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Serological Differentiation of Plant-parasitic Nematode Species with Polyclonal and Monoclonal Antibodies[1]

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Abstract: Although several attempts have been made to differentiate nematode species with polyclonal antisera, these efforts thus far have met with limited success because of extensive cross-reactivities of the sera. Since the hybridoma technique offers the opportunity to develop more specific serological reagents, some research groups have recently started to apply this technology to the problem of species identification in nematology. Monoclonal antibodies (MA) that differentiate the potato-cyst nematodes *Globodera rostochiensis* and *G. pallida*, as well as MA specific for *Meloidogyne* species, have been developed. The possibilities of developing serodiagnostic tools for identification of nematodes recovered from soil samples and the implications of such monitoring of nematode infestations in view of integrated control of plant-parasitic nematodes are discussed.

Key words: potato cyst nematode, *Globodera rostochiensis, G. pallida*, antibody, diagnosis, monoclonal antibody, hybridoma, polyclonal antibody, immunoassay.

There exists an increasing need in modern agriculture for rapid and large-scale identification of plant pathogens. This need can be explained in view of the necessity to monitor alternative crop protection methods because of increasing environmental concerns with regard to chemical crop protection. Within nematology, recent advances in biochemistry and serology may provide opportunities for such species identification. Differences between nematode species have been demonstrated at the protein level by polyacrylamide gel electrophoresis, immunodiffusion, immunoelectrophoresis, and pyrolysis gas liquid chromatography (10) and at the DNA level by restriction fragment length polymorphism (6,7). These methods, however, are often not suitable for routine applications, either because they are too laborious or they lack sensitivity. In comparison with these techniques, immunoassays offer the advantage of combining specificity, rapidity, and simplicity at a relatively low cost. These properties render immunoassays very suitable for routine applications. In this paper, we review results obtained in the identification of nematode species using conventional polyclonal antisera and monoclonal antibodies by immunoassays. We also discuss the implications of these serodiagnostic tools on integrated pest management.

IMMUNOASSAYS IN AGRICULTURE

Immunoassays have been increasingly applied in agriculture for the detection and quantification of crop diseases, pesticides, and naturally occurring compounds. Information obtained from such immunoassays can be used in determining crop rotation patterns, cultivar selection, pesticide selection, pesticide application timing, harvest dates, post-harvest handling, and other management aspects. It is expected, therefore, that the development and application of immunoassays will increase in the near future (23). Compared with medical immunoassays, however, some major differences are found in the matrices to be analyzed in agriculture. In medicine, the matrix is usually fairly simple and consists of blood serum or urine. Agricultural chemists and biologists deal with a wide variety of matrices ranging from relatively simple ground water to more complex ma-

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trices consisting of soils and macerated crop samples. Furthermore, the complex structure of fungi, bacteria, and nematodes impedes the development of specific antibodies that can be used in diagnostic tests. However, the many advantages of immunoassays over other methods for the identification of related nematode species make it worthwhile to try to overcome these problems.

Immunoassay Reagents

Before an immunoassay can be performed, antisera must be developed. However, nematode-identifying antisera produced by classical immunization protocols have been found to cross-react too strongly among nematode species (33,34) to be of value as immunological differentiating reagents. Improved sensitivity of conventional antisera has been obtained by the use of a much more refined crossed immunoelectrophoresis technique (34).

A recently described method for obtaining antibodies has the potential to overcome problems of cross-reactivity and standardization encountered with conventional antisera. With this technique, developed by Köhler and Milstein (22), a single antibody-producing cell is isolated from an immunized mouse and fused in vitro with a mouse lymphoid tumor cell. In the resulting hybrid cell (hybridoma), the characteristics of both parental cell types are combined, i.e., specific (monoclonal) antibody production and the capacity for in vitro multiplication. Thus, the humoral immune response of a mouse against a complex immunogen containing many structures, each of which gives rise to an individual antibody, can be dissected into its separate component antibodies. Concomitantly, the production of an antibody is immortalized and standardized. Moreover, rare antibody specificities, whose potential reactivities never become manifest in vivo, might be generated in vitro by cell fusion with single members of the antigen reactive B cell pool (25). As a result, hybridomas have been described which produce monoclonal antibodies able to discriminate between isoenzymes and other variants of protein antigens (4,14). Most monoclonal antibodies presently used in agriculture are directed against viruses; a few are directed against bacteria and fungi (13).

