Development of Heterodera glycines Pathotypes as Affected by Soybean Cultivars[1]

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Abstract: The reproductive abilities of four races of *Heterodera glycines* were compared on soybean cultivars by using single cyst or mass inoculations. Progeny transfers were used to determine changes in reproduction of *H. glycines*. Reproduction of all races (1, 2, 3, 4) was best on 'Lee' and poorest on PI 88788. The size of females produced and numbers of eggs/female of the different races varied with the cultivar. Races 1 and 3 appeared to contain low populations of Races 2 or 4. Races 2 and 4 were best selected by a series of transfers on 'Pickett' soybean.
Key Words: Soybean-cyst nematode, *Glycine max*, races, resistance.

The introduction of cultivars resistant to a pathogen often results in changes in the pathogen population which enable the pathogen to overcome host resistance. Such changes have occurred with cyst nematodes, including the soybean-cyst nematode (SCN), *Heterodera glycines* Ichinohe. An understanding of the effects of different cultivars on the relative reproductive rates of resulting races (pathotypes) will aid in coping with these changes.

Den Ouden (2) found the rate of reproduction (R) of *Heterodera rostochiensis* pathotypes to be variable even on cultivars susceptible to both pathotypes being compared. Pathotypes of *H. rostochiensis* varied between varieties and clones of both *Solanum tuberosum* subsp. *tuberosum* and *S. tuberosum* subsp. *andigenum* (4, 5). At high inoculum levels of *H. glycines,* Miller (7) found that reproduction was lower on susceptible soybean cultivars than with lesser inoculum levels. On resistant soybean cultivars, R increased with inoculum level. Five field populations of *H. glycines* were used by Triantaphyllou to demonstrate that R (index of parasitism) on resistant soybean cultivars increased after five to seven generations on the resistant cultivar (9). The increase in R was genetically controlled. Larval emergence from *H. rostochiensis* cysts obtained from susceptible potatoes was greater than from cysts from resistant cultivars even though the cysts were of equal volume (6).

Our studies were conducted from 1967 to 1974 to determine the relative reproduction rates of *H. glycines* on resistant and susceptible soybean after single-cyst or mass inoculation.

MATERIALS AND METHODS

Soybean [*Glycine max* (L.) Merrill] cultivars used were 'Lee', 'Pickett', 'Peking', Plant Introduction '(PI) 88788', and 'PI 90763'. Seeds were germinated in fine river sand, and plants were allowed to develop to the unifoliolate stage before transplanting and inoculation with *H. glycines.*

The populations of *H. glycines* which were used represented the four designated races (3). Race 1 came from North Carolina and was maintained on Lee soybeans; Race 2 was obtained from Virginia and was maintained on PI 79693 or Pickett soybeans; and Races 3 and 4 were collected from eastern Arkansas and were maintained on Lee and Pickett soybeans, respectively. Inoculum for all tests was obtained by the sieving method (3).

Twenty-eight days to 3 months after inoculation, females were collected from the roots and suspended in water. The suspension was poured through nested 841-μm and 250-μm sieves and the material on the 250-μm sieve was washed into a counting dish. Mature females and cysts were counted with the aid of a stereomicroscope. Reproduction was reported either as the number of females and cysts recovered or as a cyst index. The cyst index was obtained by dividing the average number of mature females and cysts recovered from a given cultivar by the average number recovered from Lee soybeans and multiplying by 100.

Single-cyst inoculations: Individual cysts of *H. glycines* Races 3 or 4 were picked from Lee, Pickett, and Peking soybeans. One cyst was placed in each of fifty, 7.5-cm pots/cultivar. Each pot contained fine river

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sand and a single plant in the first true leaf stage. These plants were grown for 35 days and then the progeny of these cysts were recovered. They were transferred as individual cysts in the same manner. After another reproductive period of 35 days, equal numbers of single cysts from each cultivar were transferred to all three cultivars.

