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Variability in Race Tests with *Heterodera glycines*[1]

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Abstract: Tests of *Heterodera glycines* on differential host plants to determine races were run in Arkansas, Illinois, and North Carolina to check the uniformity of results of the test. Methods used at the three locations varied somewhat. Results indicate that the race test is highly variable. Isolates previously identified as race 1 were identified as race 1 or race 3; those identified as race 2 were identified in these tests as race 2, 4, 9, or 14; those previously identified as race 3 were identified as race 1 or race 3; those identified as race 4 were identified in these tests as race 4 or race 14; those previously identified as race 5 were identified as race 2; and those previously identified as race 6 were identified as race 1, 2, 4, 5, or 6. Part of the variability resulted from the use of differential host plants from different sources and part from nonstandard differential host plants. Other variations may be due to inability to obtain completely uniform inoculum or to recover all nematodes that penetrated.

Key words: Heterodera glycines, race, race test, soybean cyst nematode.

Physiological variability between populations of soybean cyst nematode, *Heterodera glycines* Ichinohe (SCN), was first reported by Ross (7). This problem later resulted in the description of four races (2) with the potential for identification of 12 additional races (3,4). The races were separated using differential host plants that include the Lee soybean cultivar, a good host; two resistant cultivars, Pickett and Peking; and two resistant plant introduction lines, PI 88788 and PI 90763. Races are designated according to the combination of indices on the differential hosts. The female index, based on the relative number of females on each differential, is computed as follows:

$$\text{FI} = \frac{\text{number of females and cysts on differential}}{\text{number of females and cysts on Lee}} \times 100$$

An FI of 10 or greater is designated as susceptible (+) and less than 10 as resistant (−) (2).

Pickett has been maintained according to a cultivar description. Peking is a black-seeded hay type soybean that has been grown commercially but may not be as well defined as Pickett. PI 88788 and PI 90763 were collected in China and introduced into the United States. They have not been selected for any particular characteristic, but have been maintained at the Soybean Germplasm Laboratory, Urbana, Illinois, in such a way as to assure their genotypic characteristics.

The distinction between resistance and susceptibility to SCN is not always clear. When tested in North Carolina (probably against SCN race 1), Peking and PI 90763 were very poor hosts, i.e., resistant, and PI 88788 was a fair host for *H. glycines* (Ross, pers. comm.). Although fewer females matured on PI 88788 than on Lee, enough females matured on PI 88788 that it was rated susceptible. The resistance in Pickett was derived from Peking (1), but Pickett did not inherit all of Peking's resistance (5).

PI 88788 and PI 90763 are resistant to SCN, but there is variation within the lines. Epps (pers. comm.) selected individual PI 88788 plants which were more resistant to races 3 and 4 than the original accession. One PI 90763 plant selected for seed increase was the source of a line that has been called PI 90763R. It is agronomically and morphologically similar to PI 88788 and is more resistant to SCN race 4 than either PI 88788 or PI 90763.

Unpublished reports of variability in SCN have become so common that re-

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search was initiated to determine the reliability and variability of the race test. Information was needed also on the uniformity of seed stocks of the differentials as well as differences in reactions arising from the variability of methods used in laboratories conducting race tests.

Materials and Methods

Experiments were conducted in Arkansas, Illinois, and North Carolina to determine the reactions of selected soybean cultivars and lines to isolates of SCN from each state. Although general procedures were similar, variations occurred and are described separately for each state.

Arkansas: The first experiment included nine soybean cultivars and two plant introduction lines in addition to the standard differentials (Table 1). Seeds were germinated in vermiculite in flats. Seedlings with 2.5–3.8-cm-long radicles were transplanted, one plant per pot, into sterilized fine sand in 7.5-cm-d clay pots. There were three replications of each cultivar and line.

Inoculum of SCN isolates previously classified as races 1, 2, 3, 4, and 6 was from stock greenhouse cultures maintained on Lee, Pickett, or Bedford soybean and was prepared 2 days after transplanting test seedlings. Females and cysts were rubbed from the roots, and the suspension was poured through nested 850-μm-pore and 250-μm-pore sieves. Females, cysts, egg masses, and debris collected on the 250-μm-pore sieve were washed into a blender, blended for ca. 30 seconds, and poured once more through a 250-μm-pore sieve. Eggs, second-stage juveniles (J2), and other particles passing through this sieve were used as inoculum. About 4,000 eggs and J2 were added to each pot. Inoculated plants were maintained in the greenhouse at 28–32 C for 30 days. Females and cysts were recovered from each plant by removing the soil-root ball from the pot, washing the soil from the roots in a pail of water, rubbing the roots to dislodge attached females and cysts, and pouring the suspended soil and nematodes through nested 850-μm-pore and 250-μm-pore sieves. The material on the 250-μm-pore sieve was washed into a counting dish, and cysts and females were counted at 15× magnification.

