed with antigenic preparation from the eggs of *M. javanica* (2), reacted with antigenic preparations from the eggs of *M. incognita* (a), following electrophoretic separation.

tests aid in identifying species of root-knot nematode (8), but involve prolonged greenhouse culture of differential host plants. Therefore, a technique is clearly needed for rapid and positive identification of species of this nematode and serological approaches appear to be promising.

Hussey (5) has shown that adult females of *M. incognita* and *M. arenaria*, although closely related serologically, can be distinguished on the basis of one precipitin band unique to *M. incognita*.

Our results show that eggs and larvae of three species of *Meloidogyne* also can be distinguished serologically. These are the developmental stages most frequent in soil samples and morphologically, eggs or larvae of different species are nearly indistinguishable (14).

The occurrence of species-specific antigenic components in both eggs and larvae of *M. incognita*, *M. javanica* and *M. arenaria* suggests that serological tests could be devised for identification of species of this nematode. Fluorescent antibody techniques, for instance, have been applied in the diagnosis of such diverse microorganisms as protozoans (4), viruses (12) and bacteria (10). The bacterium *Malleomyces pseudomallei* can be detected in the soil when it constitutes only one millionth of the bacterial cells present (10).

Since quantities of *Meloidogyne* eggs (and from them, larvae) are readily available (6) for volume production of fluorescently labeled antisera rapid identification of single specimens of root-knot nematodes could be possible by this approach.

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