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Variation in Resistance of Soybean Lines to Races of *Heterodera glycines*[1]

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Abstract: The objective of this study was to determine the interrelationships of *Heterodera glycines* races based on their resistance to soybean (*Glycine max*) cultivars and lines against which they were tested. Greenhouse tests determined the numbers of females of each of eight races of *H. glycines* that developed on 277 to 522 soybean cultivars and lines. A Female Index (number of females on a test cultivar as a percentage of the number on 'Lee 74') was calculated and used in frequency distributions, correlations, and cluster analyses of the resistance reactions to the different races in an attempt to determine relationships among cultivars. Frequency distribution patterns of all cultivars and lines tested against each race were skewed in favor of resistance, and in some cases bimodality was observed. The majority of correlations between pairs of races were highly significant. Cluster analyses based on the correlations divided eight races into four clusters that explained 73% of the variation in resistance. Cluster 1 was comprised of races 2, 4, and 14; Cluster 2 was comprised of races 6 and 9; Cluster 3 was comprised of races 1 and 3; and Cluster 4 was comprised of race 5. The information obtained in this study could increase the efficiency of testing resistant soybean breeding lines for resistance to *H. glycines*.

Key words: Cluster analysis, *Glycine max, Heterodera glycines,* nematode, resistance, soybean, soybean cyst nematode, virulence group.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, causes more yield reduction of soybean, *Glycine max* (L.) Merr., in the United States than any other disease (Doupnik, 1993; Zhang et al., 1992). Use of genetic resistance is the major method for limiting yield losses due to this nematode (Epps et al., 1981).

Selection pressure results in development of virulent races of SCN and often compromises cultivars with race-specific resistance within a few years. Riggs and Schmitt (1988) identified 16 possible races of SCN based on relative female maturation on four differential soybean lines. However, breeding resistant cultivars for all 16 races is not necessary because some races occur much more frequently than others in the United States (Anand et al., 1994; Kim et al., 1997); eight of the races are seldom found in soybean fields in the United States.

Development of race-specific resistant soybean cultivars is a labor-intensive and time-consuming process. Many progeny derived from a cross must be screened to obtain a few resistant plants. These resistant plants can be identified only by a bioassay in which they are grown in soil infested with SCN. After ca. 30 days, females and cysts are extracted from the roots and soil, and counted. Hundreds of soybean plants must be screened to find one that is resistant to just one race, and the process must be repeated for each race.

A soybean field is infested with only one race of *H. glycines,* even though multiple parasitic genotypes are present. If more than one sample is taken from a field, the nematode population in each sample may be a different race, but the race in the field is a composite of all the genotypes in all the samples. Planting cultivars with different sources of resistance in different areas of the field is not practical. Therefore, a race test of the composite population from the field provides a guide to the type of resistance needed to suppress damage by the nematodes present. The race of SCN in a field should be determined before a race-specific resistant cultivar is recommended. Cultivars with resistance to several races may reduce

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chances of race shifts in the SCN population. Similarly, use of tolerant cultivars may help attain normal yields in the presence of SCN without inducing race shifts (Riggs and Schmitt, 1988).

Results from several genetic studies (Caviness, 1992; Hancock et al., 1987; Mansur et al., 1993) indicate that genes for resistance to some races of SCN may be linked to one another and that several genes for resistance may be alleles at the same locus. For example, resistance genes to races 4 and 5 in the soybean line J81-116 apparently are linked (Hartwig and Young, 1986). The objective of this study was to determine interrelationships among races of SCN relative to resistance in available soybean cultivars in order to determine whether a more efficient method of testing for resistance could be developed.

Materials and Methods

Screening procedure: During 1991–94, 524 soybean cultivars and breeding lines, mainly in Maturity Groups V–VII, were examined for resistance to *H. glycines.* Although a total of 524 cultivars and breeding lines were tested, not all were tested against every race. Not all races were tested at the same time, and, in any given test, seeds of a particular cultivar or line either might not have germinated or seed numbers may have been insufficient for all tests. Races 1–6, 9, and 14, which are found most frequently in soybean fields in the central United States (Anand and Rao-Arelli, 1989; Luedders, 1989), were used in these experiments. Race 6 was the most difficult race to maintain, and the fewest cultivars and lines were tested against it. Seeds of soybean lines were received from various sources, germinated in vermiculite, and transplanted into fine sandy soil in pots. The soil then was infested with eggs and second-stage juveniles (J2) of the appropriate race (Riggs et al., 1991). Eggs and J2 were obtained from stock cultures maintained in a greenhouse at the University of Arkansas. The four differential host soybean cultivars and lines, Pickett, Peking, PI88.788, and PI90.763 (Riggs and Schmitt, 1988), were included in each experiment, and when a test required more than one greenhouse bench they were included on each bench, to confirm race identity. Each soybean line was replicated five times for each race with one plant per replication. The number of females produced on each soybean line was converted to a Female Index (FI), defined as the number of females that developed on a soybean line expressed as a percentage of the number that developed on the susceptible cultivar, Lee 74. The average FI of each soybean line was used in statistical comparisons.