The second experiment was a race test using standard race differentials, Lee and Bedford soybean from Arkansas, and differentials from Illinois and North Carolina (Table 2). The test procedures were the same as in the first experiment. The treatments were replicated three times, one plant per replicate, and the test was repeated four times. Females and cysts were recovered at 30 days as in the first experiment.

North Carolina: Tests similar to those conducted in Arkansas were run in North Carolina using SCN isolates from North Carolina. Seeds were germinated in trays (20 × 20 × 5 cm deep) filled with vermiculite. When the cotyledons emerged, seedlings were transplanted to 7.5-cm-d clay pots, filled with a 50:50 sand : sandy soil mix. In the first experiment 14 soybean cultivars and plant introduction lines, including the standard race differentials, were tested (Table 3). There were four replications of one plant of each cultivar and line. Inoculum of races 1, 2, 3, and 4 was from greenhouse stock cultures on Lee soybean prepared as follows. Soil was gently washed from the roots, and females and cysts were removed with high water pressure onto nested 850-μm-pore and 250-μm-pore sieves. The cysts and females were elutriated to remove debris and were then crushed in a Ten Broeck tissue grinder. The resulting eggs and J2 suspension was used as inoculum. Approximately 2,000 eggs and J2 of the appropriate race of SCN were added to the soil in each pot just before transplanting. Plants were harvested 30 days later, and females and cysts were collected, as in inoculum preparation, and counted.

A second experiment was conducted using the SCN differentials from Arkansas and North Carolina plus Lee and Bedford. The experiment was similar to the first experiment except that races 1–5 were tested (Table 4). Treatments were replicated

three times and the experiment was repeated once.

Illinois: Two experiments were conducted in Illinois similar to those in Arkansas, using SCN race 3 and 4 isolates from southern Illinois which had been cultured in the greenhouse on Williams and Franklin soybean cultivars, respectively. In the first experiment seeds of the 13 soybean cultivars and plant introduction lines, including the race differentials, were planted individually in each 7.5-cm-d pot containing 210 cm^3 of infested sandy loam soil (Table 5). Soybean cultivars and lines were replicated six times in a randomized complete block design on a greenhouse bench. Numbers of white females were determined approximately 30 days after planting. Females were washed off roots and extracted from soil using the gravity-sieving technique with 850-μm-pore and 180-μm-pore sieves, and total numbers per pot were determined.

The second test followed a similar protocol, but 200 cm^3 of soil infested with either 420 race 3 cysts or 480 race 4 cysts was used. Each experimental unit consisted of three pots of each line arranged in a randomized complete block design. Soybean lines used in the second test were the race differentials used in Arkansas and Illinois plus Lee and Bedford.

Inoculum level test: In an experiment conducted in Arkansas, seeds of Lee, Pickett, Peking, PI 88788, and PI 90763 were germinated in vermiculite. Two-day-old seedlings (five replicates per host) were transplanted into a sterile 1:1 coarse sand : fine sandy soil mix. Cysts of SCN race 3 cultured on Lee soybean were crushed in a glass tissue grinder to release eggs and J2. The suspension of eggs and J2 was adjusted to a concentration of 1,600/ml (85% eggs). A 10-ml aliquant was pipetted into the soil around the seedling in each of five pots of each soybean cultivar or line. The suspension volume was doubled to give a concentration of 800 eggs and J2/ml, and 25 pots were inoculated. The dilution procedure was continued to give concentrations of 16,000, 8,000, 4,000, 2,000, 1,000, and 500 eggs and J2 per pot. Mature females from test hosts were recovered 27 days later.

TABLE 1. Average number of females on Lee and female indices on other soybean cultivars and lines inoculated with five races of *Heterodera glycines* at the Univerity of Arkansas.

