Race Comparisons of Heterodera glycines Using Crossed Immunoelectrophoresis[1]

T. W. GRIFFITH,[2] N. J. KOEHLER,[2] S. G. COKER,[2] AND R. D. RIGGS[3]

Journal of Nematology 14(4):598-599. 1982.

Four races of soybean cyst nematodes (SCN), *Heterodera glycines,* have been described in the United States (2), and many other variations have been demonstrated (4). The races have been reported to be morphologically and serologically identical (5). However, since the races interact differently when inoculated to different cultivars of soybeans, there should be some biochemical differences. This study was undertaken in an attempt to identify differences between the different races of SCN.

Nematodes were reared on 'Lee' (Race 3) or 'Pickett' (Races 2 and 4) soybean cultivars in a greenhouse. White females were sieved out by the roiling and sieving method, separated from extraneous debris, and maintained frozen until used. The packed cysts were homogenized in a Teflon-glass homogenizer in two volumes of distilled water and centrifuged at 700 $\times g$ for 15 min. The supernatant (about 10 mg protein/ml) was withdrawn and used as a source of antigens. Four rabbits were injected subcutaneously with 0.4 ml of supernatant mixed with an equal volume of Freund's Complete Adjuvant. Injections of antigen in Freund's Incomplete Adjuvant were continued every few days for one month until distinct precipitin bands were found with crossed immunoelectrophoresis. An injection of antigen was given one week prior to any additional antiserum collections. Blood obtained from each rabbit's ear was allowed to clot, centrifuged at 600 $\times g$ for 10 min, and the antiserum withdrawn and frozen.

Crossed immunoelectrophoresis was run in a 1% agarose gel in a barbital-glycine/Tris buffer, pH. 8.8, ionic strength 0.08 (1) using 5 or 10 μl of each antigen solution. In the second dimension, the separated antigens were run into agarose containing 1 ml of antiserum. In some cases, the 1 ml of antiserum was incubated with 100 μl of antigen for 1 hr at 37 C and centrifuged before use. Electrophoresis, gel washing, and protein staining with Coomassie Brilliant Blue R-250 was performed as described (6).

The pattern resulting from crossed immunoelectrophoresis of a SCN homogenate and SCN antisera was extremely complicated, with as many as 30 separate precipitin rockets appearing. The rockets were not as distinct as with precipitations that occur between human serum and its antiserum. The size and distinctness of the rockets also vary somewhat with the batch of antigen and antisera, and with the length of storage of the antisera. As a result, race identification was difficult. To simplify the pattern and look only for qualitative differences, antisera were incubated with small amounts of heterologous antigen so that most of the cross reacting antibodies were absorbed.

Tandem crossed immunoelectrophoresis tests run with SCN-3 and SCN-4 antigens against SCN-4 antisera absorbed with the heterologous SCN-3 antigens resulted in two doublet precipitin lines, with only one apparent singlet precipitin line (Fig. 1). This demonstrated the presence of a SCN-4 antigen that was not present in SCN-3. However, it is possible that the antigen was present in SCN-3, but below the detection level. In the complementary experiment (SCN-3 and SCN-4 antigens reacted with SCN-3 antisera absorbed with SCN-4 antigens), the plate showed no precipitin lines. This was reproducible and further supports the possibility of the presence of a protein unique to SCN-4.

Preliminary experiments indicate that a comparison between SCN-2 and SCN-3 is analogous, respectively, to a SCN-4 and SCN-3 comparison. SCN-4 and SCN-2 are similar in that they appear to form in response to plantings of soybeans resistant to

Received for publication 27 January 1982.

[1]Supported by Undergraduate Research Participation—NSF Grants SPI 8026623 and SPI 7927174.

[2]Department of Microbiology and Biochemistry, Northwestern State University, Natchitoches, LA 71457.

[3]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

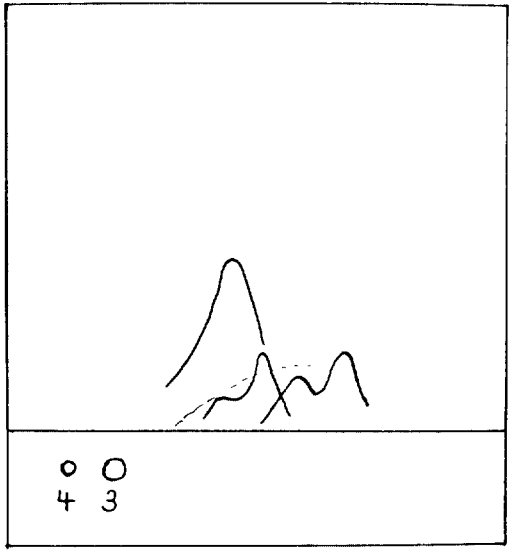

Fig. 1. Tandem crossed immunoelectrophoresis using 5 μl of *Heterodera glycines* race 4 homogenate and 10 μl of *H. glycines* race 3 homogenate run against *H. glycines* race 4 antisera absorbed with *H. glycines* race 3 homogenate. Three dominant precipitin lines form. Two are doublet patterns indicating common antigens in both samples while a singlet peak arises from the *H. glycines* race 4 homogenate.

SCN-3 and SCN-1. Although we have found only the one antigenic difference between SCN-3 and SCN-4, other differences may exist. Price et al. (3) have indicated that counter-resistance in SCN was due to several genetic factors. One would expect then to find several differences in the nematodes due to the products of these genes. However, these factors may be quantitative rather than qualitative, and we have not looked closely for quantitative differences. Some factors may be weakly antigenic or non-antigenic and therefore would not be visible through serological techniques. In addition, some factors may not be expressed in the cyst stage of development that we were using.

LITERATURE CITED

1. Axelson, N. H., J. Kroll, and B. Weeke. 1973. A manual of quantitative immunoelectrophoresis, p. 25-35. Oslo: Universitetsforlaget.

2. Golden, A. M., J. M. Epps, R. D. Riggs, L. A. Duclos, J. A. Fox, and R. L. Bernard. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (Heterodera glycines). Plant Dis. Rept., 54:544-546.

3. Price, M., C. E. Caviness, and R. D. Riggs. 1978. Hybridization of races of Heterodera glycines. J. Nematol. 10:114-118.

4. Riggs, R. D., M. L. Hamblen, and L. Rakes. 1981. Infra-species variation in reactions to hosts in Heterodera glycines populations. J. Nematol. 13: 171-179.

5. Scott, H. A., and R. D. Riggs. 1971. Immunoelectrophoretic comparisons of three plant-parasitic nematodes. Phytopathology. 61:751-752.

6. Wallenborg, Bibi, and Ulla-Brigitta Anderson. 1978. Immunoelectrophoretic techniques with the LKB 2117 Multiphor, Application Note 249. Bromma, Sweden.