Analysis: The frequency distributions of FIs of all races tested on all cultivars and lines were depicted with notched boxplots (McGill et al., 1978) (Fig. 1). The notched boxplot shows the mean (asterisk) and the median with an approximate 95% confidence interval and mild and extreme outliers. The box width is proportional to the sample size. The notched boxplot is used instead of the regular boxplot to show an approximate 95% confidence interval for the location of the population median by notches on the side of the box. Two races, the notches of which do not overlap, are considered significantly different in their central values. The notches are calculated as the median ± 1.58 (InterQuartile Range [IQR]/$\sqrt{n}$, where IQR is the middle 50% of the range and n is the number of entries) to give an approximate 95% comparison of the median (Velleman and Hoaglin, 1981).

A correlation matrix between pairs of the eight races was generated with the SAS CORR procedure (SAS version 6.12, SAS Institute, Cary, NC). These correlations were used in the SAS VARCLUS procedure to divide races into groups in a tree diagram.

Results and Discussion

Frequency distribution: The frequency distribution patterns for FI differed among races (Table 1). Lines resistant (FI <10%) to race 3 were the most abundant (48.7%, 254 of 522), and resistant lines were least frequent with races 4 and 2 (0.6%, 2 of 309 and 3.0%, 12 of 396, respectively). The skewedness of

