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Soil Infestation Density Affects the Results of *Heterodera glycines* Race Tests[1]

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Abstract: Production of females by stock populations of races 1, 2, 3, 4, 5, 6, 9, and 14 of *Heterodera glycines* on 'Lee 74', 'Pickett', 'Peking,' PI 88788, and PI 90763 soybean cultivars and lines at inoculum densities of 100, 1,000, 4,000, 5,000, and 10,000 eggs and second-stage juveniles/pot (2.2, 21.8, 87.1, 108.9, and 217.9/cm^3 soil) was tested to determine the effects of soil population density on race identification using the 16-race scheme. Tests of all races were repeated 1 to 3 times during the year, except for the race 9 test, which was conducted once. Only races 3 and 9 had the same race designation at all infestation densities in repeated tests. Races 2 and 4 consistently were identified at all except the lowest infestation densities. Race 5 was identified as race 15 at the 100 infestation density in one test, and as race 1 at the 1,000 density in another test. Race 6 had significant numbers of females on Peking and PI 90763 that resulted in female indices of 34 on Peking and 14 on PI 90763 when the infestation density was 10,000. Race 14 was consistently identified between infestation densities of 4,000 and 10,000 but was identified as race 6 or 9 at the 100 infestation density and as race 4 or 14 at the 1,000 infestation density. Race 1 was identified as race 5 in a few instances because Pickett was not resistant to this population. The results of this series of experiments suggest that an infestation density of 4,000 eggs and second-stage juveniles/pot is best for race identification. Races were very poorly differentiated at the lowest density, differentiation was inconsistent at the 1,000 infestation density, and densities higher than 4,000 had reduced numbers of females on Lee 74 and relatively high numbers on the differentials which resulted in poor race differentiation with some races.

Key words: *Glycine max, Heterodera glycines,* races, soybean, soybean cyst nematode.

Reproduction of the soybean cyst nematode, *Heterodera glycines,* by amphimixsis provides an opportunity for genetic variations between individuals or populations through genetic recombination and gene mutations during reproduction. A host plant favorable for reproduction of a few specific individual nematode genotypes will differentially increase these genotypes to form a population that has host specialization (Sidhu and Webster, 1981). A host-specific form of *H. glycines* was first recognized by Ross (1962), and new biotypes (Miller, 1967b; Riggs et al. 1968) and host specificity (Koliopanos and Triantaphyllou, 1971; Miller, 1967a) were reported later. Host-specific physiological strains of *H. glycines* were defined as races (Golden et al., 1970) and are now widespread (Riggs, 1982).

Race classification provided an avenue for professionals to communicate and define the physiological variation of populations of this economically important nematode species and its interaction with soybean. Standardized procedures for race tests and consistent race designation are essential, but significant variability in race assignment was found when race tests were run in different laboratories (Riggs et al., 1988). One factor that may contribute to variable race determination results is lack of genetic uniformity in seed of the soybean (*Glycine max* (L.) Merr.) differentials (Riggs and Schmitt, 1991). Other factors could be the procedures used in race tests, such as differences in pot size, time of infestation, nematode stage used for infestation, and temperature, which may contribute to the variability in race tests (Riggs and Schmitt, 1991).

The determination of races of *H. glycines* is based on the development of females on susceptible and resistant differentials (Golden et al., 1970; Riggs and Schmitt, 1988). Because resistance is not complete, a female index (FI) of 10 has been used arbitrarily to designate negative (resistant) (FI $<$ 10) and positive (susceptible) (FI $\geq$ 10) reactions on differential hosts to a nematode population. This relative value is determined by the actual number of females produced on differ-

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ential hosts compared to the number of females produced on a standard susceptible host 'Lee' or 'Lee 74'. Race is not a distinct genotype but the phenotype of a population (Niblack, 1992); a population of a given race may contain genetically distinct individuals that have different reproductive potential on the differential soybean hosts. When variable numbers of eggs and second-stage juveniles are used for infestation in a race test, the number of females produced on the differential hosts and Lee 74 may vary, which will affect the FI value used to identify races. Consequently, the objective of this research was to determine the effect of different infestation densities on the FI obtained in the determination of races of *H. glycines.*