Polyclonal Antisera

Bird (5) was the first to report the possibility of generating antibodies against nematodes. Since then (1964) several attempts have been undertaken to differentiate between taxonomically related plant-parasitic nematode species by serological techniques (Table 1). Nematodes in the genera *Meloidogyne, Heterodera,* and *Globodera* have been studied most extensively. Species from the other genera that have been examined are *Ditylenchus dipsaci, D. destructor, D. myceliophagus, Aphelenchoides ritzemabosi,* and *Aphelenchus avenae* (8,11, 33).

Among the many similarities in the serological reaction patterns of the antisera with nematode extracts in an Ouchterlony double diffusion technique or immunoelectrophoresis, there were some differences between taxonomically related nematodes. These studies also demonstrated that polyclonal antisera can be used to determine the phylogenetic relatedness of the different species by classical serology.

Lee (24) applied the immunodiffusion technique to the serological discrimination of *Meloidogyne* species. An antiserum raised against *M. incognita* showed no precipitation arcs with *M. hapla.* As indicated by the author, however, this may have been due to the small number of individuals used. Indeed, later studies (17,18,20,26) suggested that other *Meloidogyne* species, e.g., *M. incognita, M. javanica,* and *M. arenaria,* are serologically closely related because they show a number of common precipitin bands. Cross-absorption of the antisera also demonstrated the presence of some species-specific antigens.

Serological relationship has also been demonstrated between *Heterodera* and *Globodera* species. Thus, data from Webster and Hooper (33) indicate that *H. schachtii, H. trifolii,* and *G. rostochiensis* are related, as

TABLE 1. Summary of serological techniques used in the study of plant parasitic nematodes.

	Type of antibodies†	Stages examined	Technique used‡	Reference
Aphelenchoides ritzemabosi	P	Mixed	ODD, IE	11
Aphelenchus avenae	P	Mixed	ODD	8
Ditylenchus dipsaci	P	Mixed	ODD, IE	11
D. destructor	P	Mixed	ODD	33
D. myceliophagus	P	Mixed	ODD	33
Globodera rostochiensis	M, P	Cysts, eggs	ODD, IE, ELISA	9,29,30,33,34
G. pallida	M, P	Cysts, eggs	ODD, IE, ELISA	9,29,30,34
Heterodera betulae	P	Cysts	ODD, IE	27,31
H. carotae	P	Cysts	ODD, IE	33
H. cruciferae	P	Cysts	ODD	27,33
H. glycines	M, P	Cysts	ODD, IF	12,27
H. goettingiana	P	Cysts	ODD	33
H. schachtii	P	Cysts	ODD, IE	33
H. trifolii	P	Cysts	ODD, IE	33
Meloidogyne arenaria	P	Females, juveniles, eggs	ODD, IE	17,18,20,26
M. incognita	M, P	Females, juveniles, eggs	ODD, IE, ELISA, IF	17,18,19,20,24,26
M. javanica	P	Juveniles, eggs	ODD, IE	26

† M = monoclonal antibody; P = polyclonal antiserum.
‡ ELISA = enzyme linked immunosorbent assay. IE = immunoelectrophoresis. IF = immunofluorescence. ODD = Ouchterlony double diffusion.

are *H. cruciferae, H. goettingiana,* and *H. carotae,* without apparent serological relationship between these two groups. Later studies by Scott and Riggs (31) and Riggs et al. (27), however, could not show a serological relationship between *H. betulae* and a number of other *Heterodera* and *Globodera* species. Although *G. rostochiensis* and *G. pallida* populations can be distinguished with crossed immunoelectrophoresis (9,34), cross-reactivities of the antisera with some *Heterodera* species were observed as well. Similar results have been reported by Griffith et al. (12) for the serological differentiation of *H. glycines,* races 3 and 4 where among the common reactivities only a single antigenic difference could be demonstrated.