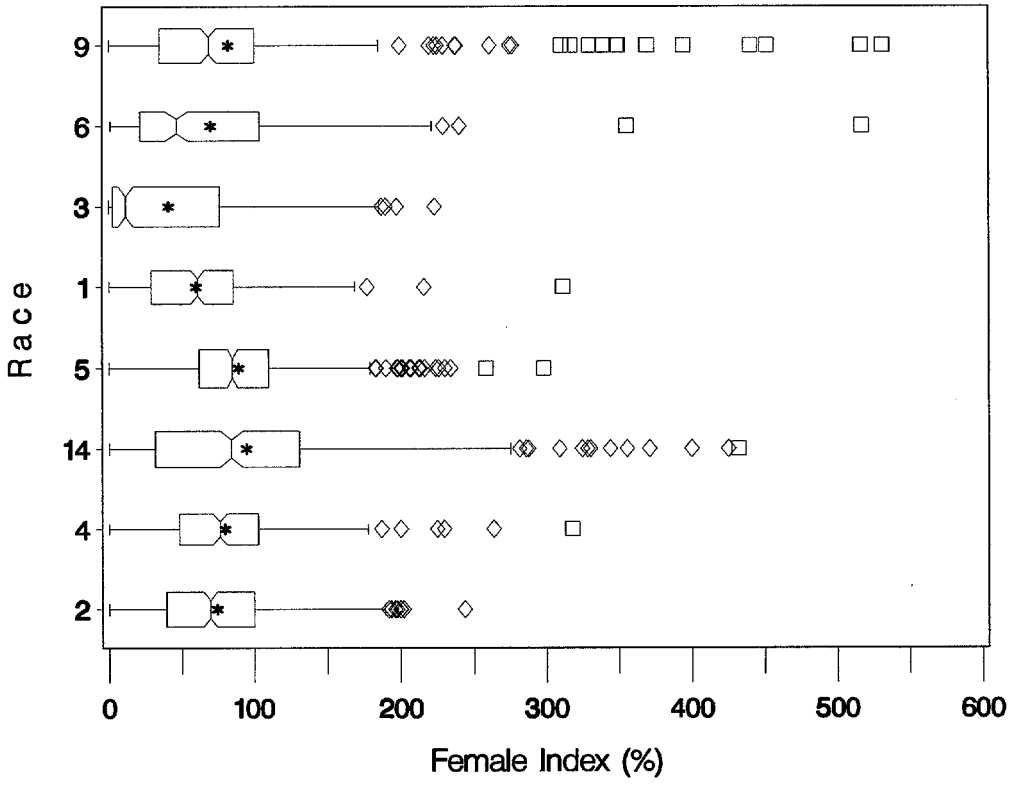

FIG. 1. Relative maturation of eight races of *Heterodera glycines* on soybean cultivars. The notched boxplot graphically represents the entire range of Female Indices (FIs) obtained by testing large numbers of soybean cultivars against the *H. glycines* races. The width of the box represents the relative number of cultivars and lines tested, the asterisk indicates the mean FI of all entries, and the notch marks the median FI from all tests within a race. The line through the box represents the population and the diamonds and squares represent different levels of outliers. The notches are calculated as median ± 1.58 (InterQuartile Range/$\sqrt{n}$ [n = number of entries]) (Velleman and Hoaglin, 1981).

TABLE 1. Summary statistics associated with frequency distribution of the Female Indices[a] of 524 soybean cultivars and lines tested against eight races of *Heterodera glycines*.

	Race							
Factors[b]	1	2	3	4	5	6	9	14
n	365	396	522	309	464	277	380	424
Mean	60.1	74.7	41.2	80.1	89.2	70.2	84.1	98.4
SD	40.9	43.0	47.2	45.0	43.8	65.9	82.6	95.3
CV (%)	68.1	57.6	114.7	56.2	49.1	93.9	98.2	96.9
Skewedness	0.95	0.78	0.99	1.28	0.96	2.02	3.49	3.65
Kurtosis	3.50	0.57	0.09	3.65	1.90	7.88	18.14	27.20
Median	60.9	69.65	11.55	76.3	84.95	46.7	69.3	84.65
IQR	311.3	243.7	223.0	317.8	298.0	516.7	762.3	1,048.0

[a] Female Index is the number of females developing on a soybean line expressed as a percentage of the number developing on the susceptible cultivar, Lee 74.

[b] Skewedness is a measure of the asymmetry of the frequency distribution for each race. Kurtosis is a measure of the extent to which the frequency distribution (curve) is "peaked"; that is, the extent of the relative steepness of the ascent in the vicinity of the mode. IQR or InterQuartile Range is the distance between the upper and lower quartiles.

the resistance distribution varied among nematode races (Table 1; Fig. 1). With race 1, the IQR had FIs between 29.0 and 85.7 with a median of 60.9; with race 2, the IQR had FIs between 39.5 and 100.0 with a median of 69.6; with race 3, the IQR fell between FIs of 2.6 and 76.2 with a median of 11.5; with race 4, the IQR fell between FIs of 48.4 and 102.7 with a median of 76.3; with race 5, the IQR fell between FIs of 62.0 and 109.8 with a median of 84.9; with race 6, the IQR fell between FIs of 21.6 and 103.6 with a median of 46.7; with race 9, the IQR fell between FIs of 35.0 and 100.0 with a median of 69.3; with race 14, the IQR fell between FIs of 31.6 and 131.4 with a median of 84.6 (Table 1; Fig. 1). The frequency distribution pattern was skewed in favor of resistance to race 3. Analysis-of-frequency distribution of FIs with Shapiro-Wilk W-statistics indicated a significant deviation from normality with every race except race 5. The frequency distributions also showed continuous variation, typical of multiple minor gene action.

Correlation analyses: The number of soybean lines used to generate correlation matrices was 109 (between races 4 and 6) to 463 (between races 3 and 5) because not all lines were tested against all races (Table 2). Most of the pairwise correlations of FIs between races were low but significant ($P < 0.05$), and all significant correlations were positive (Table 2). Race 5 was the least correlated with other races, whereas races 1 and 3 were significantly correlated with all other races tested (correlation coefficients between 0.141 and 0.486). Race 5 is characterized by having virulence genes for Pickett and PI88.788, whereas race 1 has virulence genes for PI88.788 only and race 3 has no genes for virulence on any resistant cultivar or line.

Cluster analysis: The PROC VARCLUS procedure divided the FIs of the eight races into clusters of 1 to 8. When all races were clustered together in one cluster, only about 31% of the variation was explained; when each race was in a separate cluster, 100% of the variation was explained. Four clusters (Cluster 1 = races 2, 4, 14; Cluster 2 = races 6, 9; Cluster 3 = races 1, 3; Cluster 4 = race 5) provided a good summary of data and accounted for 73% of the variations, five clusters accounted for 82%, and six clusters accounted for 90%.

