Serological Relationship of Meloidogyne incognita and M. arenaria[1]

R. S. HUSSEY[2]

Abstract: Eight to ten precipitin bands were formed in a double immunodiffusion system comparing antigens of adult females of *Meloidogyne incognita* and *M. arenaria.* Most of the precipitin bands, based on band position and coalescence, were common to both species. Antiserum specific for *M. incognita* was prepared by cross absorption. Two populations of *M. incognita* were serologically identical, whereas two populations of *M. arenaria* differed slightly with respect to one weak precipitin band. *Key words:* antigens, root-knot nematode, foot-pad injection procedure.

Serological techniques have recently been used to study relationships of nematodes (2, 3, 8, 9). Scott and Riggs (8) reported that two races of *Heterodera glycines* are serologically identical and unrelated to *H. betulae.* Webster and Hooper (9), however, were able to distinguish inter- and intraspecific differences among species of *Heterodera* and *Ditylenchus.* They reported that six *Heterodera* species could be separated into two distinct serological groups, and that these groups apparently did not have any antigens in common. Antigens from three species of *Ditylenchus* reacted only with homologous antiserum, indicating that these three species were serologically distinct.

This paper presents a study of the serological relationships between populations of *Meloidogyne incognita* and *M. arenaria* that originated from widely separated geographical regions of the world and had slightly different host ranges. A preliminary report of this work has been published (6).

MATERIALS AND METHODS

Two populations each of *Meloidogyne incognita* (from Peru and Taiwan) and *M. arenaria* (from Virginia and Greece) were propagated on tomato, *Lycopersicon esculentum* Mill. 'Rutgers', and adult females were collected as described previously (5). Antigen preparations consisted of the supernatant fluid resulting from centrifuging a nematode-buffer (1:1.5 v/v) homogenate (7).

Antisera for each population were produced in New Zealand white rabbits by a foot-pad injection procedure (Robert B. Fritz, personal communication). Antigen preparations were emulsified (1:1 v/v) with Freund's incomplete adjuvant, and 1 ml was injected into the foot-pad of each hind foot at 3- to 4-week intervals. Although good antiserum was obtained after a second injection, rabbits were usually bled 8-12 days after the third and subsequent injections. Based on the number and intensity of precipitin bands, preliminary tests indicated that the foot-pad injection procedure was superior to an intramuscular procedure for producing antiserum. Antisera were stored in 2-ml fractions at -18 C without preservative. Immunodiffusion tests were conducted in 100-mm plastic petri dishes containing 15 ml of a medium consisting of 0.8% purified Difco agar, 0.85% sodium chloride, and 0.02% sodium azide in distilled water. Circular patterns consisting of a large center well (10-mm) for the antiserum and six smaller periphery wells (7-mm) for the antigens with 7 mm between the wells were used in the double diffusion tests. Charged plates were incubated at 24.5-25.5 C in a moist chamber and examined after 48 hr. The precipitin bands were stained with Buffalo Black NBR for further examination (1).

Absorbed antisera were prepared by absorption (1:1 v/v) with heterologous antigens for 1 hr at 37 C with periodic shaking. The mixture was centrifuged in a clinical centrifuge at 1100 *g* for 10 min. The supernatant fluid was absorbed two additional times, each time reducing the volume of the heterologous antigens by half, then incubating and centrifuging each time as before. The supernatant fluid from the third absorption was concentrated by dialysis, and constituted the absorbed antisera used in the immunodiffusion tests.

RESULTS AND DISCUSSION

Eight to ten precipitin bands formed in

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[2] Research Associate, Department of Plant Pathology, North Carolina State University, Raleigh 27607. The author acknowledges the technical assistance of Lieselotte Conniff and Danny Powell and the photography of Marvin Williams.

double immunodiffusion tests that compared antigens of *M. incognita* and *M. arenaria* (Fig. 1-A, B). Most of the precipitin bands, based on band position and coalescence, were common to both species. One precipitin band unique to *M. incognita* formed when antiserum to *M. incognita* was reacted with homologous and heterologous (*M. arenaria*) antigens (Fig. 1-A). Even though this precipitin band was contiguous to another precipitin band common to both species, it was interpreted to be formed by an antigen present only in the *M. incognita* extract. When *M. arenaria* antiserum was reacted with these antigens, however, all precipitin bands that formed between the two species coalesced (Fig. 1-B). When *M. incognita*-Taiwan antiserum previously absorbed with *M. arenaria*-Virginia antigens was reacted with homologous and heterologous antigens, one strong precipitin band formed only between the homologous antigens and antiserum (Fig. 1-C). In similar tests using absorbed *M. arenaria*-Virginia antiserum, no precipitin bands formed. Throughout this study, no precipitin bands formed when normal rabbit serum was used.