These results suggest that serologically determined entities are common among related nematode species; therefore, serological techniques can potentially elucidate phylogenetic relationships. In addition, species identification may be facilitated where differences in antigenic composition between species can be demonstrated. The classical serological procedures, however, are not suited for large-scale application. As stated, such techniques are too laborious and lack sensitivity; i.e., it is doubtful whether the (cross-absorbed) antisera are still specific when used in more sensitive immunoassays like an ELISA. For instance, a substantial fraction of the soluble proteins of many *Heterodera* and *Globodera* species consists of thermostable proteins (3,29,30). These proteins probably are conservative, and they may constitute a major source of cross-reactivity in the use of conventional polyclonal antisera. Therefore, the production of species-specific monoclonal antibodies should be considered if routine identification is the ultimate goal.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (MA) can be developed for routine identification of plant-parasitic nematode species, since in principle they couple predefined specificity with the absence of (undesired) cross-reactivity. Although the application of MA for patho-

gen detection and identification has been of great value in virology and bacteriology, and to some extent also in mycology (13), nematologists only recently have discovered the potential of this technique.

Jones et al. (21) developed MA against *M. incognita* by immunizing Lou/Iap rats and Balb/c mice with soluble proteins extracted from adult female nematodes. Two hybridoma cell lines were isolated, producing antibodies with preponderant specifity for *M. incognita* antigens.

Atkinson et al. (1) raised MA that react with secretory granules formed in esophageal glands of *H. glycines*. Similar MA have been developed by Hussey (19) for *M. incognita*. Depending on the antigen that is recognized by such MA, it is possible that some of them are species specific and, thus, might be useful for diagnostic purposes.

Monoclonal antibodies have also been developed against the potato-cyst nematode species *G. rostochiensis* and *G. pallida* (30). Thermostable proteins were isolated from eggs of both species and used to immunize Balb/c mice. Among the many hybridomas isolated, three (WGP 1–3) produce antibodies that react with preferential affinity with proteins from *G. pallida*, and two (WGR 11, 12) produce antibodies which bind preferentially to *G. rostochiensis* proteins. Binding constants were determined to quantitate differences in affinity of the antibodies produced by these hybridomas for the protein antigens isolated from both *Globodera* species. Figure 1A, B shows examples of Scatchard plots (28) of monoclonal antibody WGP 3 when binding to thermostable protein antigens isolated from *G. pallida* or *G. rostochiensis*. The binding constant of an antibody is defined by the slope of the line in a Scatchard plot. When an antibody shows divalent binding, the Scatchard plot is hyperbolic and the two binding constants are defined by the slope of the asymptotes. The latter situation is observed for WGP 3, which shows divalent binding for antigens from either *G. pallida* or *G. rostochiensis*. The reactivities of WGP 3 with the antigens from both

