

Immunelectrophoretic Comparisons of Three Plant-Parasitic Nematodes

H. A. Scott and R. D. Riggs

Department of Plant Pathology, University of Arkansas, Fayetteville 72701.

ABSTRACT

Reciprocal tests showed that two races of the soybean cyst nematode are serologically identical and are unrelated to the birch cyst nematode. Immunelectrophoresis of nematode extracts, when compared to gel diffusion, resulted in greater numbers of precipitin bands. *Phytopathology* 61:751-752.

Webster & Hooper (3) showed the gel diffusion technique to be useful in investigating serological relationships among plant parasitic nematodes. Their comparisons demonstrated that *Heterodera* and *Ditylenchus* are not related. No relationships were found among species of *Ditylenchus*, and no serological differences were found among races. Within *Heterodera*, *H. schachtii*, *H. trifolii*, and *H. rostochiensis* are related, and *H. cruciferae*, *H. goettingiana*, and *H. carotae* are related, but there is no serological relationship between the two groups. Their tests demonstrated that plant-parasitic nematodes are serologically complex. Usually, one or two precipitin bands were observed in the agar, and in one test four bands were obtained.

Although gel diffusion tests are useful for characterizing mixtures of antigens, more than one antigen-antibody system can be present in a band. The technique of immunelectrophoresis is better for analysis of complex mixtures of antigens because the antigens are separated by electrophoresis before antibodies are diffused against them (1). We were, therefore, interested in continuing the investigations of serological relationships among *Heterodera* species and in evaluating the usefulness of immunelectrophoresis with these particular antigenic systems. This report describes serological studies of two races of soybean cyst nematode (SCN), *Heterodera glycines*, and the birch cyst nematode (BCN), *H. betulae*, using gel diffusion and immunelectrophoresis.

The populations of SCN used in these studies were collected near Blytheville, Ark. Races 3 (SCN-3) and 4 (SCN-4) were maintained on Lee and Pickett soybeans, respectively. BCN was obtained from river birch roots growing on Middlefork River near Fayetteville, Ark., and was cultured in the greenhouse on river birch.

SCN and BCN females were obtained by the roiling and sieving method (2). The females and associated debris collected on the 60-mesh sieve were subjected to a series of suspensions, screenings, sedimentations, and decantations to eliminate root fragments and other organic debris. From 500 to 1,000 SCN and 250-500 BCN, which served as antigens, were picked individually with tweezers, placed in 0.5 ml 0.85% saline,

crushed with a conical plunger, and frozen at -20°C . For injection, 0.5 ml antigen was thawed and emulsified with an equal volume of Freund's complete adjuvant. Two rabbits for each antigen were injected intramuscularly 6 times over a period of 20 weeks. The rabbits were bled at 2- to 4-week intervals, and the antisera frozen. Bleedings that gave the highest number of reacting bands were used.

Gel diffusion tests were made in 1% agarose in 0.0125 M veronal buffer, pH 8.8, using the Gelman-LKB apparatus (Gelman Instrument Co., Ann Arbor, Mich.). Electrophoresis samples were run in the same medium at 4 C for 50 min at 7 ma/frame in 0.05 M veronal buffer, pH 8.8. The antigen sample consisted of 3-4 fillings of the well. Following electrophoresis, antisera were added to the troughs and immunodiffusion was allowed to proceed for 48 hr. The slides were

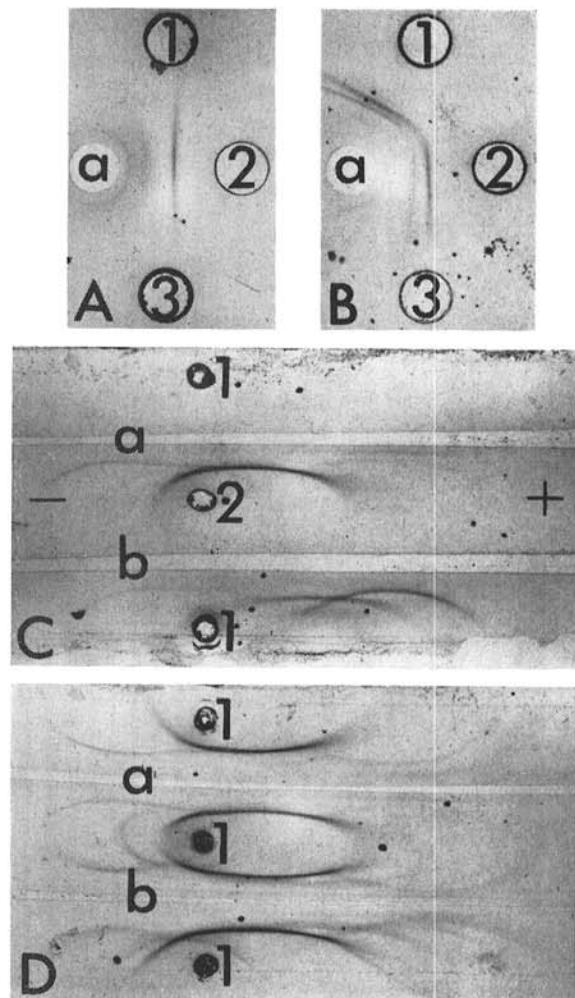


Fig. 1. Gel diffusion and immunelectrophoretic comparisons of *Heterodera glycines*, races 3 and 4 (SCN-3, SCN-4) and *H. betulae* (BCN). **A**) a = BCN antiserum; 1 = SCN-3; 2 = BCN; 3 = SCN-4. **B**) a = SCN-4 antiserum; 1 = SCN-3; 2 = SCN-4; 3 = BCN. **C**) a, b = SCN-3 and BCN antisera; 1 = BCN; 2 = SCN-3. **D**) a, b = SCN-4 and 3-antisera; 1 = SCN-3.

soaked in 2% NaCl, dried, and stained with amidoblau B according to the Gelman manual. Normal serum controls were included at intervals. In absorption tests, antisera were absorbed by adding homologous antigens (the usual volume ratios were 1:2), incubating the mixture overnight at 4 C, and removing the precipitate by low-speed centrifugation (1,250 g for 5 min). Control antiserum was appropriately diluted with saline.

In gel-diffusion tests, BCN antiserum reacted with the homologous antigen and formed one band, but did not react with either race of *H. glycines* (Fig. 1-A). Antisera for SCN-3 and for SCN-4 cross-reacted identically with these antigens, but did not react with BCN antigen (Fig. 1-B). Two bands were visible and appeared to be identical, but differed slightly in their locations in the agar. This was probably due to differences in antigen-antibody ratios. Cross-absorption of SCN-3 and -4 antisera with SCN-3 or 4 antigen removed all reacting antibody.

Immunoelectrophoresis of BCN resulted in 3-4 bands when homologous antiserum was used (Fig. 1-C). As was expected from gel diffusion tests, no cross-reaction occurred between BCN antigen and either SCN antiserum or either SCN antigen and BCN antiserum. SCN-3 and SCN-4 antigens gave several iden-

tical bands when reacted against either SCN-3 or SCN-4 antisera. Typical bands obtained are illustrated in Fig. 1-D. Cross-absorption tests removed all reacting antibody. No reactions were observed in normal serum controls.

Reciprocal serological tests showed that the soybean and birch cyst nematodes are not related serologically. Whether either species belongs to the "schachtii" or the "cruciferae" serological groups, or neither, was not established. We were unable to differentiate between two races of a species with either gel diffusion or immunoelectrophoresis; however, immunoelectrophoresis produced more precipitin bands, and may prove useful when studies are expanded to include more species and races of *Heterodera*.

LITERATURE CITED

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