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Changes in the *Heterodera glycines* Female Index as Affected by Ten-year Cropping Sequences

LAWRENCE D. YOUNG

Abstract: The objective of this experiment was to measure the change in female index (FI) of *Heterodera glycines* from bioassays on Bedford, Peking, PI 89772, and PI 90763 soybean (*Glycine max*) for 12 cropping sequence treatments over a 10-year period. Cropping sequences included continuous plantings of Forrest, Peking, and D72-8927 soybean (all resistant to race 3); Bedford, Nathan, and D75-10710 soybean (all resistant to races 3 and 14); a Bedford–corn (*Zea mays*) rotation; a rotation of Bedford, Essex (susceptible), and Forrest; and a 70:30 blend of Bedford and Forrest. The FI from bioassays with PI 89772 and PI 90763 decreased over time from 24.3 to 1.6 with treatments involving continuous Bedford, Nathan, and D75-10710 and the Bedford–corn rotation. The FI increased in bioassays using Bedford with treatments involving Bedford, Nathan, D75-10710, the Bedford–Forrest blend, and the two rotations. Results of this field experiment confirm greenhouse experiments in which reciprocal changes occur in FI on PI 89772 and PI 90673 compared with FI on Bedford.

Key words: cropping sequence, genetics, *Glycine max, Heterodera glycines,* nematode, race, resistance, rotation, soybean, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, has been reported in most states in the United States that have commercial soybean (*Glycine max* (L.) Merr.) production. The nematode is a major pest of the crop. Sixteen races of the nematode have been designated (7). *Heterodera glycines* populations are usually heterogeneous in their ability to reproduce on cultivars containing genes for resistance. Planting resistant cultivars is an effective and common control measure for the pest, although risk from this practice has been demonstrated (6,10,11,13). Most of the resistant cultivars have genes for resistance from Peking or PI 88788 or both. Some recently released cultivars also contain resistance genes from PI 437654 (1). The combined work of several workers (2, 5,6,10,11) demonstrated that sources of *H. glycines* resistance genes can be placed in two genetic groups. One group consists of Peking, PI 89772, and PI 90763. The other group contains Cloud, PI 87631-1, PI 88788, and PI 209332. Forrest and Bedford reacted similarly to their source of resistance, Peking and PI 88788, respectively. It has been suggested (5,11) that cultivars from one genetic group such as Bedford be rotated with cultivars derived from soybean in the other group.

Productivity of resistant soybean grown continuously in the same plots or in alternative cropping sequences and nematode reproduction on these soybean have been reported for an 11-year period (12). In most years, continuously cropped Bedford had higher yield than the susceptible cultivar Forrest. Yield of Bedford in rotation with two susceptible cultivars or in rotation with corn (*Zea mays* L.) was not greater than yield of continuously cropped Bedford. Cyst population density did not increase over time in plots of continuous Bedford. The objective of this study was to measure changes in the number of *H. glycines* females developing on Bedford, Peking, PI 89772, and PI 90763 in a greenhouse bioassay using soil from the cropping sequence plots over a 10-year period.

Materials and Methods

Twelve cropping treatments (Table 1) were established in 1979 in a field in which Centennial soybean (resistant to race 3 and susceptible to race 14) plants had shown severe *H. glycines* injury the previous year. The field had a combination of Center and Routon-Bonn silt loam soils and had been planted to Forrest soybean in 1977 and to alfalfa in several previous years. Each

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USDA ARS, Nematology Research 605 Airways Blvd., Jackson, TN 38301-3201.

TABLE 1. Soybean cropping treatments in a field infested with *Heterodera glycines* at Woodland Mills, Tennessee, 1979–89.

