Infra-species Variation in Reactions to Hosts in Heterodera glycines Populations

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Abstract: Eighteen hosts were inoculated with each of four races of *Heterodera glycines.* A discriminant function analysis of the reactions of these races to these hosts demonstrated that these races could be separated but not consistently. Then 33 *H. glycines* populations collected from 13 states and five obtained from Japan were tested on differential hosts. The number of variants discriminated within these 38 populations depended on the number of differentials and the rating system used. When five differentials were used with a (+) or (−) rating system there were six "races," but when 13 differentials were used with a (+) or (−) system there were 25 physiological groups. If an index rating system was used there were 36 groups. Apparently *H. glycines* is a very variable species and delineation of races varies with criteria chosen. *Key words:* soybean cyst nematodes, races, variability.

Heterodera glycines, the soybean cyst nematode (SCN), has been studied extensively in relation to host differentiation of variants. Ross (14) was the first to report on a physiological variant of *H. glycines,* and other reports subsequently appeared from Virginia (5,6,7,8,9,10,15), Arkansas (13), Tennessee and Missouri (1,2), North Carolina (4), and Japan (16). In 1969 a group of researchers met to discuss the variation in *H. glycines;* as a result the species was divided into four races (3). However, the work of Miller and co-workers in Virginia demonstrated at least 11 different races (10) based on different host ranges.

The purpose of this study was to test *H. glycines* populations from the United States and Japan on a series of differential hosts to determine the range in parasitic capabilities.

MATERIALS AND METHODS

Cultures of the original four race populations (3) were tested. One or more populations were collected from each state in which SCN was known to occur, and five populations were obtained from Japan. These populations, totaling 38, were maintained in a glasshouse on 'Lee' or 'Pickett' soybeans, depending on the source.

Thirteen soybean cultivars and five other host species (Table 1) were used as differentials. Seeds were germinated in vermiculite and transplanted into fine river sand for inoculation. There were 10 replica-

Table 1. Plants tested as hosts for different populations of *Heterodera glycines*.

Plant species	Common name	Cultivar
1. *Lespedeza stipulacea*	Korean lespedeza	
2. *L. striata*	Common lespedeza	Kobe
3. *Melilotus officinalis*	Yellow sweet clover	
4. *Cleome* sp.		Pink Queen
5. *Glycine max*	Soybean	1. Lee
		2. Pickett
		3. Peking
		4. Old Dominion
		5. Pine Dell Perfection
		6. Custer
		7. P.I. 79693
		8. P.I. 84611
		9. P.I. 88788
		10. P.I. 209332
		11. P.I. 87631-1
		12. P.I. 90763
		13. P.I. 91684
6. *Lycopersicon esculentum*	Tomato	Rutgers

tions of each host. Each SCN population was tested separately. The temperatures were maintained as close as possible to 28–32C.

Inoculum was prepared as reported previously (12). About 4,000 eggs and larvae were delivered into each pot using an automatic syringe. About 28 d after inoculation the roots and surrounding soil from each plant were processed for the recovery of mature females. Counts were made using a stereomicroscope. The counts were converted to a mature female index as follows:

$$\text{Index} = \frac{\text{number females on test host}}{\text{number females on 'Lee'}} \times 100.$$

These indices were used to analyze the relationship of these populations to each other. A step-wise discriminant function analysis of the comparative indices was used to determine the separation of the groups. In this analysis, regression equations are used to compare the data from the different groups. In a step-wise comparison the program selects the hosts which appear to be most selective for this particular separation. The program identifies all hosts which appear to have value in separating the nematode groups.