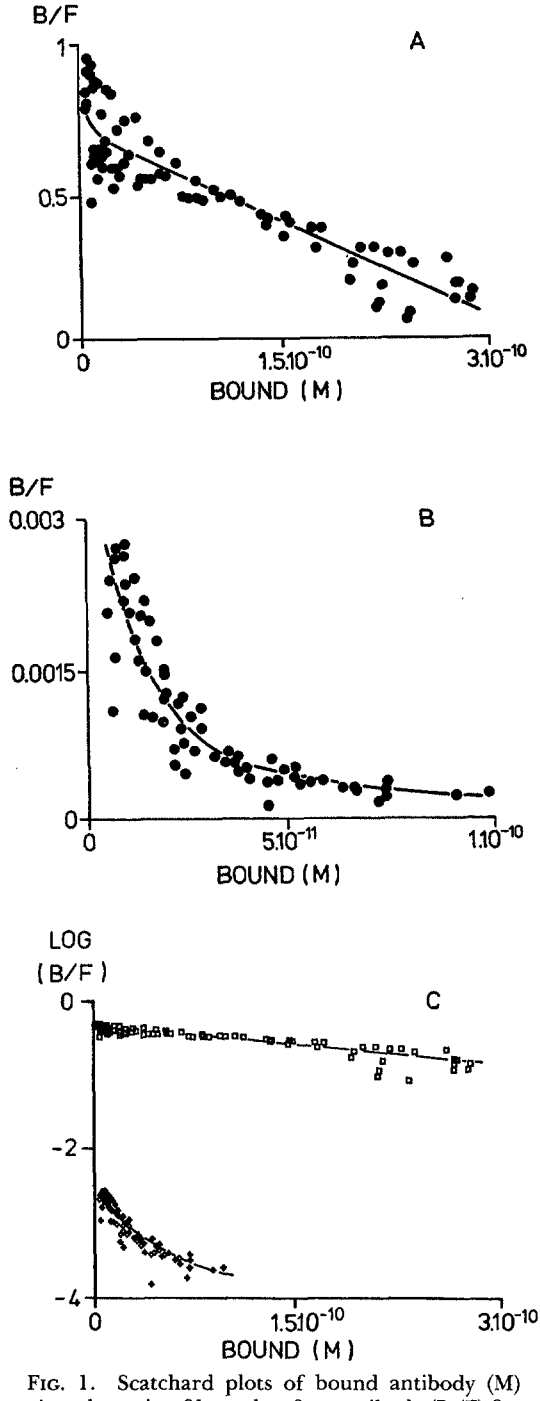

FIG. 1. Scatchard plots of bound antibody (M) against the ratio of bound to free antibody (B/F) for the interaction of monoclonal antibody WGP 3 with *Globodera* proteins. A) Reactivity with thermostable protein antigens isolated from *G. pallida*. B) Reactivity with thermostable protein antigens from *G. rostochiensis*. C) Comparison of reactivity of WGP 3 with antigens from *G. pallida* (squares) or *G. rostochiensis* (diamonds). From Schots et al. (30).

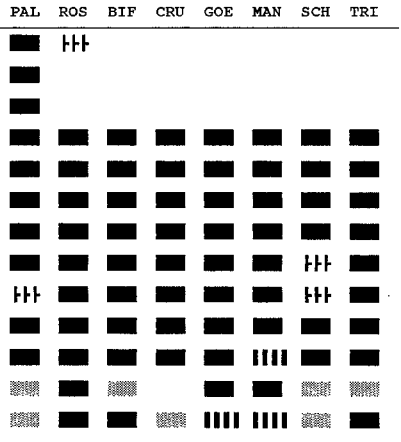

FIG. 2. Reactivity patterns of a selected panel of monoclonal antibodies raised against *Globodera pallida* (PAL) or *G. rostochiensis* (ROS), with thermostable proteins from these and six other common cyst nematodes: *Heterodera bifenestra* (BIF), *H. cruciferae* (CRU), *H. goettingiana* (GOE), *H. mani* (MAN), *H. schachtii* (SCH) and *H. trifoli* (TRI). Antibodies were tested for their ability to bind to thermostable proteins isolated from 50 eggs. White boxes = 0–20% binding; ▒ = 20–50% binding; ▒▒ = 50–80% binding; ■ = 80–120% binding; ■■ = > 120% binding. From Schots et al. (30).

potato-cyst nematode species are compared in Figure 1C. A difference of at least 100-fold in the ratio of bound to free antibodies is observed for the interaction of WGP 3 with antigens from *G. pallida* relative to that with antigens from *G. rostochiensis.*

Immunoblotting experiments showed that most of the monoclonal antibodies bind to two of four thermostable proteins with apparent molecular weights of 20.6/20.8 kD for *G. rostochiensis* and 20.5/21.0 kD for *G. pallida.* Furthermore, the reactivities of the monoclonal antibodies with thermostable protein antigens from six other common cyst nematodes were investigated (Fig. 2). All nondifferentiating monoclonal antibodies also bind to thermostable proteins from these cyst nematode species. Furthermore, at the concentrations of antibody used, two MA (WGP 2, 3) can be considered as specific for *G. pallida,* and only one MA (WGP 1) is specific for the genus *Globodera.* The latter, however, displays a higher affinity for antigens from *G. pallida* than from *G. rostochiensis.*

IMPLICATIONS AND PROSPECTS

Our studies on the reactivities, physical parameters, and epitope specificity of monoclonal antibodies with antigens from cyst nematodes suggest possible use of these antibodies for the development of a routine immunoassay for potato-cyst nematode identification, based on the following considerations: 1) the presence of potato-cyst nematodes in a soil sample can be assessed with WGP 1 because this MA does not bind to thermostable protein antigens from other common cyst nematodes; 2) the number of *G. pallida* individuals can be determined with WGP 2 or WGP 3 (numbers of *G. rostochiensis* can then be calculated from the results of this assay and that carried out with WGP 1); 3) when necessary, WGR 2 can be used to determine the total number of cyst nematode eggs in a soil sample. It has been concluded from ELISA reactivity patterns of the monoclonal antibodies that an appropriate signal will be obtained with protein quantities derived from less than one egg of either potato-cyst nematode species (Fig. 3).

