

Use of Statistics in Race Determination Tests

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, has been separated into 16 races (3,5,8). Separation is based on female or cyst (referred to as cyst) formation by SCN populations on four soybean (*Glycine max* (L.) Merr.) genotypes—'Peking', 'Pickett', PI 88788, and PI 90763—relative to reproduction on 'Lee' (susceptible) soybean. A soybean differential has a positive reaction (cyst index) to the test SCN population if the number of cysts on it equals or exceeds 10% of the number on Lee; otherwise, the reaction is negative. Often the reaction is given without numerical means and variances. The variation in number of cysts among replications of each differential, including the susceptible cultivar Lee, may be considerable. The index may be near 10 on some differentials.

The procedural steps of index calculation are often not given. Mean of a differential may be simply divided by mean of Lee, or each replication of the differential may be divided by mean of Lee. A variance for the index in the latter procedure could be determined, allowing determination of significance of the difference between the index measured and critical index value of 10. The highest number of cysts obtained on Lee among all replications (3) has been used as the divisor instead of the mean number of cysts in some studies on inheritance of SCN resistance in soybean. This has the potential of greatly altering the index value. Variation in various calculations

of cyst index on soybean differentials used for race determination of SCN was measured in this study for three soybean cultivar-SCN population combinations (Peking-TN4, Peking-TNVA, and PI 90763-TN4).

For each combination, infested soil was mixed in a cement mixer and used to fill 7.5-cm-d pots, and 100 replications of the soybean cultivar and Lee soybean were grown in a randomized complete block design. Thirty-five days after planting, white and yellow females in each pot were extracted by elutriation (1) and counted. Reaction of the two SCN populations on all the race determination differentials are given in Table 1.

Replication index and mean index were 100 times the number of cysts on a differential divided by number of cysts on Lee in each replication and by the mean number of cysts on Lee, respectively. Indices were calculated for sample sizes of $n = 1, 2, 4, 5, 10, 20, 25, 50,$ and 100 replications by sampling without replacement from the 100 replications of data for each soybean cultivar-SCN combination until all replications were used. Five randomizations of sampling were made when $n > 5$, and

TABLE 1. Mean cyst indices† followed by 95% confidence intervals of two soybean cyst nematode populations on five soybean cultivars.

Soybean	TN4	TNVA
Lee‡	198.5 ± 38.3	279.5 ± 65.0
Pickett 71	98.7 ± 23.4	87.1 ± 25.4
Peking	20.0 ± 5.5	11.5 ± 4.2
PI 88788	9.3 ± 4.1	22.4 ± 8.5
PI 90763	19.3 ± 6.2	1.8 ± 1.1

Cyst indices equal 100 times the number of cysts on the soybean cultivar divided by mean number of cysts on Lee and are means of 10 replications.

† TN4 and TNVA are race 14 and race 2, respectively, according to Riggs and Schmitt (8).

‡ Values for Lee are actual numbers of cysts.

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TABLE 2. Mean values followed by 95% confidence limits of two indices for three soybean cultivar-*Heterodera glycines* combinations and nine sample sizes.

n	Peking-TN4		PI 90763-TN4		Peking-TNVA	
	Mean index	Replication index	Mean index	Replication index	Mean index	Replication index
1	25.5	25.5	23.3	23.3	10.8	10.8
2	21.1 ± 127.8	25.5 ± 301.0	20.0 ± 131.8	23.3 ± 201.7	10.2 ± 45.6	10.8 ± 47.6
4	18.8 ± 21.0	25.5 ± 53.3	19.2 ± 19.8	23.3 ± 35.7	9.4 ± 7.3	10.8 ± 7.3
5	18.6 ± 15.9	25.5 ± 41.6	18.1 ± 14.0	23.3 ± 27.9	9.4 ± 5.7	10.8 ± 5.7
10	18.5 ± 8.8	25.5 ± 24.0	17.9 ± 7.9	23.3 ± 16.1	9.3 ± 3.3	10.8 ± 3.3
20	18.3 ± 5.8*	25.5 ± 15.7	17.5 ± 4.9*	23.3 ± 10.5*	9.2 ± 2.1	10.8 ± 2.1
25	18.1 ± 5.0*	25.5 ± 13.8*	17.5 ± 4.3*	23.3 ± 9.3*	9.1 ± 1.8	10.8 ± 1.8
50	18.1 ± 3.4*	25.5 ± 9.5*	17.4 ± 3.8*	23.3 ± 6.4*	9.1 ± 1.3	10.8 ± 1.3
100	18.0 ± 2.4*	25.5 ± 6.7*	17.4 ± 2.1*	23.3 ± 4.5*	9.1 ± 0.9*	10.8 ± 0.9