Materials and Methods

Stock cultures of *H. glycines* races 1, 2, 3, 4, 5, 6, 9, and 14, which had given consistent results in cultivar tests (Riggs et al., 1995), were tested following the procedures of Riggs and Schmitt (1988). Seeds of the differential cultivars and lines Pickett, Peking, PI 88788, and PI 90763 (Golden et al., 1970), and the standard susceptible soybean cultivar, Lee 74, were germinated in vermiculite. The cultivar Hartwig was included in all tests as a resistant standard because it is resistant to all races of *H. glycines.* Seedlings with opened cotyledons were transplanted individually to 7.5-cm-diam. clay pots filled with pasteurized fine river sand. After 48 hours, the desired number of eggs (primarily) and second-stage juveniles freshly prepared from a stock culture was injected into the rhizosphere of each test plant. Plants were grown in a greenhouse with temperature fluctuation of 23 °C to 32 °C, and nematodes were allowed to develop for 30 to 48 days (Table 1). Females on each plant were extracted by rubbing the females from the roots into a pail of water and pouring the resulting suspension through nested 850- and 250-μm-pore sieves. The females on the 250-μm-pore sieve were rinsed into a dish and counted with a stereoscopic microscope.

Five infestation densities were tested: 100, 1,000, 4,000, 5,000, and 10,000 eggs and juveniles/pot. The 4,000 was included because it has been recommended as a standard infestation level in race tests (Riggs and Schmitt, 1988). Each level in each test was replicated 5 times, and all levels of each race were tested at the same time. All tests were

TABLE 1. Dates when *Heterodera glycines* race tests were conducted.

Test number	Races tested	Date started	Date ended	Test time, days
1	1, 5, 9	26 June 1996	26 July 1996	30
	6	28 June 1996	28 July 1996	30
	2	26 June 1997	4 August 1997	39
	3	24 June 1997	4 August 1997	41
	4	30 August 1997	15 October 1997	46
	14	30 August 1997	17 October 1997	48
2	1	26 June 1997	31 July 1997	35
	2	26 June 1997	4 August 1997	39
	3	24 June 1997	4 August 1997	41
	4	30 August 1997	15 October 1997	46
	5	26 June 1997	7 August 1997	42
	6	3 July 1997	11 August 1997	39
	14	30 August 1997	17 October 1997	48
3	1	26 June 1997	31 July 1997	35
	3	24 June 1997	4 August 1997	41
	5	26 June 1997	7 August 1997	42
	2	30 August 1997	16 October 1997	47
4	1	26 June 1997	31 July 1997	35
	2	30 August 1997	16 October 1997	47

repeated 1 to 3 times at different times during the year except race 9, which was tested only once. The race of the population in each test was determined following the race scheme based on the FI (Riggs and Schmitt, 1988).

Results

In test 1 with the stock population of race 1, the number of females produced on PI 88788, the only differential host susceptible to this race, increased greatly as the infestation density increased from 100 to 10,000 (Table 2). The numbers of females on the resistant differential Pickett also increased and at the 10,000 density were great enough for a positive rating (i.e., FI ≥ 10). Few females were found on Peking and PI 90763 at any infestation level. On Lee 74, the number of females was very high and increased as infestation density increased from 100 to 5,000. However, the number of females declined at the 10,000 density. The stock population was consistently identified as race 1 at all densities except 10,000 when the population was identified as race 5. In comparison to test 1, relatively low numbers of fe-

