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Effect of Soil Temperature and pH on Resistance of Soybean to *Heterodera glycines*[1]

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Abstract: Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major pest of soybean, *Glycine max* L. Merr. Soybean cultivars resistant to SCN are commonly grown in nematode-infested fields. The objective of this study was to examine the stability of SCN resistance in soybean genotypes at different soil temperatures and pH levels. Reactions of five SCN-resistant genotypes, Peking, Plant Introduction (PI) 88788, Custer, Bedford, and Forrest, to SCN races 3, 5, and 14 were studied at 20, 26, and 32 C, and at soil pH's 5.5, 6.5, and 7.5. Soybean cultivar Essex was included as a susceptible check. Temperature, SCN race, soybean genotype, and their interactions significantly affected SCN reproduction. The effect of temperature on reproduction was quadratic with the three races producing significantly greater numbers of cysts at 26 C; however, reproduction on resistant genotypes remained at a low level. Higher numbers of females matured at the soil pH levels of 6.5 and 7.5 than at pH 5.5. Across the ranges of temperature and soil pH studied, resistance to SCN in the soybean genotypes remained stable.

Key words: abiotic factors, *Glycine max*, *Heterodera glycines*, resistance, pH, soybean cyst nematode, temperature.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is the most important pathogen of soybean in the United States (11,23). Planting SCN-resistant soybean is the most common practice used to reduce losses caused by this pest. Widespread plantings of the SCN-resistant cultivar Forrest prevented estimated losses of $405 million between 1975 and 1980 (7). Currently, more than 130 soybean cultivars with SCN resistance are available in the United States (3), the majority of which have derived SCN resistance either from PI 88788 and(or) from Peking (3). The lack of genetic diversity for resistance to SCN in soybean cultivars may have serious consequences in the event of a breakdown of resistance under adverse environmental conditions. Resistance to the root-knot nematode, *Meloidogyne incognita,* in some of the *Lycopersicon* species was found to be ineffective at soil temperatures of 32 C; consequently, the use of resistant tomato cultivars is limited to only some areas of the world (2). In wheat, resistance to soilborne wheat mosaic may be related to differential virus movement in resistant versus susceptible cultivars, with temperature influencing the expression of the resistance (21). Little is known about the stability of SCN resistance in soybean under different environmental conditions. Soil temperature and pH are among the important environmental factors that influence many functions of plants and nematodes (8,14, 16,18,19). The objective of this research was to determine the stability of SCN resistance of selected soybean genotypes across a range of soil temperatures and pH levels.

Materials and Methods

Heterodera glycines races 3, 5, and 14 (13,22) were cultured at the University of Missouri— Delta Center at Portageville, Missouri on Essex, PI 88788, and PI 90763, respectively. Their indices of parasitism, as determined from the standard race test (22), are described in Table 1. Inoculum of each race was prepared by crushing the freshly harvested white females and passing the suspension through nested 250-μm and 124-μm-pore sieves to remove debris from the egg suspension.

The soil used was a Broseley fine sandy soil (loamy, mixed, thermic Arenic Hapludalf), with an initial pH of 4.6 and a neutralizable acidity of 1.0. The dry soil was

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TABLE 1. Index of parasitism[a] of soybean cyst nematode races 3, 5, and 14 on soybean differentials.

Differentials	Race 3	Race 5	Race 14
Lee 68	100	100	100
Pickett	4	63	81
Peking	2	8	66
PI 88788	1	47	7
PI 90763	<1	3	35

[a] Average number of white females on the differential × 100/number of white females on Lee 68.

steam sterilized for all experiments. The soil pH was adjusted to 6.5 by addition of lime for temperature study.

