

Soybean Cyst Nematode, *Heterodera glycines*, in Minnesota

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## ABSTRACT

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In September 1978, *Heterodera glycines* was found approximately 550 km northwest of infestations in northern Illinois. The nematode apparently was introduced into a field in Faribault County, Minnesota, on contaminated equipment. Morphologically, it is similar to race 1, but its development on soybean differentials is dissimilar to previously described races. Subsequently, 13 other infested fields have been found.

Minnesota ranked fifth nationally in soybean (*Glycines max*) production in 1977, with an estimated  $1.54 \times 10^6$  ha harvested and a much higher than normal average production of 2.35 t/ha (6). Average Minnesota soybean production during the preceding 8-yr period was 1.67 t/ha (6). The extensive planting of soybeans for beans is a fairly recent development in Minnesota. The amount of land harvested has grown from  $2.46 \times 10^5$  ha in 1946.

The potential impact of the soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) on soybean has long been recognized by certain agricultural leaders in Minnesota. In 1967, restrictions were placed on the movement of used farm equipment to reduce the possibility that *H. glycines* would be carried passively into Minnesota on machinery that had been used in areas where SCN was well established. Despite these restrictions, the movement of such equipment from Missouri to Minnesota during the early 1970s was judged to be considerable (R. Flaskerd, *personal communication*). In August 1978, soil samples were collected from a field in Faribault County in which soybean plants showed symptoms of *H. glycines* infection. Faribault County is the center one in the southern tier of counties adjacent to the Iowa border and is one of the top soybean-producing counties in Minnesota, with  $8.03 \times 10^5$  ha harvested and an average yield of 2.66 t/ha in 1977 (6). The soil samples were subsequently found to be infested with a cyst nematode.

Our research was conducted to determine the species of cyst nematode and subsequently the race of *H. glycines* that was present, the mode of introduction, and other characteristics of the infestations.

## MATERIALS AND METHODS

The original infestation was detected by extracting and identifying nematodes from soil samples collected in late August 1978. Harpster silty clay loam (1) soil samples consisting of 8–10 2.5-cm diameter cores collected in the row to a maximum depth of 20–25 cm were processed by the Cornell pie-pan method (4). The 58-cm<sup>3</sup> subsamples were extracted at room temperature (24 C) for 6 days. The extracted nematodes were concentrated in approximately 100 ml of water. Two 8-ml subsamples were examined with the aid of a dissecting microscope, and the nematodes were identified and counted. Twenty second-stage larvae of *H. glycines* were mounted in water, heat-relaxed, and examined at  $\times 1,000$  magnification. Carefully washed roots of nearly dead soybean plants in the field in early September were examined with a dissecting microscope. Cysts extracted from moist soil by the rapid sugar flotation method (2) were fixed in 5% Formalin and sent to A. M. Golden for species confirmation.

A race determination was conducted in a greenhouse at the University of Illinois, Urbana. Infested field soil was diluted 1:1 (v/v) with heat-treated Onarga fine sandy loam to give approximately 1,500 larvae per 10-cm diameter pot at seeding. Each of the six soybean differentials was replicated twice, and each pot contained four plants. The differentials were grown in a greenhouse for 1 mo at a soil temperature of 15–25 C. Since no white females were present after 30 days, the plants were placed in a 25-C temperature

tank for 2 wk, after which the numbers of white females were determined. The entire volume of soil contained in each pot and the root washings were processed. White females were collected on the 60-mesh (250- $\mu$ m openings) screen and counted.

After the *H. glycines* infestation was confirmed, the infested field was resampled on 21 September when the "healthy" soybeans were at R-7 (yellow leaves) stage of development and the most severely affected plants were defoliated and nearly dead. Soil samples were collected from a 10-m segment of two adjacent rows. The distance between areas sampled within rows and between rows was 38 m. The only exceptions were the paired rows designated sample series A and B, which were 23 m apart. The series A samples were collected from the 22nd and 23rd rows into a portion of the field that had been planted to maize (*Zea mays*) every year from 1971 through 1977 and to the soybean cultivar Wells in 1978. The other samples were collected from the main portion of the field containing the "problem area." This portion had been in a corn-soybean rotation since 1970. The symptoms associated with plants growing in the problem area were first recognized by the grower in 1976.