TABLE 2. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in four tests with five infestation densities of Race 1.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	111 ± 156	100	932 ± 412	100	1,666 ± 429	100	2,108 ± 526	100	1,536 ± 595	100
Pickett	0	0	25 ± 29	2.7	26 ± 43	2	157 ± 119	7	241 ± 194	16
Peking	0	0	0	0	5 ± 7.5	0.3	0.6 ± 1.3	0.03	17 ± 20	1.1
PI 88788	14 ± 23	13	181 ± 132	19	184 ± 85	11	240 ± 143	11	487 ± 156	32
PI 90763	0	0	0	0	0.2 ± 0.4	0.01	0	0	0.6 ± 0.5	0.04
Hartwig	0	0	0.2 ± 0.4	0.02	0	0	0	0	0.6 ± 0.9	0.04
Race		1		1		1		1		5
					Test 2					
Lee 74	47 ± 15	100	110 ± 39	100	312 ± 149	100	325 ± 120	100	442 ± 148	100
Pickett	0.2 ± 0.4	0.4	2 ± 1.9	2	39 ± 15	13	32 ± 11	10	63 ± 22	14
Peking	0.4 ± 0.5	1	0	0	0.6 ± 0.5	0.2	1.2 ± 2.7	0.4	0.2 ± 0.4	0.05
PI 88788	17 ± 4.9	36	77 ± 29	70	185 ± 78	59	220 ± 39	68	122 ± 81	28
PI 90763	0	0	0	0	0	0	0	0	0	0
Hartwig	0	0	0	0	0.2 ± 0.4	0.1	0	0	0	0
Race		1		1		5		5		5
					Test 3					
Lee 74	17 ± 9.6	100	90 ± 18	100	434 ± 202	100	407 ± 131	100	877 ± 374	100
Pickett	0.2 ± 0.4	1	22 ± 12	24	47 ± 21	11	37 ± 7.5	9	59 ± 7.8	7
Peking	0	0	1.2 ± 1.6	0.2	2 ± 3.9	0.5	0.2 ± 0.4	0.05	1.4 ± 1.1	0.2
PI 88788	13 ± 6.6	76	93 ± 28	103	248 ± 69	57	313 ± 151	77	354 ± 94	40
PI 90763	0.2 ± 0.4	1	0.2 ± 0.4	0.2	0	0	0	0	0	0
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		1		5		5		1		1
					Test 4					
Lee 74	24 ± 11	100	163 ± 91	100	678 ± 210	100	439 ± 200	100	596 ± 273	100
Pickett	2.2 ± 2.8	9	35 ± 27	21	31 ± 14	5	36 ± 15	8	61 ± 25	10
Peking	1.2 ± 0.8	5	0.6 ± 0.5	0.4	1.6 ± 1.9	0.2	0.2 ± 0.4	0.05	1.6 ± 1.7	0.3
PI 88788	27 ± 8.5	113	124 ± 53	76	187 ± 53	28	234 ± 65	53	190 ± 169	32
PI 90763	0	0	0.2 ± 0.4	0.1	0	0	0	0	0	0
Hartwig	0.2 ± 0.4	1	0	0	0	0	0	0	0	0
Race		1		5		1		1		5

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

males were produced on tests 2–4 (Table 2). This population was identified as race 5 at the 4,000, 5,000, and 10,000 densities in test 2; at the 1,000 and 4,000 densities in test 3; and at the 1,000, and 10,000 densities in test 4.

The number of females produced by the race 2 stock population on Lee 74 and the three susceptible differentials increased slowly and almost proportionally to the infestation densities from 100 to 10,000 (Table 3). As a result, the FI on susceptible differentials were >10, and the number of females on the resistant differential PI 90763 always remained <10% of that on Lee 74 (Table 3). The population was consistently identified as race 2 at all densities except 100, when it was identified 1 time each as race 4, 5, and 9 (Table 3).

Race 3 was stable at all infestation densities in three tests (Table 4). The number of females on Lee 74 increased significantly from the lowest to the highest densities. In addition, very few females were found on the four resistant differentials at any level.