Effect of temperature: Treatments were arranged in a split-plot design with the three temperature levels (20, 26, 32 C) as the main plots and three SCN races (3, 5, and 14), two inoculation levels (0, or 1,000 eggs) and five soybean genotypes as subplots. The five soybean genotypes were as follows: PI 88788, resistant to SCN race 3 and 14 and susceptible to race 5 (6); Peking, resistant to races 3 and 5 and susceptible to race 14 (6); Forrest and Custer, resistant to race 3 (15,20) and susceptible to races 5 and 14; and Essex, susceptible to all known SCN races (5). Seeds of each soybean genotype were germinated in vermiculite, and single seedlings were transplanted to 2.5 × 20 cm plastic tubes containing steamed sterilized soil. These tubes were placed in 11.4 liter containers in water baths of 20, 26, and 32 C in a greenhouse. Five days after transplanting, the soil in the tubes was inoculated. The experiment had six replications and was repeated once. At the V2 stage of plant growth, shoots were pruned above the unifoliate node to retard vegetative growth (4,12). Plants were gently removed from the tubes at 28–30 days after inoculation. The roots were rinsed in tap water and placed on a 425-μm-pore sieve nested over a 250-μm-pore sieve. White females-cysts were dislodged from the roots by a high-pressure water stream. Cysts collected on the lower sieve were counted, using a gridded glass petri plate under a stereoscopic binocular microscope.

Effect of soil pH: The three treatments with seven replications were arranged in a split-plot design with three pH levels as main plots, and the five soybean genotypes as subplots. Soybean genotypes were PI 88788, Peking, Custer, Essex, and Bedford. Bedford is resistant to races 3 and 14 and susceptible to race 5. The soil pH was adjusted to approximately 5.5, 6.5, and 7.5 with CaOH suspended in a quantity of deionized water necessary to bring the soil to field capacity. The CaOH suspension was blended into the soil with a cement mixer by tumbling for 45 minutes. The soil pH of the amended soil, which was placed in tubes within 11.4 liter plastic containers was measured with a pH meter after air drying the soil and mixing it 1:1 with 0.1 M $CaCl_2$. Seedling preparation was done as described for the temperature experiment. Seedlings were inoculated with 0 or 3,000 eggs and juveniles of race 3, 5 or 14.

The pH was monitored at approximately 4-day intervals. Five additional tubes were established so that some plants could be sacrificed to obtain pH readings. Soil pH was maintained by adding or eliminating $CaCO_3$. The tubes at pH 5.5 were watered with deionized water. Tubes at pH 6.5 and 7.5 were watered with 5 and 10 mg/liter $CaCO_3$ solutions, respectively to maintain the desired pH level. Routine watering was done with tap water, except watering was occasionally done at pH 6.5 with deionized water. Female and cyst data on roots were collected as described for the temperature experiment.

Data analysis: Nematode data were $\log_{10}$ transformed and subjected to statistical analysis using analysis of variance (10) for a split plot design. Linear and quadratic regression equations were tested for fit on the number of female or cysts vs. temperature.

RESULTS AND DISCUSSION

Effect of temperature: The number of SCN females and cysts were affected by temperature, SCN race, and soybean genotype ($P \leq 0.01$; Table 2). The statistical interac-

TABLE 2. Mean square for soil temperature (20, 26, and 32 C) and pH (5.5, 6.5, and 7.5) effects on number of females and cysts across five soybean genotypes[a] and three *Heterodera glycines* races (3, 5, and 14).

Temperature effects		pH Effects	
Source	Mean square	Source	Mean square
Temperature	19.78**	pH	3.42*
Linear	2.36**	Error A	0.35
Quadratic	37.21**	Genotype	9.26**
Error A	0.07	Races	14.12**
Race	22.84**	Genotype × pH	0.32NS
Temperature × race	1.24**	Genotype × race	2.76**
Genotype	28.41**	Genotype × race × pH	0.19NS
Temperature × genotype	0.20**	Error B	0.14
Race × genotype	10.23**		
Temperature × genotype × race	0.38**		
Error B	0.07		

NS Not significant; * significant at $P = 0.05$; ** significant at $P = 0.01$.
[a] Soybean genotypes were PI 88788, Peking, Forrest, Custer, and Essex.

tions of temperature × race, temperature × soybean genotype, race × soybean genotype, and temperature × race × soybean genotype were significant. Linear and quadratic components of temperature effects were significant, which revealed that cyst production increased when temperature was raised from 20 to 26 C, but declined at 32 C.