Some research is still required before a routine laboratory test for the identification of the potato-cyst nematodes *G. rostochiensis* and *G. pallida* is available. Homogenization of nematodes is difficult because of the structure of the cuticle. A reproducible and simple method based on physical and (or) (bio)chemical entities must be developed. Furthermore, the effects of organic materials remaining in the cyst isolates obtained from different types of soil on the ELISA and the homogenization procedure must be investigated. Also, the interactions of soil fumigants with the protein antigens should be known; i.e., how stable are such antigens in a dead animal, and what are the effects of soil fumigants on the seroreactive properties of these proteins? Finally, the whole assay procedure must be standardized.

Once these problems are solved, an immunoassay will be available for the identification of *G. rostochiensis* and *G. pallida.* Such an assay has many advantages over other methods for the identification of

these parasites: 1) An immunoassay is very sensitive; in our hands protein equivalents of less than one egg can be detected. 2) The proportions of *G. rostochiensis* and *G. pallida* can be accurately assessed, in case both species are present in a soil sample; moreover, information on the absolute number of eggs can be obtained without extra effort. 3) The results of an immunoassay are not influenced by the physiological status of the nematodes, such as the diapause of the eggs. 4) An immunoassay can be performed rapidly within a period of 24 hours. 5) Immunoassays are cheap and computerizable; large numbers of samples can be processed in a relatively short period of time.

The introduction of immunoassays for control of plant-parasitic nematodes has implications for the cultivation of different crops. At the present time crop rotations and the nematicide applications, together with the use of resistant plant cultivars, often are essential to control nematode infestations. This strategy has several disadvantages. First, crop rotation places limitations on growing cash crops. Second, the success of resistant cultivars is restricted by the occurrence of virulent nematode populations (pathotypes, biotypes, or races). Third, nematicides, which in the Netherlands, for instance, comprise at least 60% of all pesticides used, increasingly encounter environmental concerns. In addition, several nematicides—e.g., aldicarb, oxamyl, and ethoprophos—show decreasing effectiveness after successive applications because of accelerated degradation as the result of microbial adaptation (32). Recently, decreasing effectiveness after repeated application has also been found for the fumigants 1,3-dichloropropene and methamsodium (Smelt, pers. comm.).

The possibility of developing specific MA for the identification of nematode species still might be underestimated if one assumes that morphological similarity reflects similarity at the protein level. Recent studies have indicated a major contrast in protein and morphological evolution for nematodes. Bakker and Bouwman-Smits (2) reported, at the protein level, a genetic

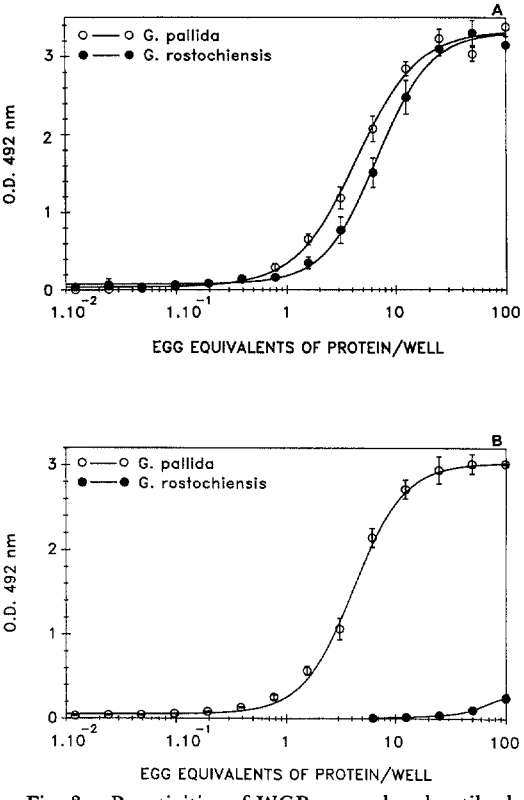

Fig. 3. Reactivities of WGP monoclonal antibodies with varying quantities of thermostable protein antigens isolated from either *G. rostochiensis* or *G. pallida*, and adsorbed to the wells of a microtiter plate. On the X-axis the antigen quantity corresponding to the number of eggs from which it was isolated is given, on the Y-axis the optical density readings at 492 nm. A) WGP 1 at 7.70×10^{-9} M. B) WGP 2 at 2.24×10^{-10} M.