Treatment designation	Year										
	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989
A	Forrest	Forrest	Forrest	Forrest	Forrest	Forrest	Forrest	Forrest	Forrest	Forrest	Forrest
B	Bedford	Bedford	Bedford	Bedford	Bedford	Bedford	Bedford	Bedford	Bedford	Bedford	Bedford
C	Nathan	Nathan	Nathan	Epps	Epps	Epps	Epps	Epps	Epps	Epps	Epps
D	D75-10710	D75-10710	D75-10710	D75-10710	D75-10710	D83-3319	D83-3319	D83-3319	D83-3319	D83-3319	D83-3319
E	Peking	PC†	PC	PC	PC	PC	PC	PC	PC	PC	PC
F	D72-8927	D72-8927	D72-8927	D72-8927	D72-8927	J82-21	J82-21	J82-21	J82-21	J82-21	J82-21
G	Corn	Bedford	Corn	Bedford	Corn	Bedford	Corn	Bedford	Corn	Bedford	Corn
H	Bedford	Corn	Bedford	Corn	Bedford	Corn	Bedford	Corn	Bedford	Corn	Bedford
I	Blend‡	Blend	Blend	Blend	Blend	Blend	Blend	Blend	Blend	Blend	Blend
J	Bedford	Forrest	Essex	Bedford	Forrest	Essex	Bedford	Forrest	Essex	Bedford	Forrest
K	Forrest	Essex	Bedford	Forrest	Essex	Bedford	Forrest	Essex	Bedford	Forrest	Essex
L	Essex	Bedford	Forrest	Essex	Bedford	Forrest	Essex	Bedford	Forrest	Essex	Bedford

Forrest, Peking, PC, D72-8927, J82-21, and Essex were susceptible to this nematode population; Bedford, Nathan, Epps, D75-10710, and D83-3319 were resistant to the nematode population.

† PC = Peking × Centennial.

‡ Blend was composed of 70% Bedford and 30% Forrest.

treatment was replicated three times in a randomized complete block design. Four substitutions in cultivar or line were made in an attempt to use more productive soybean. All substituted cultivars were closely related to the cultivar they replaced. D83-3319 (Bedford × [Forrest × (Peking × Centennial)]) was substituted for D75-10710 because D75-10710 was susceptible to stem canker caused by *Diaporthe phaseolorum* (Cook & Ellis) Sacc. var. *caulivora* K. L. Athow & R. M. Caldwell. Peking was replaced with a Peking × Centennial (PC) derived breeding line in 1980; J82-21 (Forrest × D72-8927) was substituted for D72-8927; and Epps replaced Nathan. Sources of resistance of the soybean planted in the field are listed in Table 2.

Individual plots were 16 rows wide and 24 m long, with 90 cm spacing between rows. The end of one plot was separated from the start of another by a 6-m wide alley. Data were collected from the four middle rows of each plot. Eight liters of soil were collected with a small shovel from 15 places in each plot and composited in September or October of each year except 1979. This soil was placed in pots in the greenhouse and planted with Essex, Bedford, Peking, PI 89772, and PI 90763. These plants were grown for 30 days, then white and yellow-colored females were extracted from the roots and soil by crumbling the root ball over the funnel of an elutriator (3), and hand-rubbing the roots while they were submerged in water in the elutriation funnel to help dislodge the females during elutriation. These females were placed in a gridded dish and counted with aid of a stereoscopic microscope. A female index (FI) was calculated from the counts as follows: FI = (number of cysts on Bedford, Peking, PI 89772, or PI 90763/number of cysts on Essex) × 100.

Linear regression was performed on natural log-transformed values of FI as a function of time (Y [log FI of bioassay plant] = I + bx, where I = intercept, b = slope, and x = year − 1900). Ward's minimum variance cluster analysis (8) was used to group treatment–soybean combinations. The 10-year FI mean and slope of the regression equation were assigned twice the weight given to the coefficient of determination of each treatment—soybean combination. These three variables were each normalized to an overall mean of zero and variance of one for the group of 48 treatment–soybean combinations in the cluster analysis. Slopes of the regression equations presented in Table 3 are on the natural log scale, but mean FI are untransformed values.

TABLE 2. Sources of resistance to *Heterodera glycines* of soybean used in an experiment to determine changes in female index as affected by 12 cropping sequences over a 10-year period.

