Journal of Nematology 25(1):34–37. 1993.

Susceptibility of Soybean Introductions to Races 1, 2, 3, and 4 of *Heterodera glycines*[1]

TITIK N. AENY AND R. D. RIGGS[2]

Abstract: Thirteen soybean plant introduction (PI) lines, selected for their apparent susceptibility to *Heterodera glycines,* were compared with cultivar Lee 74 as hosts of *H. glycines* races 1, 2, 3, and 4. Race 3 produced the highest average number of females of the four races. Compared to Lee 74, more ($P = 0.05$) females of *H. glycines* race 1 were extracted from PI 274420, PI 274423, and PI 317333; PI 86457 had more females of *H. glycines* race 2; and PI 86443, PI 86457, PI 261467, PI 274420, PI 274421, and PI 274423 had more females of *H. glycines* race 3. Similar numbers of females of *H. glycines* race 4 developed on all of the soybean lines and Lee 74. PI 274421, PI 274420, or PI 196159 could provide a more or equally susceptible host for *H. glycines* races 1, 2, 3, and 4 than Lee 74. One of these three lines could be substituted for Lee as the standard susceptible cultivar in the race determination test.

Key words: Glycine max, Heterodera glycines, nematode, race, soybean, soybean cyst nematode, susceptibility.

Heterodera glycines Ichinohe, the soybean cyst nematode, occurs worldwide as one of the most important parasites of soybean (*Glycine max* (L.) Merr.) (6). Soybean has become one of the main food sources for the world (7). The prevalence and importance of *H. glycines* has paralleled the expanding production of the crop, especially in Japan, Korea, The People's Republic of China, and the United States (6,7). The development and incorporation of resistance to *H. glycines* in soybean resulted in a significant step in management of the nematode. It also enhanced variability among populations of the nematode.

Variability in reproductive capacity of *H. glycines* on resistant soybean cultivars enabled scientists to develop a race scheme as a means for identifying populations of *H. glycines* (2,10). Sixteen races were characterized on four differentials compared to the susceptible standard 'Lee' (10). Use of Lee as the susceptible standard has been questioned because, at times, other cultivars or lines appear to be more susceptible (i.e., a higher level of reproduction occurs) than Lee. In addition, reproduction of *H. glycines* on 'Lee 74' was more consistent than on Lee (11); therefore, Lee 74 is used as the susceptible standard by some researchers. In greenhouse tests, certain exotic soybean lines were better hosts of *H. glycines* races 2, 3, and 14 than Lee (Riggs, unpubl.).

The objectives of this study were to determine i) the relative susceptibility of selected soybean lines to different races of *H. glycines* using Lee 74 as the susceptible standard and ii) the effect of different susceptible cultivars on female size and egg production.

MATERIALS AND METHODS

Thirteen soybean lines, which were determined to be better hosts of *H. glycines* races 2, 3, and 14 than was Lee in previous tests (Riggs, unpubl.), were obtained from the USDA Northern Soybean Germplasm Collection. Lee 74 was used as the susceptible standard. Seeds were germinated in vermiculite. When the cotyledons were fully expanded, seedlings were transplanted singly into 7.5-cm-d clay pots containing pasteurized fine sand (79% sand, 13% silt, 8% clay; 0.4% OM). Each race was tested as inoculum was available. All tests were replicated five times.

Races 1 and 2 were collected from North Carolina, and races 3 and 4 were collected from Arkansas. Races 1 and 3 were cul-

Received for publication 22 January 1992.
[1] Supported in part by funds from the Arkansas Soybean Promotion Board and in part by USAID. Approved for publication by the Director of the Arkansas Agricultural Experiment Station.
[2] Graduate Assistant and Professor, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.
Present address of first author: PS. HPT—Fakultas, Pertanian, Universitas Lampung, Bandar Lampung 35145, Indonesia.

tured on Lee 74 and races 2 and 4 on 'Pickett'. Inoculum was obtained by sieving and blending (8). Soybean seedlings were allowed to grow 2 days after transplanting to establish a root system. At that time, 10 ml of an aqueous suspension containing approximately 2,500 eggs and second-stage juveniles (J2) was added with a pipette to the sand around the base of each plant. The soil was watered immediately after the addition of the inoculum to prevent the eggs and J2 from drying.

All plants were grown in a greenhouse with ambient temperatures of 28–34 C for 28–30 days. Females were extracted from roots and soil (8). Data recorded included total numbers of females, eggs, and J2; and length and width of 10 randomly selected females from each line that was a host of a particular race.

Females were separated from debris by a modified sugar flotation method and counted (3). The females then were broken in a ground glass tissue grinder filled with water. The suspension was poured through a 37-μm-pore sieve to concentrate the eggs and J2.

Experiments were conducted with each race in a randomized complete block design with 13 soybean lines as treatments. The experiments were run three times. Data from these runs were combined for analysis because they were similar ($P = 0.05$). Means were separated with least significant differences. Correlation among female lengths, widths, and numbers of eggs were calculated. Susceptibility of the soybean lines was based on two parameters: i) number of females produced and ii) female index (10).