The results with race 4 also were very consistent (Table 5). The number of females produced on Lee 74 and the four suscep-

TABLE 3. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in four tests with five infestation densities of Race 2.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	1 ± 0.7	100	36 ± 19	100	106 ± 32	100	235 ± 77	100	292 ± 66	100
Pickett	1.2 ± 1.6	120	37 ± 17	103	116 ± 10	109	136 ± 30	58	388 ± 35	133
Peking	1.2 ± 2.7	120	17 ± 5.0	47	61 ± 22	58	70 ± 17	30	142 ± 34	48
PI 88788	0.4 ± 0.5	40	13 ± 6.6	36	35 ± 12	33	64 ± 31	27	97 ± 25	33
PI 90763	0.6 ± 0.5	60	1.2 ± 0.4	3	8.4 ±2.5	8	19 ± 5.0	8	26 ± 4.3	9
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		4		2		2		2		2
					Test 2					
Lee 74	4.8 ± 5.0	100	22 ± 6.8	100	136 ± 72	100	146 ± 46	100	235 ± 70	100
Pickett	11 ± 8.9	229	28 ± 9.1	127	102 ± 22	75	140 ± 24	96	144 ± 64	107
Peking	7.2 ± 5.0	150	10 ± 5.4	45	48 ± 35	35	56 ± 14	38	90 ± 44	38
PI 88788	1.2 ± 2.6	25	10 ± 5.4	45	62 ± 25	46	40 ± 6.8	27	113 ± 52	48
PI 90763	0	0	1.6 ± 1.3	7	9 ± 5.6	7	12 ± 7	8	22 ± 10	9
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		2		2		2		2		2
					Test 3					
Lee 74	0.2 ± 0.4	100	50 ± 22	100	127 ± 31	100	238 ± 90	100	275 ± 80	100
Pickett	0.2 ± 0.4	100	29 ± 18	58	48 ± 18	38	125 ± 42	53	130 ± 73	47
Peking	0	0	5 ± 6	10	14 ± 7.2	11	26 ± 8.1	11	43 ± 14	16
PI 88788	0.4 ± 0.5	200	7.2 ± 6.2	14	14 ± 95	11	23 ± 11	10	53 ± 28	19
PI 90763	0	0	1 ± 1	2	4.6 ± 4.2	4	14 ± 6.8	6	12 ± 10	4
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		5		2		2		2		2
					Test 4					
Lee 74	2.6 ± 3.8	100	15 ± 13	100	198 ± 85	100	71 ± 7.8	100	299 ± 116	100
Pickett	10 ± 12	384	48 ± 57	320	122 ± 74	62	70 ± 43	99	142 ± 60	47
Peking	7.4 ± 16	285	9.3 ± 10	62	40 ± 25	20	24 ± 12	34	50 ± 15	17
PI 88788	0.2 ± 0.4	8	6 ± 3.7	40	21 ± 13	11	20 ± 12	28	50 ± 43	17
PI 90763	0	0	0.2 ± 0.4	1	6 ± 6.2	0.3	1 ± 1	1	10 ± 6.6	3
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		9		2		2		2		2