distance of 0.70 between the sibling potato cyst nematode species *G. rostochiensis* and *G. pallida*, and of 0.59 between *H. glycines* and *H. schachtii*. Apparently slow morphological divergence is not rare throughout the phylum *Nematoda*, since similar results were obtained for *Radopholus similis* and *R. citrophilus* (15,16). Such large genetic distances probably will become apparent in other genera. The contrasting rates of morphological and protein evolution among nematode species suggest ample opportunities to isolate species-specific proteins from almost morphologically indistinguishable species, which may be used as immunogens when a diagnostic test based on specific monoclonal antisera is the ultimate goal.

Literature Cited

1. Atkinson, H. J., P. D. Harris, E. J. Halk, C. Novitski, J. Leighton-Sands, P. Nolan and P. C. Fox. 1988. Monoclonal antibodies to the soya bean cyst nematode, *Heterodera glycines.* Annals of Applied Biology 112:459–469.

2. Bakker, J., and L. Bouwman-Smits. 1988. Contrasting rates of protein and morphological evolution in cyst nematode species. Phytopathology 78:900–904.

3. Bakker, J., A. Schots, L. Bouwman-Smits, and F. J. Gommers. 1988. Species specific and thermostable proteins from second-stage larvae of *Globodera rostochiensis* and *G. pallida.* Phytopathology 78:300–305.

4. Berzofsky, J. A., G. Hicks, J. Fedorko, and J. Minna. 1980. Properties of monoclonal antibodies specific for determinants of a protein antigen, myoglobin. The Journal of Biological Chemistry 255: 11188–11191.

5. Bird, A. F. 1964. Serological studies on the plant parasitic nematode, *Meloidogyne javanica.* Experimental Parasitology 15:350–360.

6. Curran, J., D. L. Baillie, and J. M. Webster. 1985. Use of genomic DNA restriction fragment length differences to identify nematode species. Parasitology 90:137–144.

7. De Jong, A. J., J. Bakker, M. Roos, and F. J. Gommers. 1989. Repetitive DNA and hybridization patterns demonstrate extensive variability between the sibling species *Globodera rostochiensis* and *G. pallida.* Parasitology 99:133–138.

8. El-Sherif, M., and W. F. Mai. 1968. The use of immunodiffusion in nematode identification. Nematologica 14:593–595.

9. Fox, P. C., and H. J. Atkinson. 1985. Immunochemical studies on pathotypes of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida.* Parasitology 90:471–483.

10. Fox, P. C., and H. J. Atkinson. 1986. Recent developments in the biochemical taxonomy of plant-parasitic nematodes. Agricultural Zoology Reviews 1: 301–331.

11. Gibbins, L. N., and G. S. Grandison. 1968. An assessment of serological procedures for the differentiation of biological races of *Ditylenchus dipsaci.* Nematologica 14:184–188.

12. Griffith, T. W., N. J. Koehler, S. G. Coker, and R. D. Riggs. 1982. Race comparisons of *Heterodera glycines* using crossed immunoelectrophoresis. Journal of Nematology 14:598–599.

13. Halk, E. L., and S. H. De Boer. 1985. Monoclonal antibodies in plant-disease research. Annual Review of Phytopathology 23:321–350.

14. Hollander, Z., and E. Katchalski-Katzir. 1986. Use of monoclonal antibodies to detect conformational alterations in lactate dehydrogenase isoenzyme 5 on heat denaturation and on adsorption to polystyrene plates. Molecular Immunology 23:927–933.

15. Huettel, R. N., D. W. Dickson, and D. T. Kaplan. 1983. Biochemical identification of the two races of *Radopholus similis* by starch-gel electrophoresis. Journal of Nematology 15:338–344.