When four clusters were used to represent the variation, Cluster 1 (races 2, 4 and 14) accounted for the highest proportion of

TABLE 2. Correlation coefficients (upper numbers) and number of comparisons (lower numbers) between Female Indices[a] of pairs of eight races of *Heterodera glycines* tested on 277 to 522 soybean lines.

	Race							
Race	1	2	3	4	5	6	9	14
1	1.000							
	365							
2	0.198*	1.000						
	266	396						
3	0.486*	0.236*	1.000					
	364	396	522					
4	0.158*	0.310*	0.260*	1.000				
	268	231	308	309				
5	0.146*	0.177*	0.141*	0.177*	1.000			
	364	337	463	309	464			
6	0.161*	0.100*	0.467*	0.195*	0.049	1.000		
	198	229	277	109	219	277		
9	0.157*	0.189*	0.196*	0.108*	0.073*	0.514*	1.000	
	340	255	380	286	381	201	381	
14	0.415*	0.266*	0.237*	0.127*	−0.014	0.186*	0.192*	1.000
	269	350	426	250	368	235	286	426

[a] Female Indices (the number of females developing on a soybean line expressed as a percentage of the number developing on the susceptible cultivar, Lee 74) of each race on each resistant soybean line were used to generate the correlation matrix.

variation (55.6%). Of the 220 lines tested against all three races, 19 lines were resistant to race 14, 4 were resistant to race 2, and 1 was resistant to race 4 as well as races 2 and 14. Cluster 2 (races 6 and 19) represented 24.7% of the variation. Fewer soybean lines were resistant to race 6 or 9. Of the 144 lines tested against both races, 7 lines were resistant to race 6, and 19 lines were resistant to race 9; 7 lines were resistant to both races. All seven lines resistant to race 6 were also resistant to race 9. Cluster 3 (races 1 and 3) accounted for the lowest proportion of variation (0.1%). This group was tested against many soybean cultivars and lines that were resistant to both races. Of the 365 lines tested against both races, 53 lines were resistant to race 1 and 171 lines were resistant to race 3; 49 lines were resistant to both races (Table 3). Cluster 4 had only race 5, and it explained 19.6% of the variation. Only 5 of the 463 lines tested were resistant to race 5.

The VARCLUS procedure performs hierarchical clustering of variables based on a correlation matrix (all variables are treated as equally important). Therefore, VARCLUS can be used to reduce the number of variables. The clusters are chosen to maximize the variation represented by the first principal component of each cluster. The first principal component is a weighted average of the variables that explains as much variance as possible. It also attempts to divide a set of numeric variables into nonoverlapping clusters in such a way that each cluster can be interpreted as essentially unidimensional. Cluster component scores and tree diagrams of hierarchical clusters show variations within clusters. Variable cluster analysis has been used to identify virulence groups among races of various pathogens (Chen et al., 1993; Zhang et al., 1992).

The VARCLUS oblique component analysis of the FI of all of the cultivars and lines tested against the respective races of *H. glycines* is shown in the tree diagram (Fig. 2). A given number of cluster components does not generally explain as much variance as the same number of principal components in the full set of variables, but the cluster components are usually easier to interpret than the principal components, even if the latter are rotated. By default, VARCLUS begins with all variables in a single cluster. It then repeats the following steps: (i) a cluster is chosen for splitting; (ii) the chosen cluster is split by finding the first two principal components, performing an oblique rotation and assigning each variable to the rotated component with which it has the higher squared correlation; and (iii) variables are iteratively reassigned to clusters to maximize the variance represented by the cluster components. Races 6 and 9 were the first to be grouped together followed by races 1 and 3, then races 2 and 4. Race 14 was closely related to the last group, and race 5 was not closely related to any other group but was closest to race 4.

Races 6 and 9, which formed Cluster 2, both parasitize the soybean cv. Pickett; race 9 also parasitizes cv. Peking, which was the

TABLE 3. Number of soybean lines in each category of phenotype susceptibility for each race of *Heterodera glycines*.