Soybean	Source of resistance
Bedford	Peking, PI 88788
D72-8927	Peking, PI 90763
D75-10710	Peking, PI 88788
D83-3319	Peking, PI 88788
Epps	Peking, PI 88788
Essex	None
Forrest	Peking
J82-21	Peking, PI 90763
Nathan	Peking, PI 88788
PC	Peking

Results and Discussion

Cluster analysis divided the 48 regression equations (12 treatment means for the 10 years × 4 bioassay plants) for the treatment–soybean combinations into three clusters that appeared biologically explainable (Table 3). One cluster had FI ≤ 15 and negative slopes that were significantly different from zero ($P < 0.01$). The treatments in this cluster were bioassay soybean PI 89772 and PI 90763 grown in soil from plots of continuous production of the following resistant soybean: Bedford, Nathan (Epps), and D75-10710 (D83-3319), and the rotation of Bedford and corn.

The second cluster contained only the bioassay host Bedford. The soil was collected from plots of continuous Bedford, Nathan (Epps), D75-10710 (D83-3319), and Bedford–Forrest blend; the rotation of Bedford and corn; and two of the three

TABLE 3. Clustering of 10-year cropping sequences effects on number of females of *Heterodera glycines* based on Wards Clustering Method (8) of female indices, slope of linear regression equations, and coefficient of determination.

Treatment†	Bioassay host	Female index‡	Slope§	Coefficient of determination
		Cluster 1		
C Nathan	PI 89772	5.8	−.24**	.73
B Bedford	PI 89772	6.3	−.25**	.86
G Corn(Bedford)	PI 89772	7.1	−.18**	.60
C Nathan	PI 90763	7.4	−.25**	.94
H Bedford(Corn)	PI 89772	7.8	−.21**	.58
B Bedford	PI 90763	8.8	−.27**	.81
D D75-10710	PI 89772	9.9	−.23**	.65
D D75-10710	PI 90763	11.5	−.31**	.74
H Bedford(Corn)	PI 90763	15.0	−.34**	.73
G Corn(Bedford)	PI 90763	15.0	−.21**	.64
		Cluster 2		
K Forrest(Essex)(Bedford)	Bedford	17.4	.17**	.64
J Bedford(Forrest)(Essex)	Bedford	18.7	.18**	.56
I Blend	Bedford	26.7	.26**	.67
G Corn(Bedford)	Bedford	47.6	.22**	.71
H Bedford(Corn)	Bedford	50.5	.18**	.65
C Nathan	Bedford	55.2	.14**	.88
B Bedford	Bedford	70.4	.12*	.61
D D75-10710	Bedford	77.7	.17**	.47
		Cluster 3		
E Peking	Bedford	7.7	.02	.05
F D72-8927	Bedford	8.1	.03	.07
A Forrest	Bedford	10.4	.04	.04
J Bedford(Forrest)(Essex)	PI 89772	11.3	−.06	.18
K Forrest(Essex)(Bedford)	PI 89772	11.6	−.01	.05
L Essex(Bedford)(Forrest)	PI 89772	12.7	−.06	.21
I Blend	PI 89772	14.0	−.08	.35
I Blend	PI 90763	15.6	−.07	.28
K Forrest(Essex)(Bedford)	PI 90763	16.8	−.01	.01
C Nathan	Peking	17.9	−.10*	.51
L Essex(Bedford)(Forrest)	Bedford	18.1	.08	.10
L Essex(Bedford)(Forrest)	PI 90763	19.0	−.15**	.48
D D75-10710	Peking	19.0	−.06	.17
J Bedford(Forrest)(Essex)	PI 90763	19.2	−.02	.04
E Peking	PI 89772	19.6	.00	.00
A Forrest	PI 89772	20.9	−.03	.04
B Bedford	Peking	21.2	−.11*	.32
F D72-8927	PI 89772	25.9	.00	.00
E Peking	PI 90763	26.5	.07	.25
J Bedford(Forrest)(Essex)	Peking	27.7	.03	.05
I Blend	Peking	28.3	−.09	.33
H Bedford(Corn)	Peking	28.8	−.18**	.71
A Forrest	PI 90763	29.1	.01	.00
K Forrest(Essex)(Bedford)	Peking	30.1	.05	.12
L Essex(Bedford)(Forrest)	Peking	30.4	−.07	.15
G Corn(Bedford)	Peking	30.6	−.07	.11
F D72-8927	PI 90763	35.7	−.08	.27
E Peking	Peking	37.7	−.00	.00
A Forrest	Peking	40.2	.07	.25
F D72-8927	Peking	54.9	−.04	.18

† Treatment A = Forrest; B = Bedford; C = Nathan (Epps, 1982–89); D = D75-10710 (D83-3319, 1984–89); E = selection of Peking × Centennial (Peking in 1979); F = D72-8927 (J82-21, 1984–89); G and H = rotation of corn and Bedford; I = blend of 70% Bedford and 30% Forrest; and J, K, and L = Bedford, Forrest, and Essex grown in rotation in a field infested with *H. glycines*, 1979–89.