16. Huettel, R. N., D. W. Dickson, and D. T. Kaplan. 1984. *Radopholus citrophilus* sp. n. (Nematoda), a sibling species of *Radopholus similis.* Proceedings of the Helminthological Society of Washington 51:32–35.

17. Hussey, R. S. 1972. Serological relationship of *Meloidogyne incognita* and *M. arenaria.* Journal of Nematology 4:100–104.

18. Hussey, R. S. 1979. Biochemical systematics of nematodes—a review. Helminthological Abstracts, Series B, Plant Nematology 48:141–148.

19. Hussey, R. S. 1989. Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species. Journal of Nematology 21:392–398.

20. Hussey, R. S., J. N. Sasser, and D. Huisingh. 1972. Disc-electrophoretic studies of soluble proteins and enzymes of *Meloidogyne incognita* and *M. arenaria.* Journal of Nematology 4:183–188.

21. Jones, P., D. J. Ambler, and M. P. Robinson. 1988. The application of monoclonal antibodies to the diagnosis of plant pathogens and pests. Brighton Crop Protection Conference—Pests and Diseases—1988, vol. 2. BCPC Registered Office, Thornton Heath, Surrey, U.K. Pp. 767–776.

22. Köhler, G., and C. Milstein. 1975. Continous cultures of fused cells secreting antibody of predifined specificity. Nature 256:495–497.

23. Lankow, R. K., G. D. Grothaus, and S. A. Miller. 1987. Immunoassays for crop management systems and agricultural chemistry. Pp. 228–260 *in* H. M. LeBaron, R. O. Mumma, R. C. Honeycutt, J. H. Duesing, J. F. Philips, and M. J. Haas, eds. Biotechnology in agricultural chemistry. Washington, D.C.: American Chemical Society.

24. Lee, S. H. 1965. Attempts to use immunodiffusion for species identification of *Meloidogyne.* Nematologica 11:41 (Abstr.).

25. Metzger, D. W., L. -K. Ch'ng, A. Miller, and E. E. Sercarz. 1984. The expressed lysozyme-specific B cell repertoire. I. Heterogeneity in the monoclonal anti-hen egg white lysozyme specificity repertoire, and its difference from the in situ repertoire. European Journal of Immunology 14:87–93.

26. Misaghi, I., and M. A. McClure. 1974. Antigenic relationship of *Meloidogyne incognita, M. javanica* and *M. arenaria.* Phytopathology 64:698–701.

27. Riggs, R. D., L. Rakes, and M. L. Hamblen. 1982. Morphometric and serologic comparison of a number of populations of cyst nematodes. Journal of Nematology 14:190–199.

28. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. Annals of the New York Academy of Sciences 51:660–672.

29. Schots, A., J. Bakker, F. J. Gommers, L. Bouwman-Smits, and E. Egberts. 1987. Serological differentiation of the potato-cyst nematodes *Globodera pallida* and *G. rostochiensis:* Partial purification of species-specific proteins. Parasitology 95:421–428.

30. Schots, A., T. Hermsen, S. Schouten, F. J. Gommers, and E. Egberts. 1989. Serological differentiation of the potato-cyst nematodes *Globodera pallida* and *G. rostochiensis.* II. Preparation and characterization of species specific monoclonal antibodies. Hybridoma 8:401–413.

31. Scott, H. A., and R. D. Riggs. 1971. Immu-

noelectrophoretic comparisons of three plant-parasitic nematodes. Phytopathology 61:751–752.

32. Smelt, J. H., S. J. H. Crum, W. Teunissen, and M. Leistra. 1987. Accelerated transformation of aldicarb, oxamyl and ethoprophos after repeated soil treatments. Crop Protection 6:295–303.

33. Webster, J. M., and D. J. Hooper. 1968. Serological and morphological studies on the inter- and intraspecific differences of the plant-parasitic nematodes *Heterodera* and *Ditylenchus*. Parasitology 58:879–891.

34. Wharton, R. J., R. M. J. Storey, and P. C. Fox. 1983. The potential of some immunochemical and biochemical approaches to the taxonomy of potato cyst nematodes. Pp. 235–248 *in* A. R. Stone, H. M. Platt, and L. K. Khalil, eds. Concepts in nematode systematics. London: Academic Press.