Soil samples were collected in late September and October 1978 by personnel from the Division of Plant Industry, Minnesota Department of Agriculture, at the entrance points to fields that were planted to soybeans in 1978. The sampled fields were located in concentric rings 1.6, 5, and 11 km from the problem field. Sampling procedures were essentially those described previously. The first 25 samples were processed by the pie-pan method by personnel of the Plant Nematology Laboratory, University of Minnesota. An additional 12 samples from Faribault County were processed by Division of Plant Industry personnel using a soil-processing machine (5).

## RESULTS

Soil samples collected in late August 1978 contiguous to soybean plants with no, moderate, or severe symptoms (stunting, chlorosis, and weediness) contained, respectively, 28, 2,300, and 2,676 infective second-stage *H. glycines* larvae per 116 cm<sup>3</sup> of soil. White females were attached to the roots of plants

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collected on 10 September. Cysts containing larvae examined by A. M. Golden were identified as *H. glycines* (personal communication).

Morphological characters of larvae are shown in Table 1. With regard to tail length (3), the Minnesota isolate most closely resembled race 1.

A comparison of the responses of the Minnesota population and races 1, 2, 3, and 4 of *H. glycines* to the standard soybean differentials (3) is shown in Table 2. The responses of the Minnesota isolate to the differentials used were very similar to those of *H. glycines* race 4, except that the isolate did not develop on the cultivar Peking. In two additional

tests not reported in detail here, the Minnesota *H. glycines* isolate also did not reproduce on PI 90763.

The distribution of *H. glycines* in the Faribault County field as indicated by second-stage larvae followed a predictable pattern if the hypothesis is valid that the original SCN inoculum was introduced near field location 1 in series B and the progenies of that inoculum were then passively distributed primarily within the series by cultivation (Table 3). Other plant parasitic nematodes, chiefly *Helicotylenchus pseudorobustus* (Steiner) Golden and *Pratylenchus hexincisus* Taylor and Jenkins, were not abundant even in the portion of the field that had

been repeatedly cropped to maize. Nine of the first 25 samples collected by Division of Plant Industry personnel contained infective second-stage *H. glycines* larvae. Those populations were generally small ( $\bar{x}$  258, range 48–945). The 14 infestations of *H. glycines* in Minnesota confirmed by December 1978 are in a circle approximately 22 km in diameter located along the Iowa border in Faribault County.

## DISCUSSION

The Minnesota population of *H. glycines* differs from previously described races of this species morphologically and in its ability to develop on soybean differentials. The possibility that this population is a new race that originated somewhere in the vicinity of the infested field is plausible. The field probably became infested with inoculum carried on equipment and introduced in the greatest quantity near the entrance point. The progenies from the original inoculum were spread more rapidly in the direction of cultivation (Table 3, series B) than in other directions. No used equipment from a recognized SCN-infested area was ever used in the field. Although the field is adjacent to a highway, it is unlikely that drivers of out-of-state equipment would choose the entrance point to the field as a place to turn around. Local spread on equipment such as anhydrous ammonia applicators or sugar beet harvesting equipment is the best explanation of the origin of this infestation, rather than the expected long-distance spread from an area with a history of SCN infestation.

The pathogenicity of the Minnesota population of *H. glycines* to susceptible soybean cultivars growing under otherwise ideal cultural conditions has not been determined. The smallest populations of second-stage larvae were associated with the weakest as well as some of the most vigorously growing plants (Table 3). Very large numbers of *H. glycines* were