	Susceptibility level[a]			
	R	MR	MS	S
Race 1			Race 3	
R	49	3	0	1
MR	30	5	2	5
MS	51	6	10	18
S	41	16	21	105
Race 2			Race 4	
R	2	2	1	3
MR	0	5	8	12
MS	0	11	16	30
S	0	9	27	105
Race 4			Race 14	
R	1	0	0	0
MR	1	0	2	4
MS	3	5	7	14
S	17	18	18	50
Race 6			Race 9	
R	7	0	0	0
MR	8	16	5	6
MS	4	3	12	14
S	0	0	19	50
Race 2			Race 14	
R	2	0	1	6
MR	8	7	5	22
MS	10	23	11	55
S	13	14	25	139

[a] R = resistant (FI = 0–9%), MR = moderately resistant (FI = 10–30%), MS = moderately susceptible (FI = 31–60%), S = susceptible (FI > 60%).

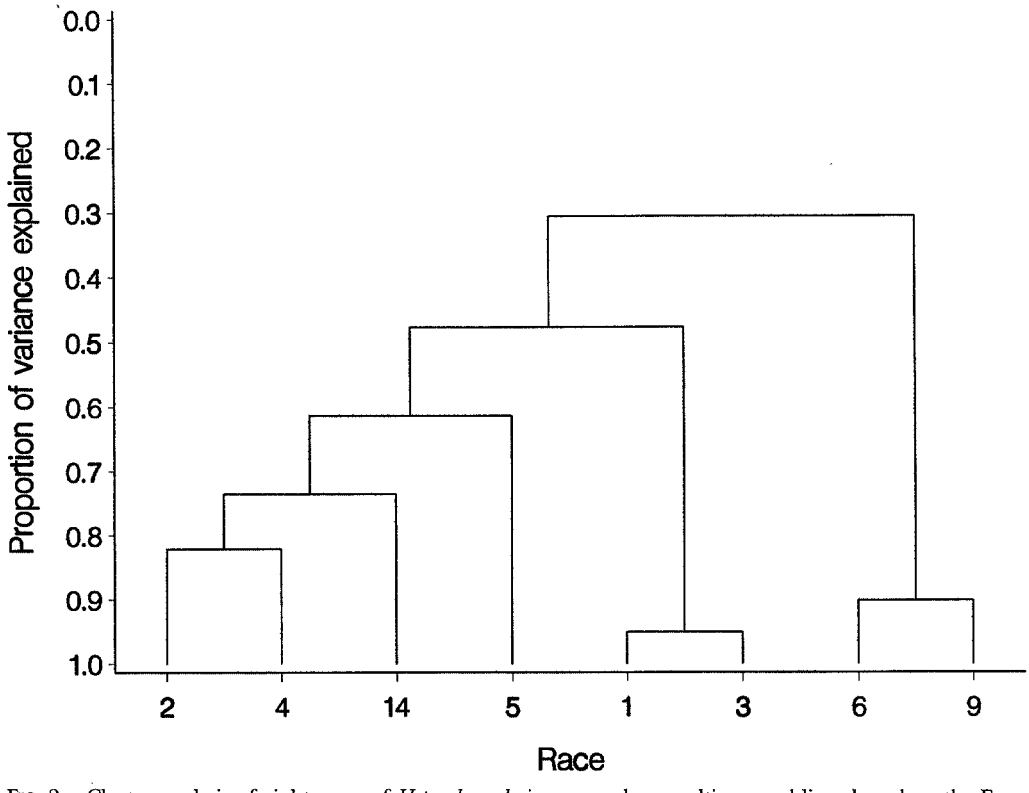

FIG. 2. Cluster analysis of eight races of *Heterodera glycines* on soybean cultivars and lines based on the Female Index (number of females on a cultivar or line ÷ number of females on Lee 74 × 100).

source of resistance for Pickett. Races 1 and 3, forming cluster 3, differ only in the ability to parasitize PI88.788. Race 3 differs from all other races in that it does not parasitize any of the differentials. Race 6 differs from race 3 only in that it parasitizes Pickett. Races 2, 4, and 14 in cluster 1 all parasitize Pickett and Peking; race 2 does not parasitize PI90.763, and race 14 does not parasitize PI88.788, both of which are parasitized by race 4. Race 5 represents a separate cluster or is an outlier in that it parasitizes Pickett, with resistance from Peking, and PI88.788, which represents a different mechanism of resistance (Kim et al., 1987). Resistance in Peking is a hypersensitive type that is associated with considerable tissue necrosis, whereas resistance in PI88.788 results in a breakdown of the nuclear envelope but with little or no tissue necrosis. Cluster analysis placed 20 races of *Puccinia striiformis* in six virulence clusters, which agreed closely with virulence, chronological appearance, and geographic distribution of the races (Chen et al., 1993).