Cultivar or line	Race 1	Race 2	Race 3	Race 4	Race 6
Lee	664†	89	144	368	148
Pickett	< 1	230	1	52	82
Peking	0	47	1	47	9
PI 88788	< 1	35	1	53	8
PI 90763	0	11	< 1	34	0
PI 97063R	5	9	2	1	50
PI 209332	< 1	14	1	2	59
Bedford‡	18	60	6	2	94
Centennial§	< 1	285	3	87	78
Custer§	< 1	149	1	70	23
Essex	82	228	77	72	102
Fayette‡	14	28	8	4	44
Forrest§	1	225	1	78	48
Franklin§	0	167	1	72	14
Jeff‡	65	30	2	5	103
Mack‖	13	127	1	81	114

† Figures for Lee are actual female counts, other figures are female indices (percentage of Lee).
‡ Resistant to SCN races 3 and 4.
§ Resistant to SCN races 1 and 3.
‖ Resistant to SCH race 3.

RESULTS

Arkansas: Most of the hosts used in this test reacted to the five SCN isolates as expected based on FI (Table 1); however, deviations from the expected occurred with races 1 and 2. PI 88788 was resistant to Arkansas race 1 (Table 1), and PI 90763 had an index of 11 against race 2. Of the differential hosts only Pickett had an index >10 for race 6. Commercial cultivars had FI of 14–114 for this race, with Franklin and Custer being more resistant than the others.

In the second experiment, host differentials from three sources gave highly variable results when inoculated with Arkansas race 2. Three of five tests with Arkansas hosts indicated race 2 as expected (Table 2); the other two had race 4 reactions but the indices on PI 90763 were 10 and 12, only slightly above the negative level. The differentials from North Carolina gave very different results (Table 2). Only one of five

TABLE 2. Average number of females on Lee or Williams and female indices on soybean differentials and Bedford or Fayette for five tests each of four races of *Heterodera glycines* on differential host plants used in Arkansas (AR), North Carolina (NC), and Illinois (IL). Tested at the University of Arkansas.

Host source	Test	Females	Female indices on differential					Race designation
			P—F†	Peking	PI 88788	PI 90763	B—FA‡	
				Race 2				
AR	1	1,520	104	16	12	3	11	2
	2	1,395	94	11	12	5	13	2
	3	573	120	40	42	10	35	4
	4	691	130	37	23	12	14	4
	5	807	174	38	31	7	22	2
NC	1	1,004	43	22	6	4	8	9
	2	760	61	26	8	6	18	9
	3	632	15	188	8	3	7	9
	4	638	58	23	5	4	11	9
	5	419	110	49	14	9	21	2
IL	1	752	75	19	10	5	16	2
	2	912	75	27	4	4	10	9
	3	269	58	53	7	12	10	14
	4	351	56	52	12	7	16	2
	5	359	267	68	15	13	19	4
				Race 3				
AR	1	533	3	< 1	< 1	< 1	3	3
	2	280	4	0	< 1	< 1	1	3
	3	563	1	< 1	< 1	< 1	7	3
	4	347	1	1	< 1	< 1	2	3
	5	156	4	1	2	0	10	3
NC	1	323	3	< 1	1	< 1	46	3
	2	220	4	1	2	1	6	3
	3	573	1	< 1	1	< 1	2	3
	4	221	2	1	4	0	2	3
	5	240	1	< 1	2	< 1	6	3
IL	1	507	1	1	1	0	5	3
	2	200	1	2	1	2	6	3
	3	347	2	1	2	0	4	3
	4	560	2	1	1	< 1	1	3
	5	600	0	1	1	< 1	3	3
				Race 4				
AR	1	680	31	51	63	67	10	4
	2	960	97	25	25	26	2	4
	3	467	147	63	66	74	7	4
	4	987	77	30	23	26	8	4
	5	493	162	43	48	50	9	4
NC	1	733	100	44	3	30	8	14
	2	573	63	56	3	39	24	14
	3	920	82	36	4	33	8	14
	4	813	92	38	3	39	10	14
	5	740	108	23	4	33	7	14
IL	1	880	65	33	2	35	3	14
	2	920	64	30	3	261	5	14
	3	520	105	51	4	46	18	14
	4	827	126	44	2	39	5	14
	5	653	41	38	2	31	12	14
				Race 6				
AR	1	560	25	3	4	< 1	40	6
	2	461	35	7	4	< 1	37	6
	3	323	28	3	2	0	57	6
	4	288	46	7	2	1	60	6
	5	167	30	2	2	0	45	6

TABLE 2. Continued.