RESULTS

An attempt was made to determine whether the four races could be actually separated on the basis of host differentials. A discriminant function analysis of the mature female indices was used to determine the separation of the groups. The results of one test indicated that the races could be readily separated with overlap only between races 2 and 4 (Fig. 1 A). The separation of the data from a second test was just as distinct, and there was the same overlap between races 2 and 4 (Fig. 1 B). However, the program used a different series of hosts in the second separation. When the program was run using data from the first test but using the two *Lespedeza* species and the 13 soybean lines, the separation was not as good (Fig. 1 C). Data from a third test, which also included the *Lespedeza* spp. and the 13 soybean lines, resulted in the distinct separation of all four races (Fig. 1 D). Again, a different series of hosts was used by the program for the separation. This demonstrated that the races could be separated, usually to a reliable degree, but there were variations in the host groups which would separate. This indicated inconsistency. To further test the separability, the data from two tests were processed using only the differential soybean lines proposed by Golden et al. (3). The separation was not as good as had been obtained earlier, and different hosts were used by the program in separation of the two sets of data (Fig. 2, A & B).

When the 39 populations of SCN were compared on the four soybean cultivars used by Golden et al. (3) to distinguish the four races (Table 2), no population gave the reaction reported for race 1. Five populations reacted as reported for race 2, while 17 gave the race 3 reaction, and 6 reacted as race 4. In addition there were three other combinations of reactions that were not reported by Golden et al. (3) in the race separation. One of these reactions (a) was represented by five populations, one (b) by two populations, and one (c) by three populations. Thus six groups of reactions were represented, but the race 1 reaction as described by Golden et al. (3) was not observed even though the population (population 13) referred to as race 1 by Golden et al. (3) was included.

The 39 SCN populations were also compared for reproduction on 12 soybean cultivars and lines. The results were analyzed in two ways (Tables 3 and 4). In the first analysis the reproduction of each population on each of the 12 cultivars or lines was compared to its reproduction on 'Lee.' If a population reproduced on a cultivar or line at a level which was ≥ 10% of the reproductive level on 'Lee,' the combination was rated postive (+). If the reproduction ratio was < 10%, then it was rated negative (−).

With this rating scheme, 25 physiological groups were distinguishable (Table 3). In the second analysis (Table 4) each population-host combination was given an index rating based on a comparison with the reproduction on 'Lee.' The index scheme was as follows: 0 = no reproduction; 1 = 1–10% of the reproduction on 'Lee,' 2 = 11–50%; 3 = 51–100%; and 4 = >100%. Under this classification scheme, 36 physiological groups could be distinguished.

Table 2. Separation of 38 populations of *H. glycines* into races based on criteria of Golden et al. (3).

Popu-lation	Origin	Reaction to soybean cultivar*				Race
		Pickett	Peking	P.I. 90763	P.I. 88788	
1. 1	Arkansas	—	—	—	—	3
2. 1A	"	—	—	—	—	3
3. 1B	"	—	—	—	—	3
4. 1C	"	—	—	—	—	3
5. 2	"	+	+	—	+	2
6. 2A	"	+	+	—	+	2
7. 2B	"	+	+	—	+	2
8. 2D	"	+	+	—	+	2
9. 3	Japan	—	—	+	—	a
10. 3AL	"	+	+	+	+	4
11. 3AP	"	+	+	+	+	4
12. 3BL	"	+	+	+	+	4
13. 3BP	"	+	+	—	—	b
14. 4	Tennessee	—	—	—	—	3
15. 4A	"	+	—	—	—	c
16. 5	Louisiana	—	—	—	—	3
17. 6	Virginia	+	—	—	—	c
18. 6A	"	+	+	+	+	4
19. 6B	"	+	+	+	+	4
20. 6C	"	—	—	—	—	3
21. 7	Kentucky	—	—	—	—	3
22. 8	Florida	—	—	—	—	3
23. 9	Mississippi	—	—	—	—	3
24. 10	Missouri	—	—	—	—	3
25. 10A	"	+	+	—	+	b
26. 10B	"	+	+	—	—	3
27. 11	Illinois	—	—	—	—	4
28. 11A	"	+	+	+	+	3
29. 11B	"	—	—	—	—	a
30. 12	Indiana	—	—	+	—	3
31. 12A	"	—	—	—	—	3
32. 13	N. Carolina	—	—	+	—	a
33. 13C	"	—	—	+	—	a
34. 15	S. Carolina	—	—	—	—	3
35. 15A	"	—	—	+	—	a
36. 16	Alabama	—	—	—	—	3
37. 16A	"	—	—	—	—	3
38. 16B	"	+	—	—	—	c