The efficiency of testing soybean lines for resistance to multiple races of *H. glycines* could be increased by screening for resistance to the races in a particular order. Considering the soybean lines used in our study, if the cultivars and lines that were screened against races 1 and 3 (Cluster 3) were screened first against race 1, 53 of them would have been selected as resistant to race 1; and of the 53 lines, 92% (49 lines) would be resistant to race 3 (Table 3). If the screening order were reversed, however, the efficiency would be much lower: 171 lines would be selected as resistant to race 3; then, of the 171 lines, 29% (49 lines) would be resistant to race 1. Consequently, screening with race 1 first would result in a reduction in the number of soybean lines that would need to be screened against race 3, thus increasing the efficiency. This screening order would mean that the race 3 resistance in 122

of the lines would remain unknown, a situation that might not be important in the southern states where race 3 is scarce, but could be important in the northern states where race 3 predominates.

Among the cultivars and lines that were screened against races 6 and 9 (Cluster 2), all 7 lines resistant to race 6 were also resistant to race 9, but only 7 of 19 lines resistant to race 9 were resistant to race 6. In contrast, among the cultivars and lines that were screened against races 2, 4, and 14 (Cluster 1), the order would not be as important. Only two cultivars or lines resistant to race 4 and 9 were resistant to race 2, whereas 33 were resistant to race 14. Screening race 4 first would delete potentially important lines resistant to races 2 and 14. Race 2 could be screened first because both lines resistant to race 4 were also resistant to race 2, and fewer lines would need to be screened against race 14. Consequently, the suggested order of screening is race 1, 3, 6, 9, 2, 14, 4, and 5.

Our results demonstrate that host resistance levels are correlated among some races of SCN. Races are differentiated by hosts that carry different genes for resistance. Thus, assuming gene-for-gene correspondence, such correlations suggest close associations of genes for resistance in the soybean lines.

Use of an intermediate level of resistance may prevent economic losses while reducing the chance of race shifts in the SCN population. This approach would be similar to the use of tolerant lines as recommended by Reese et al. (1988). Therefore, in order to develop soybean lines with resistance to as many races as possible, it may be important to consider intermediate levels of resistance as well as high levels of resistance. For example, Peking is classified as susceptible to race 4, but it is much more resistant than Lee or Pickett (Hancock et al., 1987). Because few lines are available that have FI < 10 to race 4 or to race 2, consideration should be given to using lines that have moderate resistance.

In practice, in the northern United States where races 1 and 3 predominate (Anand et al., 1994; Luedders, 1989), lines should be screened against races 1 and 6 first, then against races 3 and 9, with only lines resistant or moderately resistant to races 1 and 6 being screened against races 3 and 9, respectively. In contrast, in the southern states where races 2, 4, 5, 6, 9, and 14 comprise 87% of the SCN populations (Luedders, 1989), initial screening should be against races 6 and 2, then resistant and moderately resistant lines should be screened against races 14, 4, and 5. Although many lines are resistant to races 3 and 6, little benefit would be derived from selecting them because 90% of the SCN populations in the southern United States have virulence genes for Pickett (Anand et al., 1994; Luedders, 1989). The procedure proposed in this study would be especially valuable for those who are screening many soybean breeding lines against several races of SCN. For example, if 1,000 lines are to be screened against six races of SCN with the usual three replications, the total number of pots would be 18,000. Several months, large areas of greenhouse space, and hundreds of working hours of skilled technicians would be required. With the above recommended procedure, if the 1,000 lines were first screened against race 1, only about 14%, or 140 lines, would be screened against race 3. Screening against race 3 would require only 420 pots rather than 3,000, for a total of 3,420 rather than 6,000, or a 43% reduction. Similar reductions would occur with the other clusters of SCN races.

Soybean lines used in this study were not collected randomly but were breeding lines from various seed companies and public breeders actively seeking resistance to SCN in their programs. Consequently, the results could be biased because they used common sources of resistance. Peking, because it has agronomic characteristics that are superior to those of most *H. glycines*-resistant lines, is used widely as a resistant parent in many breeding programs. Resistance from Peking was found in a yellow-seeded SCN-resistant cultivar early in the breeding program to provide resistant cultivars to use in *H. glycines*-infested soil (Brim and Ross, 1966). The race groups and frequency distribution

patterns postulated in this study should be reconfirmed whenever new resistance sources are used extensively in a breeding program. The information obtained in this study should be valuable in developing cultivars with multiple resistance to many races of SCN.

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