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

TABLE 4. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in three tests with five infestation densities of Race 3.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	14 ± 4	100	187 ± 59	100	525 ± 262	100	358 ± 90	100	667 ± 252	100
Pickett	0	0	0	0	0.4 ± 0.9	0.1	10 ± 15	3	0	0
Peking	0	0	0	0	10 ± 21	2	0	0	0	0
PI 88788	0.4 ± 0.5	3	5 ± 5.1	3	4 ± 2.5	1	2 ± 2.5	1	4 ± 2.5	1
PI 90763	0	0	0	0	0	0	0.2 ± 0.4	0.1	4 ± 8	1
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		3		3		3		3		3
					Test 2					
Lee 74	6 ± 6.7	100	176 ± 53	100	184 ± 82	100	234 ± 140	100	701 ± 405	100
Pickett	0	0	0.4 ± 0.5	0.2	0	0	0.2 ± 0.4	0.1	20 ± 39	3
Peking	0	0	0.6 ± 1.3	0.3	0	0	0	0	1.8 ± 2.7	0.3
PI 88788	0.2 ± 0.4	3	0.6 ± 0.9	0.3	4 ± 2.9	2	5 ± 9	2	16 ± 5.0	2
PI 90763	0	0	0	0	0	0	0	0	0	0
Hartwig	0	0	0.4 ± 0.9	0.2	0	0	0	0	0	0
Race		3		3		3		3		3
					Test 3					
Lee 74	24 ± 11	100	130 ± 51	100	166 ± 73	100	282 ± 171	100	402 ± 176	100
Pickett	0	0	0	0	0	0	0.6 ± 1.3	0	3.6 ± 8.0	0
Peking	0	0	0.2 ± 0.4	0.1	0	0	0	0	0	0
PI 88788	0.2 ± 0.4	1	0.2 ± 0.4	0.1	2.4 ± 1.3	1	3 ± 3	0.1	11 ± 11	3
PI 90763	0.4 ± 0.9	2	0	0	0	0	0	0	0.2 ± 0.4	0.05
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		3		3		3		3		3

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

tible differentials increased as infestation density increased. On each differential, FI was >10 at all densities except 100 in test 1, where the number of females on PI 90763 was too low and the population was identified as race 2.

Race 5 consistently was identified at all inoculum densities in test 1 but was identified as race 15 at the 100 density in test 2 and as race 1 at the 1,000 density in test 3 (Table 6). Few females were found on the resistant differentials in any test. However, susceptible Pickett had relatively low numbers in test 3, resulting in race misidentification at the 1,000 density.

In test 1 with race 6, very high numbers of females were produced on Lee 74 and the susceptible differential Pickett (Table 7). On the resistant differential Peking, the numbers of females produced resulted in a positive rating, indicating race 9, at the 1,000 and 5,000 densities in test 1 and at the 4,000 density in test 2. On PI 90763, the numbers of females produced resulted in a positive rating, indicating race 10, at the 100 density in test 1, and indicating race 14 at the 10,000 density in test 1 and at the 5,000 and 10,000 densities in test 2. Therefore, this population was identified as race 6 only at the 4,000 density in test 1 and at the 100 and 1,000 densities in test 2.

In tests with race 9, the number of females on Lee 74 at the 100 density was higher than in tests with other races at the same level (Table 8). Also, the number of females generally increased on Lee 74 and Pickett as infestation density increased to 5,000 and then was lower at the 10,000 density. Numbers of females were low on resistant PI 88788 and PI 90763 at the 1,000 and 4,000 densities but were relatively higher at the 100, 5,000, and 10,000 densities. Consistent

TABLE 5. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in four tests with five infestation densities of Race 4.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	13 ± 3.9	100	37 ± 8.3	100	174 ± 31	100	295 ± 82	100	502 ± 112	100
Pickett	16 ± 9.8	123	47 ± 22	127	262 ± 85	151	264 ± 42	89	445 ± 52	89
Peking	3 ± 15	23	16 ± 5.4	43	65 ± 36	37	97 ± 21	33	211 ± 155	42
PI 88788	2 ± 1.5	15	17 ± 6.1	46	36 ± 13	21	51 ± 12	17	109 ± 36	22
PI 90763	0.6 ± 0.9	5	15 ± 11	41	32 ± 8.6	18	36 ± 25	12	52 ± 26	10
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		2		4		4		4		4
					Test 2					
Lee 74	10 ± 7.8	100	30 ± 15	100	185 ± 49	100	306 ± 194	100	276 ± 76	100
Pickett	8 ± 6.2	80	34 ± 24	113	166 ± 37	90	313 ± 94	102	333 ± 103	121
Peking	2 ± 1.6	20	13 ± 3.4	43	67 ± 15	36	110 ± 35	36	147 ± 53	53
PI 88788	1 ± 1.4	10	13 ± 6.9	43	52 ± 28	28	62 ± 27	20	100 ± 13	36
PI 90763	1 ± 1.2	10	14 ± 15	47	22 ± 5.4	12	31 ± 5.8	10	39 ± 16	14
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		4		4		4		4		4

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Numbers of females on a differential/number of females on Lee 74 × 100.

identification of this population as race 9 was obtained at all infestation densities.

