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Distribution of Soybean Cyst Nematode in Nebraska[1]

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Abstract: A survey of 552 soybean fields in 20 counties in Nebraska in 1986–88 revealed 35 fields infested with the soybean cyst nematode (SCN), *Heterodera glycines.* Identification was confirmed with a greenhouse bioassay, using 'Lee 74' soybean, and by the application of a DNA hybridization probe derived from SCN mitochondrial DNA. Most of the SCN-infested fields were located on the Missouri River floodplain and in the southeastern corner of the state.

Key words: Glycines max, Heterodera glycines, Nebraska, soybean, soybean cyst nematode, survey.

Until 1986 Nebraska was the only major soybean (*Glycines max* L. Merrill) producing state without a known infestation of the soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe (3). In the fall of 1986 a heavily infested field (>50 cyst/cm^3 soil) was identified on the Missouri River floodplain in the southeast corner of the state (4). This level of infestation suggested a well-established population of long duration and indicated that other SCN-infested fields might occur elsewhere in Nebraska. At that time a 2-year survey was initiated to determine the distribution of SCN in 20 eastern Nebraska counties, where approximately 52% of Nebraska soybeans are grown. A greenhouse bioassay technique was used to enhance detection of field populations of low density. This paper reports the results of that survey.

Materials and Methods

A preliminary sampling for SCN was conducted in eight fields along the Missouri River flood plain in the fall of 1986. Soil samples from 544 soybean fields were taken during July and August of the 1987 and 1988 growing seasons (Fig. 1). In 1987 sampling of 130 soybean fields was concentrated in eight southeastern and east central Nebraska counties bordering the Missouri River. In 1988 sampling was expanded to include an additional 174 fields in the eight original counties plus 240 fields in the 12 neighboring counties to the west and north. Estimated field size ranged from 3 to 100 ha.

In each field, 25–50 soil cores, 2.5 cm d × 20 cm deep, from within the root zone were collected in a random pattern throughout the field and combined to make one 500-cm^3 composite sample. From each composite field sample, 100 cm^3 soil was mixed with approximately 1,500 cm^3 sterilized sand in 20-cm-d pots and planted with four 'Lee' soybeans. After 10 weeks of greenhouse growth at 25–28 C, potential cyst occurrence was determined by root examination and extraction from soil through flotation and isolation on a 250-μm-pore sieve.

If cysts were recovered in the greenhouse bioassay, the remaining soil from the original field sample was washed and carefully examined for the presence of cysts. In some cases only a few brown cysts were recovered from both the bioassay technique and by processing the original field sample. Since these could have been unrelated cyst species, unable to reproduce on soybean but carried along in the soil sample during the bioassay, we examined the identity of these cysts through DNA hybridization.

Cysts samples to be examined by DNA hybridization were handpicked into a microfuge tube and ground in an MSB-proteinase K solution with a pellet pestle (1). The nematode lysate was incubated at 65 C for 30 minutes, treated with 5 μl RNAse (10 mg/ml) for 30 minutes at 37 C, and phenol extracted; the DNA was concen-

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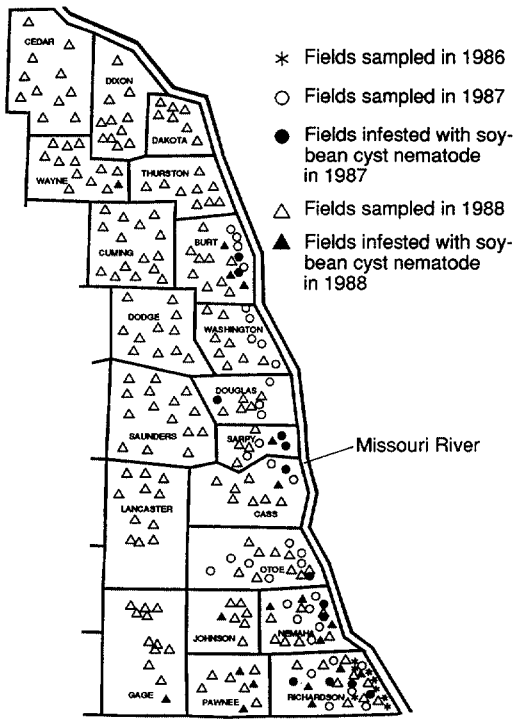

Fig. 1. Soybean cyst nematode surveys in Nebraska, 1986–88. Symbols do not indicate the actual number of fields sampled or infested.

trated by ethanol precipitation. The cellular DNA was cleaved with the restriction enzyme *Hin*dIII and electrophoretically fractionated on 0.65% agarose gels for 3 hours at 80 volts. The DNA was transferred to nitrocellulose filters and hybridized with an SCN mitochondrial DNA probe (1).