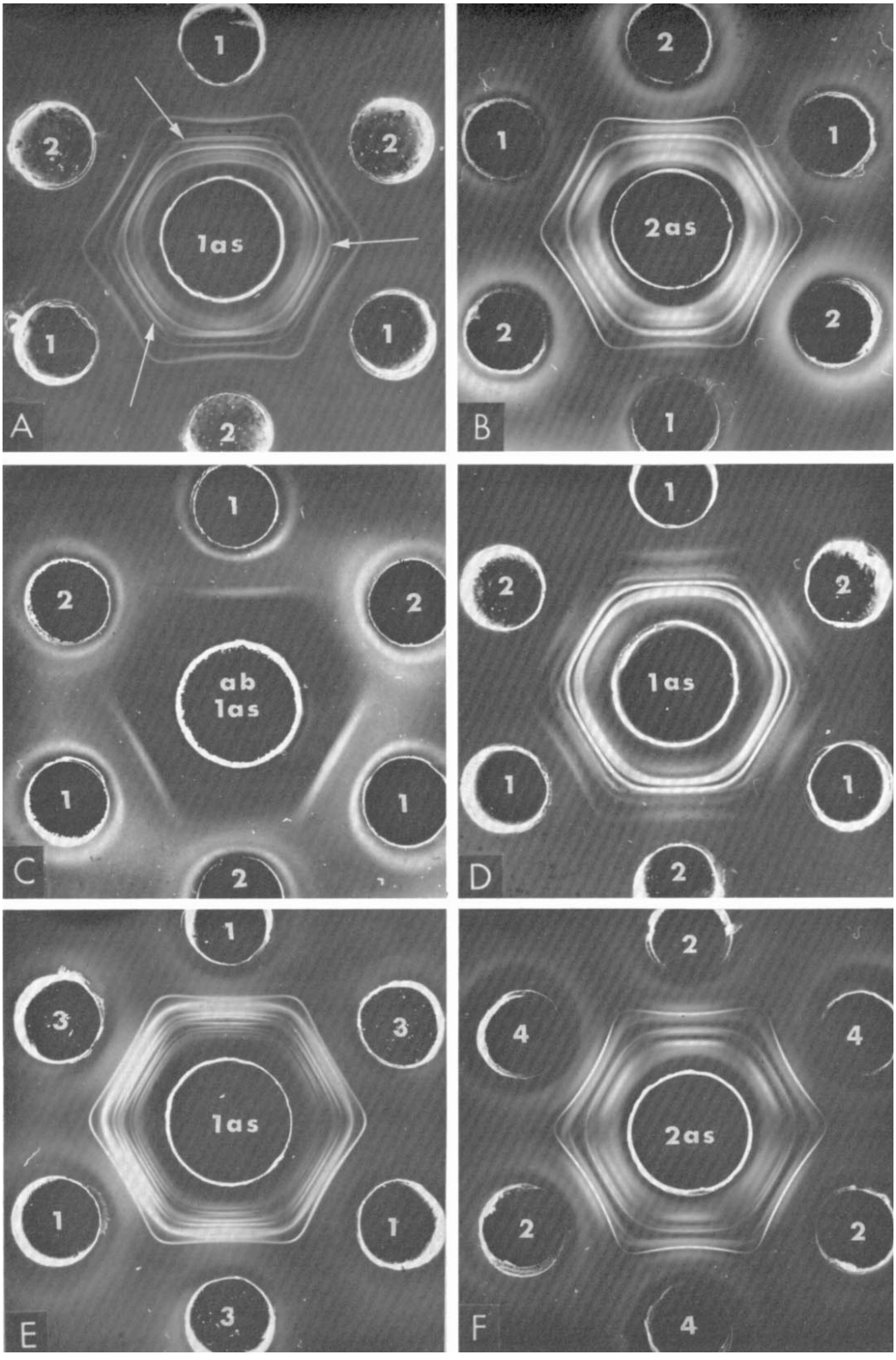
The large number of common precipitin bands that formed in the double diffusion tests indicates a close serological relationship between the two *Meloidogyne* species. Webster and Hooper (9), however, found that three species of *Ditylenchus* were serologically distinct, and that six species of *Heterodera* could be separated into two distinct serological groups.