Female numbers were low at all infestation densities on all differentials and on Lee 74 with the race 14 population (Table 9). PI 88788 was resistant at all infestation densities in the two tests with the race 14 population except at the 1,000 density in test 2 (Table 9). Susceptible differentials had increased numbers of females as infestation density increased. At the 1,000 density, the number of females on Lee 74 was low in both tests, which resulted in FI > 10 on PI 88788 in the second test and identification as race 4. Tests with the 100 density produced very low numbers of females, and the population was identified as race 6 in test 1 and as race 9 in test 2.

Discussion

Race determination is based on resistant and susceptible responses of four differentials to a test population (Riggs and Schmitt, 1988). In the race test a differential is susceptible to a given population if the FI ≥ 10 (Golden et al., 1970). In contrast, susceptibility of a soybean breeding line or cultivar is rated at different levels with FI ≤ 9 being resistant, FI of 10–30 being moderately resistant, FI of 31–60 being moderately susceptible, and FI ≥ 61 being susceptible (Schmitt and Shannon, 1992). Thus, enough females must be produced on Lee 74 to make the determination of the reaction of a given differential or cultivar credible. At the 100 infestation density, numbers of females extracted from Lee 74 were almost always low and the FI were very variable. In these tests, the numbers of females produced on Lee 74 at the higher (5,000–10,000) infestation densities were not always proportional to the infestation levels. With six of the seven races, the average percentage of females on Lee 74 relative to the infestation level was lower when a density of 10,000 was used than when lower densities were used. However, the number of females produced on resistant differentials relative to the infestation density remained at about the same level at all densities, which resulted in FI > 10 in some cases. In three of four tests with race 1, for example, the population was repeatedly identified as race 5 at

TABLE 6. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in three tests with five infestation densities of Race 5.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	16 ± 8.6	100	124 ± 9.2	100	154 ± 57	100	794 ± 325	100	465 ± 149	100
Pickett	1.8 ± 1.6	11	34 ± 24	27	49 ± 38	32	276 ± 124	35	340 ± 161	73
Peking	0	0	0	0	0	0	0.2 ± 0.4	0.03	4.8 ± 2.7	1
PI 88788	7.8 ± 5.0	49	53 ± 24	43	150 ± 36	97	582 ± 203	73	316 ± 155	68
PI 90763	0	0	0	0	0	0	0	0	7.2 ± 4.5	2
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		5		5		5		5		5
					Test 2					
Lee 74	19 ± 6.9	100	174 ± 69	100	216 ± 80	100	252 ± 54	100	452 ± 141	100
Pickett	4.5 ± 3.9	23	28 ± 30	16	41 ± 29	19	55 ± 31	22	132 ± 91	29
Peking	1.2 ± 1.6	6	0	0	3.6 ± 2.5	2	1.2 ± 2.7	0.4	6.6 ± 5.3	1
PI 88788	7.8 ± 6.2	41	59 ± 26	34	149 ± 71	69	146 ± 55	60	348 ± 110	77
PI 90763	6.2 ± 3.5	33	0.6 ± 1.3	0.3	0	0	0	0	0	0
Hartwig	0.6 ± 1.3	3	0	0	0	0	0	0	0	0
Race		15		5		5		5		5
					Test 3					
Lee 74	17 ± 6.2	100	223 ± 39	100	201 ± 45	100	330 ± 65	100	552 ± 125	100
Pickett	3.6 ± 2.5	21	20 ± 9.3	9	88 ± 42	44	41 ± 36	12	79 ± 46	15
Peking	0	0	0	0	9 ± 5.2	4	3.6 ± 2.5	1	7.8 ± 7.5	1
PI 88788	7.2 ± 5.4	42	66 ± 25	30	145 ± 49	72	132 ± 70	40	270 ± 59	52
PI 90763	0.2 ± 0.4	1	3 ± 6.7	1	1.6 ± 2.2	1	0	0	0	0
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		5		1		5		5		5