*— = less than 10% of the reproduction on 'Lee'; + = 10% or more of the reproduction on 'Lee.'
†The race numbers correspond in reaction to the races designated by Golden et al. (3). The letters represent reactions which were not known at the time the four races were designated. Populations 1, 2, 6A, and 13 correspond to the populations named 3, 4, 2, and 1 by Golden et al. (3).
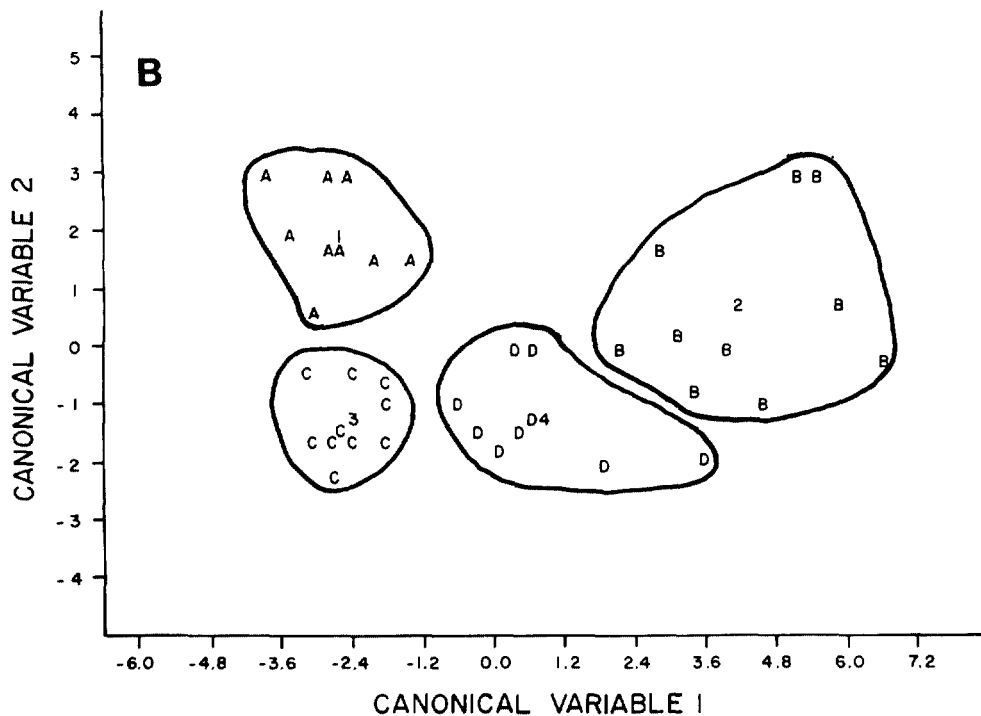