Host-race tests (2,5) were conducted on one population each from Richardson and Burt Counties. Both the Richardson and Burt County field populations were located within 1.6 km of the Missouri River, although the Burt County field was approximately 193 km upstream from Richardson County.

Results and Discussion

Thirty-five fields in 11 counties sampled positive for SCN (Table 1). Most of the positive fields were located within the Missouri River floodplain (Fig. 1) and had soils consisting of silt loam or silty clay loam texture. Because many of these fields were located within 1.6 km of the river, it is

Table 1. Soybean fields infested with soybean cyst nematode (SCN) identified by county in Nebraska 1986–88.

County	1986		1987		1988		1986–88	
	Fields sampled	SCN-inf. fields	Fields sampled	SCN-inf. fields	Fields sampled	SCN-inf. fields	Total Fields sampled	Total SCN-inf. fields
Burt			20	2	20	3	40	5
Cass			10	1	24	1	34	2
Cedar					20	0	20	0
Cuming					20	0	20	0
Dakota					20	0	20	0
Dixon					20	0	20	0
Dodge					20	0	20	0
Douglas			7	1	12	0	19	1
Gage					20	1	20	1
Johnson					20	1	20	1
Lancaster					20	0	20	0
Nemaha			20	2	25	4	45	6
Otoe			20	1	24	0	44	1
Pawnee					20	3	20	3
Richardson	8	3	20	4	34	4	62	11
Sarpy			13	2	15	1	28	3
Saunders					20	0	20	0
Thurston					20	0	20	0
Washington			20	0	20	0	40	0
Wayne					20	1	20	1
Total	8	3	130	13	414	19	552	35

possible that periodic flooding and migratory water fowl helped spread the nematode among locations. Not all positive fields, however, were confined to the floodplain. Isolated discoveries in Wayne, Douglas, Johnson, Pawnee, and Gage Counties were geographically distant from other known infested fields.

Host-race tests conducted on SCN populations from two floodplain counties indicated that at least two races are present in Nebraska. The Burt County population was determined to be race 4 (positive on all differentials), whereas the Richardson County population was found to be race 3 (negative on all differentials). Neither field had ever been planted with a SCN-resistant cultivar. This physiological variation indicates that genetic differentiation exists among these floodplain populations that is difficult to explain on the basis of selection pressure imposed by resistant soybean cultivars.

Approximately 5% of the field samples contained cysts that contained large numbers of apparently viable eggs but did not amplify under greenhouse bioassay conditions. Typically 1–5 of these cysts were recovered from the composite field sample. None of these cyst samples produced a positive hybridization signal, whereas tests of cysts from samples that did amplify in the greenhouse bioassay showed distinct positive hybridization. This suggests that some fields contain a non-SCN cyst species that is unable to reproduce on soybean. No further attempts were made to identify this species.

Overall, the results of the survey indicate that SCN is not widespread throughout the major soybean growing region of Nebraska, but is well established in some locations along the Missouri River floodplain and in the southeastern counties of the state.

Literature Cited

1. Besal, E. A., T. O. Powers, A. D. Radice, and L. J. Sandall. 1988. A DNA hybridization probe for the detection of soybean cyst nematode. Phytopathology 78:1136–1139.

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 Disease Reporter 54:544–546.

3. Miller, L. I. 1985. Economic importance of cyst nematodes in North America. Pp. 373–385 *in* F. Lamberti and C. E. Taylor, eds. Cyst nematodes. New York: Plenum Press.

4. Powers, T. O., and D. S. Wysong. 1987. First report of soybean cyst nematode (*Heterodera glycines*) in Nebraska. Plant Disease 71:1146.

5. Riggs, R. D., and D. P. Schmitt. 1988. Complete characterization of the race scheme for *Heterodera glycines*. Journal of Nematology 20:392–395.