Immunoprecipitin patterns resulting from multiple antigen systems often are difficult to interpret. Differences in precipitin bands can be obscured due to the complex nature of a precipitin pattern or to diffuse precipitin bands. These problems primarily arise from unbalanced antigen-antibody systems. When multiple antigen systems are used, each reactant cannot be adjusted to its optimum concentration. In the present study, the greatest number and the best overall sharpness of the precipitin bands were obtained when nematode antigen preparations were used undiluted. Two bands near the antiserum well, however, were sharpest when the antigen preparations were diluted 1:1 (v/v), whereas the other bands in the pattern were very weak (Fig. 1-D). The strong precipitin bands coalesced and were not helpful in distinguishing between the two *Meloidogyne* species. This procedure may prove useful when comparing antigens of other nematode species or genera. Perhaps more striking serological differences would be obtained by making comparisons with certain purified nematode proteins. Partial purification of *Fomes annosus* antigens resulted in simplification of a precipitin pattern, and permitted an easier interpretation of the interaction of precipitin bands (4).

Comparative studies of proteins of these two *Meloidogyne* species by polyacrylamide-gel electrophoresis revealed qualitative and quantitative differences in their protein composition (7). *Meloidogyne incognita* and *M. arenaria* were distinguished reliably by their non-specific esterase, malate dehydrogenase, and α-glycerophosphate dehydrogenase patterns, and less reliably by their soluble-protein patterns. Many of the soluble proteins of these two species were antigenically similar as determined by immunodisc electrophoresis.

The usefulness of serology in distinguishing populations or races of nematodes is still uncertain. In tests comparing Taiwan and Peru populations of *M. incognita*, nine distinct precipitin bands formed (Fig. 1-E). All precipitin bands, however, coalesced, indicating that these two populations were serologically identical. Similar results were obtained with antiserum to either the Peru or Taiwan

FIG. 1. Double immunodiffusion tests comparing antigens of adult females of *Meloidogyne incognita* populations, from Peru and Taiwan, and *M. arenaria* populations, from Greece and Virginia. Nematode antigens are in the peripheral wells and antisera are in the center wells. **A.** A test comparing *M. incognita*-Peru (1) with *M. arenaria*-Virginia (2) against antiserum (1as) to *M. incognita*-Peru. Arrow points to precipitin band characteristic for *M. incognita*. **B.** A test comparing the same antigens as in A showing no difference when antiserum (2as) to *M. arenaria*-Virginia is used. **C.** A test comparing the same antigens as in A against antiserum (ab 1as) to *M. incognita*-Peru absorbed three times with *M. arenaria*-Virginia antigens showing a reaction only with the homologous combination. **D.** A test similar to A, except that antigen preparations are diluted 1:1 (v/v) with buffer. **E.** A test comparing the Peru (1) and Taiwan (3) populations of *M. incognita* against antiserum to the Peru population showing no differences. **F.** A test comparing the Virginia (2) and Greece (4) populations of *M. arenaria* against antiserum to the Virginia population showing no difference in major precipitin bands.


1
2
2
1as
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A
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population. Use of absorbed antisera also did not reveal any serological differences. When similar tests were conducted with the Virginia and Greece populations of *M. arenaria*, a spur formed with one very weak precipitin band, indicating a slight difference in this particular antigen. All other precipitin bands in this pattern coalesced (Fig. 1-F). Scott and Riggs (8) were not able to detect any serological differences between two races of *Heterodera glycines*. Slight serological differences, however, were detected among races of *D. dipsaci* (9).

Serological techniques provide reliable procedures for determining differences or similarities in *Meloidogyne* antigens; hence, these techniques have great potential for elucidating the phylogenetic relationships of *Meloidogyne* species, and may facilitate the identification of certain of these species.

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