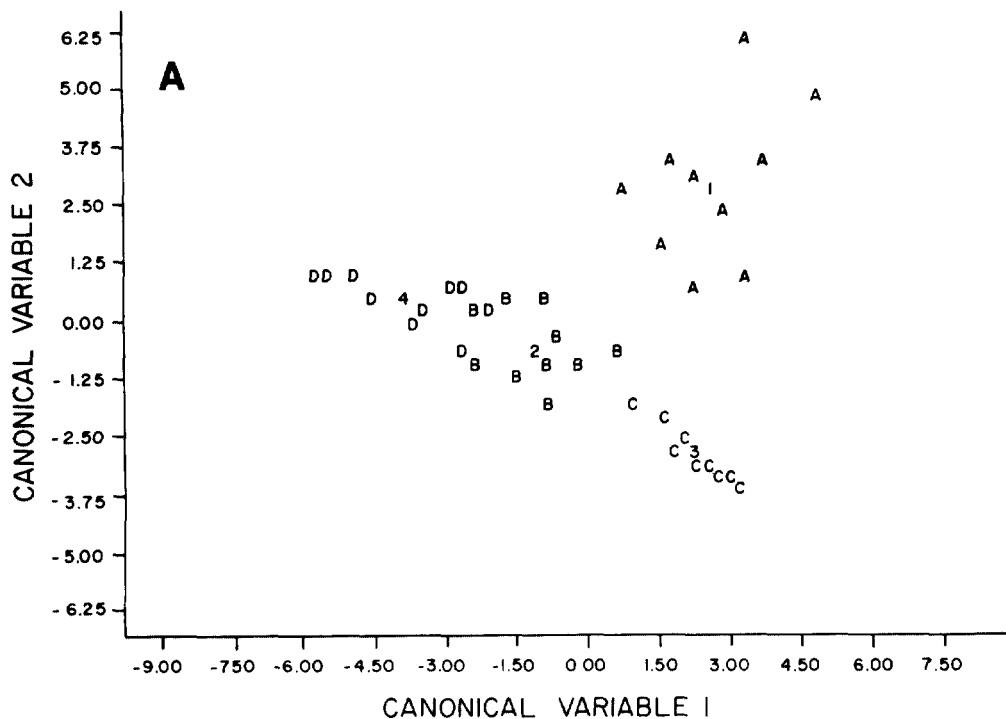Fig. 1. Discriminant function analysis of the mature female indices of host tests with various host combinations. (A) Test of 18 hosts, but program used indices of 'Korean' lespedeza, tomato, 'Pickett' soybean, P.I. 209332 and P.I. 89631-1. (B) A separate test of 18 hosts, but program used indices of 'Kobe' lespedeza, 'Pickett' soybean, 'Custer,' P.I. 79693, P.I. 90763 and P.I. 91684. *(Continued on next page.)*