Results and Discussion

More females ($P = 0.05$) of race 3 were recovered per plant (191) than with races 1, 2, or 4 (83, 83, and 76, respectively), regardless of the soybean line. Race 3 is considered to be the "wild type" because most of the original populations in Arkansas were race 3 before resistance was introduced (Riggs, unpubl.). In addition, this race does not reproduce on any resistant germplasm (2,10). The number of females averaged over races was less on Lee 74 than on any of the other soybean lines (Table 1). Female numbers were greater ($P = 0.05$) on PI 86457, PI 274420, PI 274421, PI 274423, and PI 317334B than on Lee 74. The lines were not equally susceptible to the *H. glycines* races. Lines PI 274420, PI 274423, and PI 317333 had more ($P = 0.05$) females of race 1; PI 86457 had more

TABLE 1. Female counts of races 1, 2, 3, and 4 of *Heterodera glycines* on Lee 74 and 13 soybean lines at 28–30 days after inoculation.

Cultivar or PI line	Race 1	Race 2	Race 3	Race 4	Mean
Lee 74	44 ± 21	69 ± 18	110 ± 30	86 ± 35	77 ± 15
PI 86075	35 ± 9	64 ± 27	158 ± 68	69 ± 35	69 ± 23
PI 86443	87 ± 38	56 ± 10	225 ± 52	90 ± 22	115 ± 24
PI 86457	45 ± 17	151 ± 75	218 ± 66	65 ± 19	120 ± 31
PI 196159	86 ± 49	106 ± 36	165 ± 60	101 ± 34	114 ± 24
PI 200471	63 ± 29	120 ± 56	185 ± 49	71 ± 21	110 ± 24
PI 200479	56 ± 21	92 ± 24	171 ± 54	72 ± 23	98 ± 20
PI 261467	62 ± 28	50 ± 19	220 ± 77	68 ± 22	100 ± 28
PI 274420	122 ± 53	42 ± 23	280 ± 146	104 ± 30	137 ± 45
PI 274421	103 ± 46	89 ± 41	215 ± 98	80 ± 17	122 ± 32
PI 274423	153 ± 83	44 ± 25	217 ± 60	82 ± 30	124 ± 32
PI 317333	124 ± 66	65 ± 20	199 ± 63	56 ± 20	111 ± 28
PI 317334	109 ± 73	112 ± 58	207 ± 68	56 ± 23	122 ± 32
PI 358314	69 ± 41	96 ± 46	107 ± 51	57 ± 35	82 ± 22
LSD ($P = 0.05$)	66	55	103	38	39

Numbers are means ± standard deviation of 15 replications.

(P = 0.05) females of race 2; and PI 86443, PI 86457, PI 261467, PI 274420, PI 274421, and PI 274423 had more (P = 0.05) females of race 3 than Lee 74 (Table 1).

The lines on which the highest average numbers of females matured differed among the races. For example, PI 274423 had the highest female count of race 1 but had next to the lowest number of females of race 2. Conversely, PI 86457 had the highest number of females of race 2 but the third lowest number of females of race 1. These results demonstrate that susceptible soybean lines react differently to different *H. glycines* races as reported by others (4,5).

Variation in the quantity of *H. glycines* females that mature may be related to differences in viability of eggs and infectivity of juveniles in the inoculum (13). Differences in numbers of females among races also may be attributed to differential egg hatch on different lines. Efficient hatching of nematode eggs have been reported to be stimulated by exudates from roots of susceptible cultivars and less efficiently by those from resistant ones (1). However, Schmitt and Riggs (12) reported a higher hatch rate on a resistant cultivar.

Similar female indices were demonstrated between races 1 and 3 and 2 and 4, as shown earlier by Riggs et al. (9). Female indices of *H. glycines* race 1 on PI 86443, PI 200471, PI 274420, PI 274421, and PI 274423 were about the same as those of race 3 on the same lines, even though the number of females recovered was much larger with race 3. Female indices of SCN race 4 on some soybean lines tended to be closer to the indices of race 2 than to those of races 1 and 3. An exception to this trend occurred with race 2 on PI 196159, PI 200471, PI 200479, and PI 317334B. These indices were similar to those of race 3. Female indices were not used to determine susceptibility because actual number of females gave a good measure of susceptibility.

Egg production: Race 3 was 2.5–3.2 times more fecund than races 1, 2, and 4 (Table 2). The mean egg production on the PI lines was similar for races 1, 2, and 4. Numbers of eggs varied so much that apparent differences were not statistically significant.

The number of eggs extracted from *H. glycines* race 1 females obtained from Lee 74 and the 13 soybean lines were positively correlated (P = 0.05) with the number of females per plant. This relationship probably indicates that the food supply was abundant for the population level. The correlations also were positive with races 2, 3, and 4, but coefficients were low.

Female length and width (Table 3) were negatively correlated with female numbers

TABLE 2. Numbers of *Heterodera glycines* eggs from inoculations of races 1, 2, 3, and 4 on Lee 74 and 13 soybean lines.