Mean index and replication index were calculated as 100 times the number of females on the soybean cultivar divided by mean number of females on Lee and number of females on Lee in each replication, respectively. Means followed by asterisks were different from 10 ($P = 0.05$), the critical value for distinguishing positive and negative reactions.

Indices are means of 100, 50, 25, 20, 50, 25, 20, 10, and 5 samples of size n for n = 1, 2, 4, 5, 10, 20, 25, 50, and 100, respectively.

means in Table 2 are for all samples of size n from all randomizations. Thus there were 100, 50, 25, 20, 50, 25, 20, 10, and 5 samples for n = 1, 2, 4, 5, 10, 20, 25, 50, and 100, respectively. Confidence intervals (95%) were determined for the indices. An index based on the maximum number of cysts on Lee (maximum index) was also calculated for n = 100 as 100 times the number of cysts on a cultivar divided by the maximum number of cysts obtained on Lee among all replications for that soybean cultivar-SCN combination.

Replication index was always the same because all 100 replications were used with each sample size for the three soybean cultivar-SCN combinations (Table 2). Mean index was smaller and had smaller confidence intervals than replication index. The smaller confidence interval reflects a compression in variability because the divisor (number of females on Lee in each pot) was variable in replication index relative to a constant divisor (mean number of females on Lee) in the mean index. The mean indices with n = 100 for Peking-TN4 and PI 90763-TN4 were 18.0 and 17.4, respectively, but maximum indices were 8.0 and 6.9, respectively. Mean indices indicated positive reactions, but maximum indices indicated negative reactions. Maximum index for Peking-TNVA was 3.7. Use of maximum index is questionable because

its value is determined by the most extreme number of cysts obtained on Lee which could reflect variability in initial inoculum or environmental conditions for individual pots.

Indexing on a replication basis or using a small number of replications introduced considerable positive bias as can be seen in Table 2. For the Peking-TN4 combination, replication index was 25.5, compared with 18.0 for the mean index with 100 replications. Increasing the sample size from two to four replications decreased the mean index by 11%. This bias, however, decreased quickly as replications increased. Mean index decreased only 1% as sample size increased from 5 to 10. Mathematical treatment of this bias of mean and variance of an index as a function of number of replications has been published (2,6,7).

Golden et al. (2) recommended 10 replications of each differential be grown. The critical value of 10% of reproduction on Lee for differentiation between a negative and positive reaction was outside the 95% confidence intervals of mean indices for PI 90763-TN4 and Peking-TN4 only when at least 20 replications were used.

Variability encountered in this study was similar to that encountered by the author in determining the races of SCN populations in field soil. The mean index should be used in determining reproduction of a

SCN population on the soybean differentials because it minimizes variance while retaining meaning between experiments. Converting the index to a negative or positive reaction allows a SCN population to be classified to race, but the method is imprecise unless the index is large or a large number of replications are used. A positive reaction indicates an index value from 10 to infinity. Variance of the index needs to be considered in addition to the index value. One option is to declare a positive reaction only when the index is significantly greater than 10. Riggs et al. (8) reported that the three authors sometimes classified the same SCN population as different races. Often this occurred when the index for a differential was near 10 and they used only three replications. If they had declared a positive reaction only when the index was significantly greater than 10, they might have avoided the differences in race classification. A less decisive option is to report the index with its confidence interval and number of replications and allow individual researchers to interpret significance of the data. This is the minimum information that should be reported.

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