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

the 10,000 density. At the same time, at the 100 infestation density the race 2 population was identified as another race in three of four tests and the race 14 population was identified as another race in both of the tests. However, in tests with races 1, 3, 5, and 9, the numbers of females produced on Lee 74 infested with the 100 density were sufficient to correctly identify the race. The poor or variable results with the 100 or 1,000 density suggest that a minimum level of infection units is required to ensure the production of a reasonable number of females on Lee 74 for a valid race determination. Because the variation in race identification in these tests mostly occurred at the 100 density, densities as low as 100 should not be used in race tests.

Selection pressure by resistant differentials was obvious at high levels of infective units. In tests with race 1, Pickett was susceptible at the 10,000 density, whereas at lower infestation densities it was resistant. In tests with race 6, Peking and PI 90763, normally resistant, were susceptible at the 5,000 and 10,000 infestation densities. Such change may be expected because genetic diversity among the individuals of the same population allows increases in the number of females on the resistant lines. At the high population levels, the number of individuals that could mature on the resistant lines would be higher than at the low population levels. Also at high infestation levels a number of the nematodes penetrating roots of the susceptible Lee 74 may not be able to mature because of the crowded conditions. The combination of higher population levels on resistant differentials and lower numbers maturing on Lee 74 could result in FI > 10 and misidentification of races. Previous research supports these results in that low

TABLE 7. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in two tests with five infestation densities of Race 6.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	47 ± 29	100	1,415 ± 1,359	100	3,730 ± 1,734	100	1,810 ± 2,035	100	2,081 ± 952	100
Pickett	95 ± 79	202	1,302 ± 591	92	1,325 ± 332	36	1,814 ± 947	100	2,282 ± 1,199	110
Peking	3 ± 6.7	6	136 ± 84	10	170 ± 112	5	422 ± 164	23	536 ± 322	26
PI 88788	0	0	0.2 ± 0.4	0.01	11 ± 7.8	0.3	12 ± 12	1	29 ± 21	1
PI 90763	9 ± 6.4	19	35 ± 32	2	60 ± 57	2	125 ± 47	7	258 ± 95	12
Hartwig	0	0	0	0	0.6 ± 0.6	0.02	0	0	0.4 ± 0.5	0.02
Race		10		9		6		9		14
					Test 2					
Lee 74	7.2 ± 6.2	100	89 ± 25	100	175 ± 55	100	355 ± 147	100	250 ± 79	100
Pickett	7.8 ± 6.2	108	64 ± 37	72	218 ± 85	125	354 ± 108	100	342 ± 45	137
Peking	0	0	4.6 ± 5.5	5	17 ± 7.8	10	66 ± 32	19	85 ± 39	34
PI 88788	0	0	3.4 ± 6.5	4	5.4 ± 1.3	3	5 ± 4.3	1	2.4 ± 3.3	1
PI 90763	0	0	5.6 ± 9.0	6	11 ± 8.1	6	43 ± 32	12	36 ± 25	14
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		6		6		9		14		14

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

infestation densities often produced the greatest number of females on the susceptible cultivars and lines (Miller, 1966). In contrast, more females were produced on resistant PI 90763 and PI 209332 as infestation densities increased. Misidentification of races might not occur because of increased numbers of females on resistant differentials if the number on Lee 74 increased with infestation density. Unfortunately, the combination of responses of susceptible Lee 74 and the resistant differentials may affect some races more than others. For example, female production would be more likely to increase on Pickett than on Peking or PI 90763, which have higher levels of resistance to *H. glycines*.