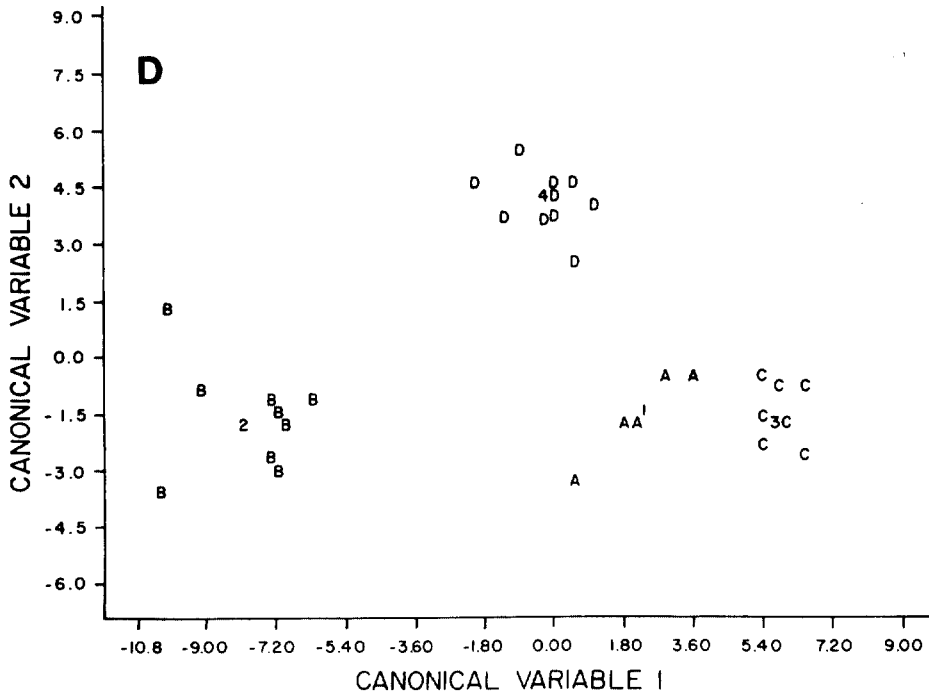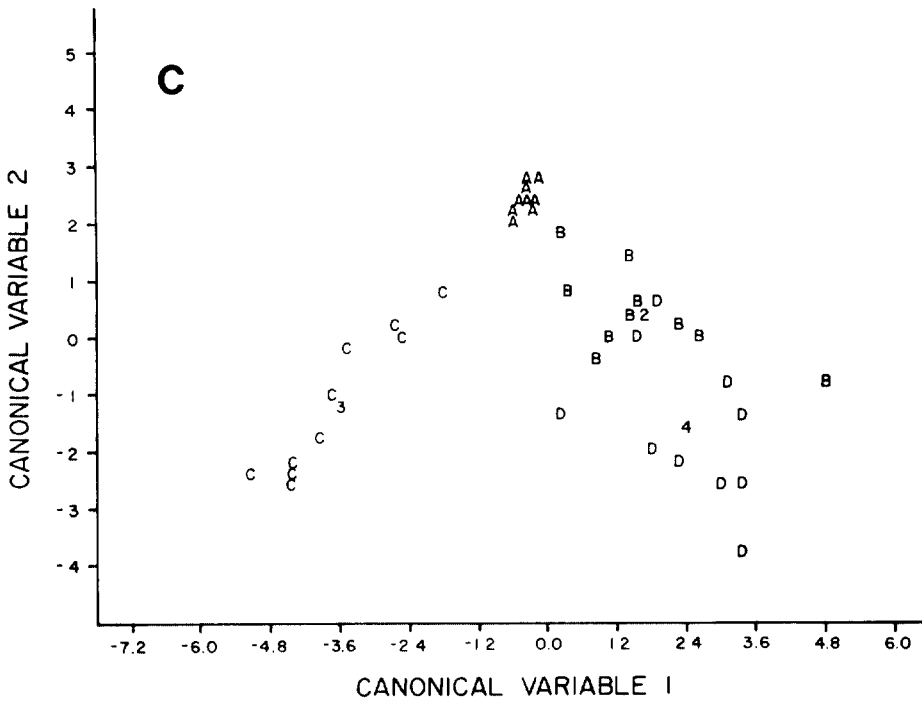
Fig. 1 *(continued)*. (C) Analysis of indices from test A using data from 13 soybean lines and two lespedeza species. (D) Analyses of a third test using the 13 soybean lines and two lespedeza species.
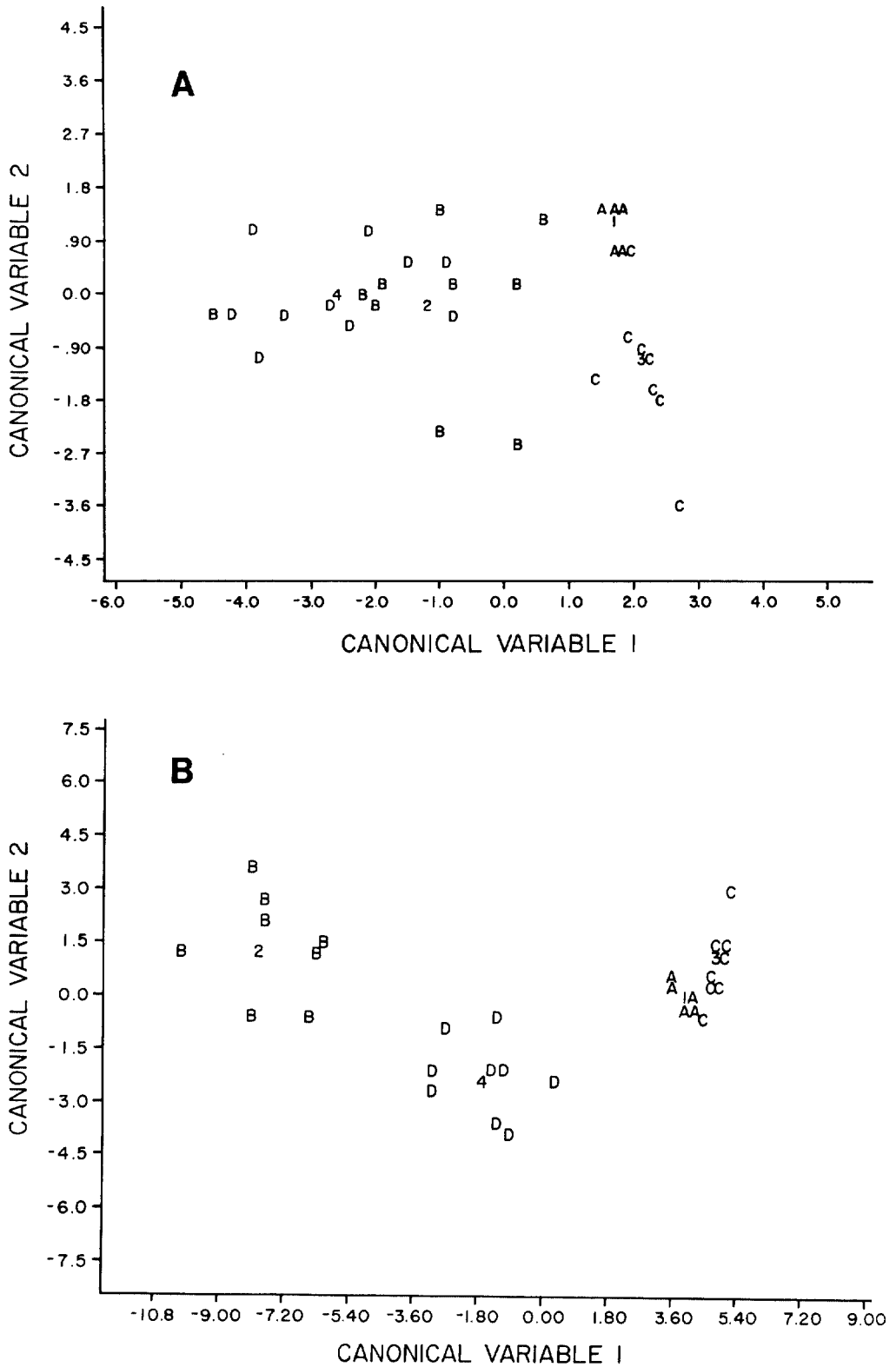
Fig. 2. Discriminant function analysis of mature female indices from two tests (A, B) involving the soybean varieties used as differentials (3).