**Table 1.** Morphological characters of 20 second-stage larvae of a Minnesota population of *Heterodera glycines*

Character	Mean	Standard deviation	Range
Stylet length ( $\mu\text{m}$ )	24.3	0.7	23.0–25.2
Opening of dorsal esophageal gland posterior to knobs ( $\mu\text{m}$ )	4.6	0.6	3.6–5.4
Tail length ( $\mu\text{m}$ )	53.7	3.2	47.6–59.5
Length of clear area in tail ( $\mu\text{m}$ )	28.5	2.2	25.9–32.1
Stylet knobs			
Rounded: 1 specimen			
Flat anterior surface to slight anterior indentation: 8 specimens			
Definite anterior indentation: 11 specimens			

**Table 2.** Development of races and the Minnesota population of *Heterodera glycines* on soybean differentials

Race or population	Differentials					
	Pickett 71	Peking	PI 88788	PI 90763	D75-10710	Essex
1	– <sup>a</sup>	–	+	–	–	+
2	+	+	+	–	+	+
3	–	–	–	–	–	+
4	+	+	+	+	–	+
Minnesota	+(84%) <sup>b</sup>	–(2%)	+(17%)	?	–(4%)	+(100%)

<sup>a</sup>+ = number of white females  $\geq 10\%$  of the number on Essex; – = number of white females  $< 10\%$  of the number on Essex (after Golden et al [3]).

<sup>b</sup>Percentage of development of white females compared with development on Essex.

**Table 3.** Distribution of *Heterodera glycines* larvae (*H.g.*) and other plant parasitic nematodes in relation to soybean growth and weed infestations<sup>a</sup>

Field location	Series A				Series B				Series C				Series D				
	Nematode no.		Growth rating		Nematode no.		Growth rating		Nematode no.		Growth rating		Nematode no.		Growth rating		
	<i>H.g.</i>	Other	Soybean <sup>b</sup>	Weed <sup>c</sup>	<i>H.g.</i>	Other	Soybean	Weed	<i>H.g.</i>	Other	Soybean	Weed	<i>H.g.</i>	Other	Soybean	Weed	
1	127	30	1	1	484	0	2.5	1	29	0	0.5	2	119	10	2.5	1	
2	0	41	4	0	1,921	40	4.0	1	Wet area, no soybeans				Wet area, no soybeans				
3	13	121	4	0	2,493	14	4.0	1	528	23	2.5	1	894	8	2.0	2	
4	0	111	3	0	2,306	10	4.0	1	784	0	2.5	0	164	6	2.5	1	
5	843	80	4	0	4,344	0	3.5	1	242	0	3.5	1	55	0	2.5	2	
6	375	86	5	0	1,906	0	3.5	2	292	26	2.5	2	354	6	3.0	1	
7	56	103	4	0	3,122	0	3.0	2	660	24	3.0	1	128	21	1.0	3	
8	110	105	4	0	3,337	36	2.5	2	217	18	3.0	1	39	13	2.5	2	
9	599	15	2	2	5,621	22	3.0	2	852	0	2.5	3	166	47	5.0	1	
10	599	0	1	1	972	4	3.0	1	699	31	2.5	1	77	19	4.5	0	
11	5	207			494	19	3.0	0	371	55	5.0	1	695	31	4.0	0	
12		Sugar beets, 1978				811	7	3.0	0	105	73	4.5	0	293	23	3.5	0
13									529	6	3.5	0	357	22	2.5	0	

<sup>a</sup>Distances between sampling sites were 38 m in all directions except for series A and B, where the rows were 23 m apart. Series A was planted to maize each season from 1971 through 1977 and to soybean cultivar Wells in 1978. With the exception of a small area of sugar beets, the remaining portion of the field (series B–D) was in a maize-soybean rotation beginning in 1970.

<sup>b</sup>0 = dead plants 30 cm tall; 5 = excellent growth, plants  $\geq 90$  cm tall.

<sup>c</sup>0 = no weeds, 1 = few weeds, 2 = moderate weeds, 3 = severe weeds.

associated with the roots of plants growing either moderately well or strongly and with no evidence of stunting, premature chlorosis, or reduced yield (Table 3). The area where the symptomatic plants were located was, in addition to being infested with the nematode, low, wet, compacted, and deficient in potassium and, especially, phosphorus. Studies to determine if the Minnesota population is a new race and to answer questions posed by its discovery are continuing.

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