Cultivar or PI line	Race 1	Race 2	Race 3	Race 4	Mean
Lee 74	2,748	6,384	14,446	13,992	9,393
PI 86075	4,068	2,904	22,488	4,198	8,414
PI 86443	4,536	4,896	20,292	5,712	8,859
PI 86457	3,516	6,240	22,848	5,844	9,612
PI 196159	4,524	5,496	12,720	9,960	8,175
PI 200471	4,380	5,904	20,472	7,020	9,444
PI 200479	3,468	7,248	17,632	7,308	8,914
PI 261467	2,292	5,496	12,216	5,592	6,399
PI 274420	5,616	6,264	15,600	5,964	8,361
PI 274421	7,008	3,912	14,364	7,072	7,777
PI 274423	7,752	4,704	17,808	4,623	9,334
PI 317333	6,516	4,872	9,708	4,623	6,430
PI 317334B	5,712	4,992	8,388	5,336	6,107
PI 358314	2,364	3,912	4,920	5,700	4,224
Mean	4,750	5,142	15,342	6,166	
LSD(P = 0.05)	5,629	3,282	10,734	5,877	3,993

Numbers are means of 10 replications.

in race 1 ($r = -0.67$ and -0.71, respectively) and positively correlated for race 4 ($r = 0.71$ and 0.75, respectively). Correlations were negative and low for race 2 and positive and low for race 3. Smaller females may be expected when large numbers of females are produced on a root system because the food supply would be limited, thus limiting size of the nematodes.

The use of number of eggs produced by females after 28–30 days to determine host susceptibility may not be as reliable as using the number of females produced because some females may not be mature. Time of egg production should be evaluated as an indicator of susceptibility.

Three or four of the 13 soybean lines may be more susceptible than Lee 74, but consistent reproduction across races still was not achieved. Physiological differences between races or even populations within races may prevent achievement of consistent reproduction.

If female counts are used as the criterion for susceptibility, PI 274420, PI 274423, PI 274421, and PI 86457 would be good candidates for a standard if the race scheme is modified. However, in each case, the number of females was less for one of the races than on Lee 74. More females of all races occurred on PI 196159 than on Lee 74.

TABLE 3. Measurements† of *Heterodera glycines* females on Lee 74 and 13 soybean lines.

Cultivar or line	Length (μm)	Width (μm)
PI 274420	672 ± 24	454 ± 18
PI 274423	659 ± 29	442 ± 20
PI 274421	685 ± 18	462 ± 12
PI 317334B	631 ± 28	454 ± 18
PI 86457	654 ± 19	447 ± 15
PI 86443	630 ± 19	445 ± 13
PI 196159	678 ± 21	465 ± 13
PI 317333	659 ± 17	452 ± 16
PI 200471	638 ± 22	457 ± 12
PI 261467	632 ± 16	453 ± 18
PI 200479	632 ± 17	455 ± 15
PI 358314	660 ± 18	453 ± 14
PI 86075	630 ± 18	466 ± 13
Lee 74	692 ± 16	490 ± 20
LSD ($P = 0.05$)	29.2	22.2

† Means are from measurements of 40 individuals, 10 from each of four races (races 1–4).

Additional studies with more *H. glycines* races are needed. Selections within each line should be made to determine whether the variation between plants can be reduced and the number of females per plant increased. None of these lines is being proposed to replace Lee as the standard susceptible cultivar against which the race differentials are rated. They are presented as candidates in case a change in the race determination scheme is made or as an additional check.

LITERATURE CITED

1. Caballero, L. G., E. A. Osmanski, and P. M. Tefft. 1986. Factors influencing plant-induced egg hatching in *Heterodera glycines*. Journal of Nematology 18:636 (Abstr.).

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. Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

4. Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1982. Screening soybean for resistance to *Heterodera glycines* Ichinohe using monoxenic cultures. Journal of Nematology 14:593–594.

5. Miller, L. I. 1971. Physiologic variation within the Virginia-2 population of *Heterodera glycines*. Journal of Nematology 3:318 (Abstr.).

6. Noel, G. R. 1992. History, distribution, and economics. Pp. 1–13 *in* R. D. Riggs and J. A. Wrather, eds. Biology and management of soybean cyst nematode. St. Paul, MN: American Phytopathological Society Press.

7. Riggs, R. D. 1977. Worldwide distribution of soybean cyst nematode and its economic importance. Journal of Nematology 9:34–39.

8. Riggs, R. D., and M. L. Hamblen. 1962. Soybean-cyst nematode host studies in the family Leguminosae. Arkansas Agricultural Experiment Station Report Series 110.

9. Riggs, R. D., M. L. Hamblen, and L. Rakes. 1977. Development of *Heterodera glycines* pathotypes as affected by soybean cultivars. Journal of Nematology 9:312–318.

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

11. Riggs, R. D., and D. P. Schmitt. 1991. Optimization of the *Heterodera glycines* race test procedure. Journal of Nematology 23:149–154.

12. Schmitt, D. P., and R. D. Riggs. 1991. Influence of selected plant species on hatching of eggs and development of juveniles of *Heterodera glycines*. Journal of Nematology 23:1–6.