Table 3. Separation of 38 populations of *H. glycines* into groups based on reproduction on 12 soybean cultivars and lines.*

Popu-lation	Reaction to soybean cultivars and lines†												Group
	Pi	Pe	OD	PD	Cu	79	84	87	88	90	91	20	
1. 1‡	—	—	+	+	—	+	+	—	—	—	+	—	1
2. 1A	—	—	—	+	—	+	+	+	—	—	+	—	2
3. 1B	—	—	+	+	—	+	+	—	—	—	+	—	1
4. 1C	—	—	+	—	—	+	+	—	—	—	+	—	3
5. 2	+	+	+	+	+	+	+	—	+	—	+	—	4
6. 2A	+	+	+	+	+	+	+	—	+	—	+	—	4
7. 2B	+	+	+	+	+	+	+	—	+	—	+	—	4
8. 2D	+	+	+	+	+	—	+	+	+	—	+	—	5
9. 3	—	—	+	+	—	+	+	+	—	+	+	+	6
10. 3AL	+	+	+	+	+	+	—	+	+	+	+	—	7
11. 3AP	+	+	+	—	+	+	—	+	+	+	+	—	8
12. 3BL	+	+	+	+	+	+	+	+	+	+	+	—	9
13. 3BP	+	+	—	+	+	—	+	—	—	—	+	—	10
14. 4	—	—	—	+	—	+	+	—	—	—	+	—	11
15. 4A	+	—	+	+	+	+	+	—	—	—	+	—	12
16. 5	—	—	+	+	—	+	+	—	—	—	+	—	1
17. 6	+	—	—	+	—	—	+	—	—	—	+	—	13
18. 6A	+	+	+	+	+	+	+	+	+	+	+	—	14
19. 6B	+	+	+	+	+	+	+	+	+	+	+	—	14
20. 6C	—	—	—	+	—	—	—	—	—	—	+	—	15
21. 7	—	—	+	+	—	+	+	+	—	—	+	—	16
22. 8	—	—	—	+	—	—	—	—	—	—	+	—	15
23. 9	—	—	+	+	—	+	+	—	—	—	+	—	1
24. 10	—	—	+	+	—	+	+	+	—	—	+	—	16
25. 10A	+	+	—	—	+	+	+	—	+	—	+	—	17
26. 10B	+	+	+	+	—	—	+	—	—	—	+	—	18
27. 11	—	—	+	+	—	—	+	+	—	—	+	—	19
28. 11A	+	+	+	+	+	+	+	+	+	+	+	—	14
29. 11B	—	—	+	+	—	+	+	—	—	—	+	—	1
30. 12	—	—	+	+	—	—	+	+	—	+	+	—	20
31. 12A	—	—	+	+	—	+	+	—	—	—	+	—	19
32. 13	—	—	+	+	—	+	+	+	—	+	+	+	6
33. 13C	—	—	+	+	—	+	—	+	—	+	+	+	21
34. 15	—	—	+	+	—	—	—	+	—	—	+	—	22
35. 15A	—	—	+	+	—	—	—	+	—	+	+	—	23
36. 16	—	—	—	+	—	—	+	—	—	—	+	—	24
37. 16A	—	—	+	+	—	+	+	—	—	—	+	—	1
38. 16B	+	—	+	+	—	—	+	+	—	—	+	—	25