Many race tests that are reported do not include the level of infestation used (Anand and Brar, 1983; Riggs et al., 1982; Young, 1989). Probably most race tests on field populations of *H. glycines* are run without consideration of the number of infective units in the soil or of the number of eggs

TABLE 8. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in one tests with five infestation densities of Race 9.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
Lee 74	61 ± 53	100	365 ± 498	100	1,013 ± 1,084	100	1,552 ± 1,354	100	821 ± 329	100
Pickett	152 ± 105	249	450 ± 445	123	350 ± 84	35	1,374 ± 1,108	89	704 ± 357	86
Peking	11 ± 15	18	44 ± 28	12	382 ± 291	38	230 ± 165	15	542 ± 302	66
PI 88788	3 ± 5.1	5	0.4 ± 0.5	0.1	0.4 ± 0.5	0.04	10 ± 5.4	1	19 ± 8.9	2
PI 90763	4 ± 5.4	7	3.6 ± 5.4	1	12 ± 15	1	24 ± 15	2	70 ± 36	9
Hartwig	0	0	0.8 ± 1.1	0.2	2.4 ± 5.4	0.2	0	0	0.2 ± 0.4	0.02
Race		9		9		9		9		9

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

TABLE 9. Numbers of females produced on the race differentials for *Heterodera glycines* and standard deviation, the female indices (FI), and race designations in two tests with five infestation densities of Race 14.

	Infestation densities[a]									
	100		1,000		4,000		5,000		10,000	
Differentials	Females	FI[b]	Females	FI	Females	FI	Females	FI	Females	FI
					Test 1					
Lee 74	3.6 ± 2.3	100	30 ± 7.3	100	172 ± 62	100	173 ± 34	100	331 ± 63	100
Pickett	1.8 ± 1.1	50	30 ± 6.0	100	118 ± 26	69	204 ± 29	118	388 ± 117	117
Peking	0.2 ± 0.4	6	17 ± 5.0	57	40 ± 20	23	36 ± 17	21	68 ± 27	21
PI 88788	0.2 ± 0.4	6	1.5 ± 1.6	5	6.6 ± 6.5	4	6.6 ± 3.2	4	3.8 ± 2.6	1
PI 90763	0.2 ± 0.4	6	16 ± 11	53	50 ± 15	29	49 ± 16	28	163 ± 26	49
Hartwig	0	0		0	0	0	0	0	0	0
Race		6		14		14		14		14
					Test 2					
Lee 74	4 ± 3.7	100	28 ± 7.8	100	112 ± 13	100	179 ± 31	100	342 ± 84	100
Pickett	2.2 ± 1.6	55	37 ± 11	132	137 ± 37	122	181 ± 47	101	382 ± 107	112
Peking	0.8 ± 1.1	20	13 ± 3.9	46	40 ± 20	36	67 ± 30	37	148 ± 31	43
PI 88788	0	0	3 ± 4.2	11	4.6 ± 3.5	4	9 ± 5.2	5	16 ± 6.8	5
PI 90763	0.2 ± 0.4	5	9.6 ± 3.3	34	49 ± 24	44	45 ± 6	25	127 ± 36	37
Hartwig	0	0	0	0	0	0	0	0	0	0
Race		9		4		14		14		14

[a] Eggs + second-stage juveniles used to infest soil.
[b] FI = Number of females on a differential/number of females on Lee 74 × 100.

present that will hatch during the test. Results from the present studies indicate that race tests run under such conditions may not be reliable. This research supports the use of an optimum infestation density in identifying races of *H. glycines* and further indicates that 4,000 eggs and juveniles/7-cm-top diam.-clay pot (45.9 cm^3), as used in earlier tests (Kim et al., 1997), appeared to be the best overall density for our experimental conditions. Lower or higher infestation levels were more likely to result in misidentification of the race of a given population, whether a stock culture or a field population. Each location that conducts race determinations for *H. glycines* should determine the optimum infestation density for their conditions.

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