For race 3, temperature and soybean genotypes influenced the development of female and cyst production (Table 3). The mean number of females and cysts that developed in 30 days was almost 2 to 6 fold greater at 26 C than at 20 and 32 C (Table 3). Partitioning of temperature effects into linear and quadratic components revealed a significant quadratic effect and a lack of

TABLE 3. Number of *Heterodera glycines* females and cysts produced on soybean genotypes by races 3, 5, and 14 at three temperatures.

Temp. (C)	Essex	Forrest	Custer	Peking	PI 88788	Mean[a]
			Race 3			
20	89	3	0	0	1	18.6 b
26	255	6	1	1	8	54.2 a
32	125	3	0	0	1	25.4 b
Mean[a]	156 a	3 b	0.3 b	0.3 b	3.3 b	
			Race 5			
20	58	5	2	1	43	21.8 c
26	200	39	21	10	203	94.6 a
32	126	5	1	1	93	45.2 b
Mean	128 a	16 b	8 b	4 b	113 a	
			Race 14			
20	20	24	12	3	3	12.4 c
26	128	97	98	50	9	76.4 a
32	72	49	64	37	1	44.6 b
Mean	73 a	57 a	58 a	30 b	8 c	
			Across races			
20	55	10	5	1	16	17.4 c
26	194	46	44	20	80	76.8 a
32	108	20	41	102	36	43.4 b
Mean	128 a	16 b	8 b	4 b	113 a	

[a] Means followed by the same letter in rows and columns are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

fit to the linear relationship (data not presented). The differences between the number of females and cysts that developed at 20 and 32 C were slight.

The effects of temperature, genotype, and their interactions were significant for races 5 and 14 ($P \leq 0.01$; Table 3). Greatest numbers of females and cysts were produced at 26 C and lowest at 20 C across the five soybean genotypes ($P \leq 0.05$; Table 3).

Effect of soil pH: Cyst production was influenced by soil pH, plant genotype, SCN race, and the interaction of race × genotype ($P \leq 0.05$; Table 2). Interactions between genotype × pH, or genotype × pH × SCN race on cyst production were not significant. Across all genotypes and SCN races, mean numbers of cysts produced were affected by soil pH (Table 4). The numbers at pH 6.5 and 7.5 were greater than at pH 5.5 ($P = 0.05$). Cyst production was much greater on Essex than on the resistant genotypes.

Cyst development was found to be temperature dependent as expected for poikilothermic organisms; however, resistance, based on cyst indices remained effective at the three temperatures tested. Jin et al. (16) found that a recessive resistance gene in barley was ineffective against pathotype QCC of *Puccinia graminis tritici* at temperatures greater than 27 C. Similarly, while working with resistance genes in soybean to *Phytophthora megasperma,* Classen and Ward (9) reported that resistance was not effective at high temperatures because heat prevented the synthesis of anti-fungal compounds. These types of responses to SCN in this study were not apparent.

Soybean cyst nematode production was greater at pH 6.5 and 7.5 than at 5.5. This could have several explanations. First, a soil pH of 6.5 to 7.5 is better than 5.5 for plant growth of soybean (17) in the soils used, which should result in a greater root system and consequently greater number of potential nematode infection sites. A second explanation relates to the development of a thicker suberized layer on soybean roots grown at a lower pH compared with those grown at high pH levels as fewer nematodes (*Pratylenchus alleni*) were found to enter the roots at soil pH of 4.0 compared with those at pH of 6.0 and 8.0 (8). The lower SCN production at pH 5.5 could be due to an increased suberized layer in the roots acting as a barrier to penetration by SCN juveniles. Third, the SCN reproduction and(or) development at pH 5.5 could have been adversely affected by the low pH (1).

In conclusion, resistance of soybean genotypes to SCN remained effective at temperatures of 20, 26, and 32 C and at soil pH 5.5, 6.5, and 7.5. Thus, resistance was not temperature or pH sensitive at the levels tested.

TABLE 4. Mean number of females and cysts of three races of *Heterodera glycines* per plant on five soybean genotypes at three pH levels.

Genotype	pH 5.5	pH 6.5	pH 7.5	Mean
Essex	237	558	416	404 a
Bedford	14	110	132	85 b
Custer	11	90	96	99 b
Peking	63	91	115	90 b
PI 88788	125	87	98	104 b
Mean	90 b	187 a	172 a	

Means followed by the same letter in a column or row are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

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