*Pi = 'Pickett,' Pe = 'Peking,' OD = 'Old Dominion,' PD = 'Pine Dell Perfection,' Cu = 'Custer,' 79 = P.I. 79693, 84 = P.I. 84611, 87 = P.I. 87631-1, 88 = P.I. 88788, 90 = P.I. 90763, 91 = P.I. 91684, 20 = P.I. 209332.

†+ = number of females recovered/pot equal to or greater than 10% of the number recovered from 'Lee.' — = less than 10% of the number on 'Lee.'

‡For origin of populations see Table 2.

DISCUSSION

Results of studies with the four races of SCN appear to indicate that this nematode is extremely variable. The average reaction of a population can be characterized at a given time by host tests. The reaction, however, is not stable and may change during the course of maintenance for inoculum production.

Miller (6) demonstrated that different races of the SCN could be recovered from the same field and that numerous physiological groups could be distinguished (10). While the four races of SCN described by Golden et al. (3) are useful in designating certain similar populations from different areas, the introduction of additional cultivars resistant to race 4 has increased the awareness that this nematode is considerably more variable than that previously de-

Table 4. Separation of 38 populations of *H. glycines* into groups based on relative number of females recovered per pot.

Popu-lation	Pi	Pe	OD	PD	Cu	79	84	87	88	90	91	20	Group
1. 1†	1‡	1	2	2	1	2	2	1	1	1	4	1	1
2. 1A	1	0	1	3	0	3	2	2	1	1	4	1	2
3. 1B	1	1	2	2	1	2	2	1	1	1	4	1	1
4. 1C	1	0	2	1	0	3	2	1	1	0	2	0	3
5. 2	3	3	2	2	3	3	3	1	2	1	3	1	4
6. 2A	4	3	2	2	2	2	2	1	2	1	2	1	5
7. 2B	4	2	3	4	3	4	2	1	2	0	4	1	6
8. 2D	2	3	2	3	3	1	2	2	2	1	4	1	7
9. 3	1	1	3	3	1	2	3	3	1	2	3	2	8
10. 3AL	3	2	3	3	2	3	1	4	3	3	4	1	9
11. 3AP	4	2	2	1	3	2	1	3	3	2	3	1	10
12. 3BL	2	2	2	2	2	2	2	2	2	2	3	1	11
13. 3BP	4	2	1	2	3	1	2	1	1	1	4	1	12
14. 4	1	1	1	2	1	3	2	1	1	1	2	1	13
15. 4A	4	1	2	2	2	2	2	1	1	1	3	1	14
16. 5	1	0	2	2	1	2	2	1	0	1	4	1	15
17. 6	2	1	1	2	1	1	2	1	1	1	3	1	16
18. 6A	4	2	2	3	3	3	3	2	2	2	3	1	17
19. 6B	3	2	2	2	2	3	3	2	2	2	4	1	18
20. 6C	1	1	1	2	0	1	1	1	1	1	2	1	19
21. 7	1	1	2	3	1	2	2	2	1	1	3	1	20
22. 8	1	1	1	2	1	1	1	1	1	1	3	1	21
23. 9	1	0	2	2	1	2	2	1	1	1	3	1	22
24. 10	1	1	2	2	1	2	2	2	1	1	2	1	23
25. 10A	2	2	1	1	2	2	2	1	2	1	2	1	24
26. 10B	4	2	2	2	1	1	2	1	1	1	4	1	25
27. 11	1	0	2	2	1	1	3	2	1	1	4	1	26
28. 11A	4	3	3	2	3	2	2	2	2	2	4	1	27
29. 11B	1	0	2	2	1	2	2	1	1	1	2	1	22
30. 12	1	0	2	2	1	1	2	2	1	2	3	1	28
31. 12A	1	1	2	22	1	1	2	2	0	1	2	1	29
32. 13	1	0	3	3	1	2	3	2	1	2	4	2	30
33. 13C	1	1	2	3	1	2	1	2	1	4	4	2	31
34. 15	1	1	2	4	0	1	1	2	0	1	2	1	32
35. 15A	1	1	2	3	1	1	0	2	1	2	3	1	33
36. 16	1	1	1	2	1	1	2	1	1	1	2	1	34
37. 16A	1	1	2	3	1	3	2	1	1	1	3	1	35