‡ Female index (untransformed values) = the number of *H. glycines* white and yellow females on Peking, Bedford, PI 90763, or PI 89772/females developing on Essex in a greenhouse test (mean of 10 years, 1980–89) × 100.

§ Slopes of linear regression equations for natural log-transformed values of female index as a function of time for each treatment–soybean combination.

* = $P > 0.01$ and <0.05, and ** = $P < 0.01$.

sets of the Bedford, Forrest, and Essex rotation. These treatments had positive slopes ($P < 0.05$) and FI values between 17 and 78. Slopes of all equations in cluster 2 were similar. The relatively smaller FI values of treatments I, J, and K (Table 1) are likely due to less selection pressure on Bedford because it occurred in the rotated plots every third year and constituted only 70% of the germplasm in the blend. Cluster 2 could be subdivided into the two groups with treatments B, C, D, G, and H in one group and treatments I, J, and K in the other group.

The third cluster contained treatments with slopes near zero, except for four. Three of the four treatments with significant negative slopes in this cluster were with Peking as the bioassay host grown in soil from plots of continuous Bedford, continuous Nathan (Epps), and one set of the Bedford-corn rotation. The other treatment with a significant negative slope involved the bioassay host PI 90763 grown in soil from one set of plots with the Bedford, Forrest, and Essex rotation. This same set of rotation plots (treatment L) also did not appear in cluster 2 with the other two sets of this rotation. Thus, this set of rotation plots had a different response than the other two sets of the same rotation with both the Bedford and Peking bioassay plants. The four treatments in cluster 3 with significant negative slopes have slopes and FI values similar to treatments in cluster 1. They probably were not included in cluster 1 because the mean FI was larger (17.9 to 28.8) and the slopes had smaller absolute values (0.11 to 0.18).

The *H. glycines* population at the beginning of the experiment was probably race 9 because that was the race present at one end of the field planted to Forrest in 1979 (10 [Table 1—Woodland Mills population]). Race determination tests were conducted in 1989 for each plot of the continuous Forrest, continuous Bedford, and blend treatments. All plots of the continuous Bedford and blend treatments contained race 2. Two plots of the continuous Forrest contained race 9 if Lee was used as the susceptible check. The other plot contained race 14. Where Essex (bioassay check) was used as the susceptible check, all continuous Forrest plots contained race 14.

Fewer white and yellow females developing on PI 89772 and PI 90763 grown in soil collected from plots planted with Bedford every year or in rotation with corn or other soybean lines or cultivars is similar to results obtained in greenhouse experiments to select *H. glycines* populations that reproduce on Bedford (2,10). The greater development (i.e., larger FI) on the Bedford bioassay of *H. glycines* in soil in which Bedford was continuously cultured also occurred in the greenhouse selection experiments (2,10). In the selection experiments, host suitability of PI 89772 and PI 90763 to *H. glycines* decreased, but increased on Bedford. Conversely, the opposite effect also occurred. The reciprocal nature of host suitability of PI 89772 and PI 90763 to *H. glycines* compared with that of PI 88788 and Bedford may be due to an allelic series at a common locus in these soybean. Thomas et al. (9) proposed an allelic series with dominance in the order of Peking > PI 90763 > PI 88788 (one of the sources of resistance in Bedford). The reciprocal nature of host suitability between the two genetic groups was the basis of proposals to rotate cultivars with resistance from these two genetic groups in order to manage *H. glycines* population densities below the damage threshold (5,11). The data being reported indicates such rotations could be helpful; however, specific statements about rotations of Bedford and cultivars derived from PI 89772 or PI 90763 cannot be made because none of them were included in the experiment.

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