Host source	Test	Females	Female indices on differential					Race designation
			P—F†	Peking	PI 88788	PI 90763	B—FA‡	
NC	1	283	107	8	69	1	112	5
	2	217	83	10	83	1	88	2
	3	805	26	3	19	< 1	40	5
	4	448	37	4	37	< 1	75	5
	5	329	36	4	30	0	56	5
IL	1	557	8	6	32	0	57	1
	2	565	18	7	50	1	62	5
	3	635	11	8	40	0	56	5
	4	680	7	5	11	< 1	20	4
	5	469	9	4	45	< 1	45	1

Numbers are averages of three replications. Susceptible soybeans were Lee for AR and NC sources and Williams for IL.
† Pickett (P) was used for AR and NC sources and Franklin (F) for IL.
‡ Bedford (B) was used for NC and AR sources and Fayette (FA) for IL. Races 6, 9 and 14 are being described (5).

tests indicated race 2, whereas the other four indicated race 9 because of low FI on PI 88788. Using the differentials from Illinois, two of the five tests indicated race 2 but in both cases PI 88788 was just above the positive level (Table 2). Results from the other three tests indicated the isolates were races 4, 9, and 14.

The three sources of differentials consistently had race 3 reactions when inoculated with race 3 (Table 2). Race 4 in the five tests with the Arkansas differentials reacted as race 4; however, on the North Carolina and Illinois differentials it reacted as race 14 because of low FI on PI 88788.

When race 6 was tested on the Arkansas differentials, it reacted as race 6 in all five tests (Table 2). On the North Carolina differentials it reacted as race 2 in one test and race 5 in the other four tests (Table 2). When the Illinois differentials were used, the race 6 population was classed as race 5 in two tests, race 1 in two tests, and race 4 in one test. One race 5 had an index on Franklin of 11, just high enough to be positive.

North Carolina: The SCN isolates from North Carolina did not react as expected for two of the four races in the first experiment (Table 3). Race 1 reacted as race 2, and race 2 as race 4. Greater numbers of females of race 3 matured on Bedford, Fayette, Jeff, and Mack than expected (Table 3). More females of race 4 were recovered from Bedford, Fayette, and Jeff and fewer from Essex than expected for this race. The nine nondifferential cultivars were all susceptible to race 2.

When the race tests were run on North Carolina and Arkansas differentials in the second experiment, race 1 from North Carolina reacted as race 1 on North Carolina differentials but was race 3 on the Arkansas differentials (Table 4). An isolate which had previously given a race 2 reaction was race 2 on the North Carolina differentials in the first test and race 4 in the second test (Table 4). Conversely, on the Arkansas differentials it was race 4 in the first test and race 2 in the second test. An SCN race 3 isolate was race 1 on the North

TABLE 3. Average number of females on Lee and female indices on other soybean cultivars and lines inoculated with four races of *Heterodera glycines* at North Carolina State University.

Cultivar or line	Race 1	Race 2	Race 3	Race 4
Lee	152†	50	104	62
Pickett	25	98	1	86
Peking	15	92	0	119
PI 88788	89	96	6	55
PI 90763	9	78	2	24
Bedford	27	50	24	11
Centennial	26	138	1	34
Custer	24	110	0	37
Essex	154	132	93	21
Fayette	28	140	12	66
Forrest	26	146	1	23
Franklin	11	282	1	71
Jeff	51	86	60	23
Mack	34	120	46	57

† Figures for Lee are actual female counts; other figures are female indices (percentage of Lee). Numbers are averages of four replications.

TABLE 4. Average number of females on Lee and female indices on the standard differential host plants and Bedford for two tests of five races of *Heterodera glycines* on differentials from Arkansas (AR) and North Carolina (NC). Tested at North Carolina State University.

Host source	Test	Females	Female indices on differential					Race designation
			Pickett	Peking	PI 88788	PI 90763	Bedford	
				Race 1				
	1	97	3	1	28	0	58	1
NC	2	107	1	0	37	0	47	1
	1	71	3	0	1	0	89	3
AR	2	99	5	0	0	0	85	3
				Race 2				
	1	71	95	100	66	9	86	2
NC	2	7†	130	39	70	20	39	4
	1	59	116	98	75	15	70	4
AR	2	9†	62	38	30	0	93	2
				Race 3				
	1	152	1	< 1	63	3	32	1
NC	2	15†	0	0	14	4	14	1
	1	129	1	0	1	0	54	3
AR	2	9†	0	0	0	0	30	3
				Race 4				
	1	191	155	63	58	34	61	4
NC	2	122	110	43	23	20	43	4
	1	207	107	82	77	22	59	4
AR	2	89	144	116	99	66	47	4
				Race 5				
	1	46	75	42	70	1	75	2
NC	2	27	153	68	38	0	113	2
	1	65	70	25	21	2	97	2
AR	2	16†	171	29	38	4	96	2

† Test of questionable value because of low numbers of females on Lee.