38. 16B	2	1	2	3	1	1	3	2	1	1	4	1	36

*Pi = 'Pickett,' Pe = 'Peking,' OD = 'Old Dominion,' PD = 'Pine Dell Perfection,' Cu = 'Custer,' 79 = P.I. 79693, 84 = P.I. 84611, 87 = P.I. 87631-1, P.I. 88788, 90 = P.I. 90763, 91 = P. I. 91684, 20 = P. I. 209332.

†For origin of populations see Table 2.

‡0 = 0 index units, 1 = 1-10, 2 = 11-50, 3 = 51-100, 4 = 100+.

scribed (3). Work on the inheritance of resistance to SCN (17,18), and on the inheritance of the parasitic capabilities of the nematode (11), further supported these conclusions. Thomas (17) studied the inheritance of resistance to the four races of SCN and proposed that at least 10 genes were involved. When the inheritance of resistance is this complicated, it indicates a high degree of variability in the pathogen. Price (11) studied the inheritance of parasitic capabilities in SCN races. He proposed that a multiple allelic series of genes was involved and that there was a possibility of complementary effects. He did not determine how many alleles were involved but proposed that extreme variation was possible under these conditions. There appeared to be an extremely wide range of parasitic capabilities with small increments of variation over the entire range, depending on how many criteria (host differential or

index combinations) were used in separating the variants. Therefore, the number of distinguishable groups depended upon the criteria applied.

The separation of SCN into four races by Golden et al. (3) provided a convenient reference point for breeding work and other studies. However, the present studies show that more than four races exist even when the original differentials are used. To continue to refer to only four races is impractical and inaccurate. However, the expansion to 25 or more races would complicate the breeding program for resistance to SCN so much as to be prohibitive. A procedure which would provide a measure of the variability (races) and still be practical could be designed. For example, as cultivars are introduced with new levels of genes for resistance, these could be added to the differential list and SCN populations which reproduce on these cultivars would be designated as races. Under the present situation, Bedford would become a differential and those populations which reproduce on Bedford would be called race 5. When resistance to race 5 is introduced, this would become a differential. The best procedure for determining SCN races should be decided by a group of five to seven nematologists and plant breeders who have been involved in SCN work.

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