Carolina differentials and race 3 on the Arkansas differentials (Table 4). The race 4 isolate reacted as race 4 in all tests (Table 4). The isolate originally identified as race 5 was race 2 on both sets of differentials in this test. All races in both tests matured readily on Bedford.

Illinois: The first experiment in Illinois did not give the expected results. Reproduction on PI 88788 by race 3 was > 10 and by race 4 was < 10; therefore, race 3 was called race 1 and race 4 was called race 14 (Table 5). The FI of race 3 was > 10 on Fayette, Jeff, and Mack. Race 4 had FI > 10 on Fayette and Jeff. The second experiment had many seeds that did not germinate or emerge, and the data are not included.

Inoculum level tests: The number of females recovered from Lee was generally related to the number of eggs and J2 in the inoculum (Table 6). The number of females recovered, however, did not double with each increase in inoculum from 500 to 4,000/pot, and the numbers recovered from the 8,000 and 16,000 inoculum levels were similar. The number of females at the 8,000 level was more than double the number from the 4,000 level. Despite this lack of consistent maturation on Lee, the FI were very consistent on each differential.

DISCUSSION

Several sources of variation occurred among the three locations where the tests were conducted. The differentials used at Illinois were different from those used at Arkansas and North Carolina, and the

TABLE 5. Average number of females on Lee and female indices on other soybean cultivars and lines inoculated with two races of *Heterodera glycines* at University of Illinois.

Cultivar or line	Race 3	Race 4
Lee	800	620
Pickett 71	4	103
Peking	1	52
PI 88788	20	8
PI 90763	1	35
Centennial	4	101
Custer	1	158
Essex		53
Fayette	23	16
Forrest	2	104
Franklin	1	81
Jeff	39	32
Mack	16	

Figures for Lee are actual female counts, other figures are female indices (percentage of Lee). Numbers are averages of six replications.

TABLE 6. Average number of females on Lee and female indices on differentials inoculated at six levels with *Heterodera glycines* race 3.

Inoculum level†	Females on Lee	Female indices on differentials			
		Pickett	Peking	PI 88788	PI 90763
500	126	0.3	0.0	0.4	0.3
1,000	150	0.1	0.2	3.0	0.0
2,000	282	0.1	0.0	0.5	0.0
4,000	346	0.1	0.2	0.4	0.1
8,000	987	0.0	0.1	0.1	0.1
16,000	1,014	0.0	0.1	0.1	0.1

Numbers are averages of five replications.
† Number of eggs and second-stage juveniles per pot (85% were eggs).

methods used for the race test at each location were not the same. The method of inoculum preparation may affect the number or the activity of the J2 in the soil, and this could affect the FI on the differentials. Differences in extraction procedure also could affect the results, as small females from resistant plants may be more difficult to extract. Cultivars may give different results because of the variability of both the host and the parasite (6). At each location two races did not perform according to previous tests. At Arkansas and North Carolina races 1 and 2 did not give expected FI, and at Illinois races 3 and 4 did not develop on the differentials as expected. This could be due to variation in test conditions, genetic differences in seeds, or changes in the nematode population.

The most obvious variation in the experiment comparing sources of differentials was in PI 88788. There was no variation in race 3, but in races 4 and 6 the variation was mainly due to differences in PI 88788 from the different locations. Plant mortality at Illinois prevented race determinations in many instances. Poor plant emergence is one of the problems associated with direct seeding in infested soil.

A standard race test has been established for SCN (2). The original race description designated Lee as the standard susceptible cultivar and Pickett, Peking, PI 88788, and PI 90763 as the differentials. Results of these tests suggest that further standardization and adherence to the standards is needed. The five cultivars and lines should be purified by single seed culture, tested for reaction to SCN races, and maintained at a single location in a manner that would assure purity. All race tests at all locations could then be run with seed from one source. In addition, we propose that the differential host seeds be germinated in vermiculite or other media and transplanted into the test medium. Seedlings should be transplanted when the radicle is about 2–4 cm long and should be selected for uniformity. Inoculum should consist of eggs and (or) J2 added just before or just after transplanting. Breaking the cysts with a Ten Broeck homogenizer probably damages the eggs and J2 less than a blender. Mature female counts should be made 30 days after inoculation. Temperature of the test medium should be maintained at 24–30 C if possible and should not go below 20 C or above 33 C for an extended period of time. Use of infested soil may not affect test results, but there may be more problems in getting a full complement of plants.

The results of these tests point to the need to include pure and defined differentials in any test of breeding lines or cultivars or other test where race designation is important. This would insure that the proper race is reported with the results.

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