Mass inoculations: Cysts of Races 3 or 4 from stock cultures were collected *en mass* and broken in a Waring blender to release the eggs and larvae which comprised the inoculum (8). The eggs and larvae were suspended in water and a measured volume was applied to each of 10 pots/cultivar (Lee, Pickett, and Peking) in the sequence given in Figure 1. The incubation period was about 5 weeks from inoculation to recovery of females and reinoculation. In the fourth transfer, the nematodes were reinoculated to the same host from which they were taken and were left for 3 months after which they were again each inoculated to all three cultivars.

Reproductive potential of races: In these tests, reproduction of the four races was determined on all five soybean cultivars (Lee, Pickett, Peking, PI 88788, PI 90763). The inoculum was prepared as previously, but was applied with an automatic pipette which delivered the same volume of inoculum to each pot. In the first test, a calculated 200 or 1,000 eggs and larvae were applied to each of 10 pots of each cultivar. In the second test, 1,000-2,000 eggs and larvae were applied to each of five pots of each cultivar.

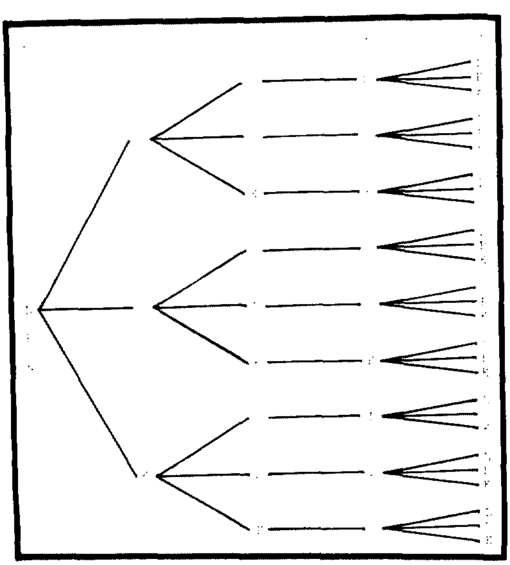

FIG. 1. Diagramatic scheme of inoculation sequence with *Heterodera glycines* used on three soybean cultivars. L = Lee, P = Pickett, K = Peking; 1, 2, 3, 4, 5 at top are transfer numbers.

In these tests, the mature females were recovered 5 weeks after inoculation. The data taken included: *(i)* number of mature females/pot; *(ii)* measurements of the length and width of 10 females from each race for each cultivar and counts of the numbers of eggs/female. The females of each race recovered from each cultivar were then used to inoculate three pots each of the five cultivars. After another 5 weeks, the females were recovered and the number per pot counted. In the second test, the females of each race recovered from each cultivar were reinoculated to the cultivar from which they were recovered. These plants were maintained for 3 months, after which the reproduction was determined and measurements were taken.

RESULTS

Single cyst inoculations: In general, Lee plants had more infections than Pickett or Peking, but cysts from Pickett produced the most infections on all hosts (Fig. 2). The largest number of cysts/plant infected were on Lee inoculated with cysts from Pickett. Peking was a poor host even when the populations had been maintained on it for several generations.

Mass inoculations: In the inoculation sequences with Race 3, all transfers in the continuous Lee (L) series (L, LL, LLL, LLLLL) resulted in similar cyst indices on Pickett (P) (3, 0.2, 0.3, 0.3) and Peking (K) (0.1, 0.01, 0.02, 0.01) (Table 1). Only When Pickett or Peking was the host for two consecutive transfers was there an increase in the reproduction on either Pickett or Peking. For example, in sequence LLPPP there was 41% as much reproduction as on LLPPL, in comparison with 0.3% as much in sequence LLLLP. In the same sequence, Peking (LLPPK) had 14% as much reproduction as Lee (LLPPL). The highest cyst index on Pickett and Peking was in sequence LPKKP (68%) and LPKKK (52%), in comparison with LPKKL. One crop of Pickett in the sequence was necessary for reproduction on Peking.

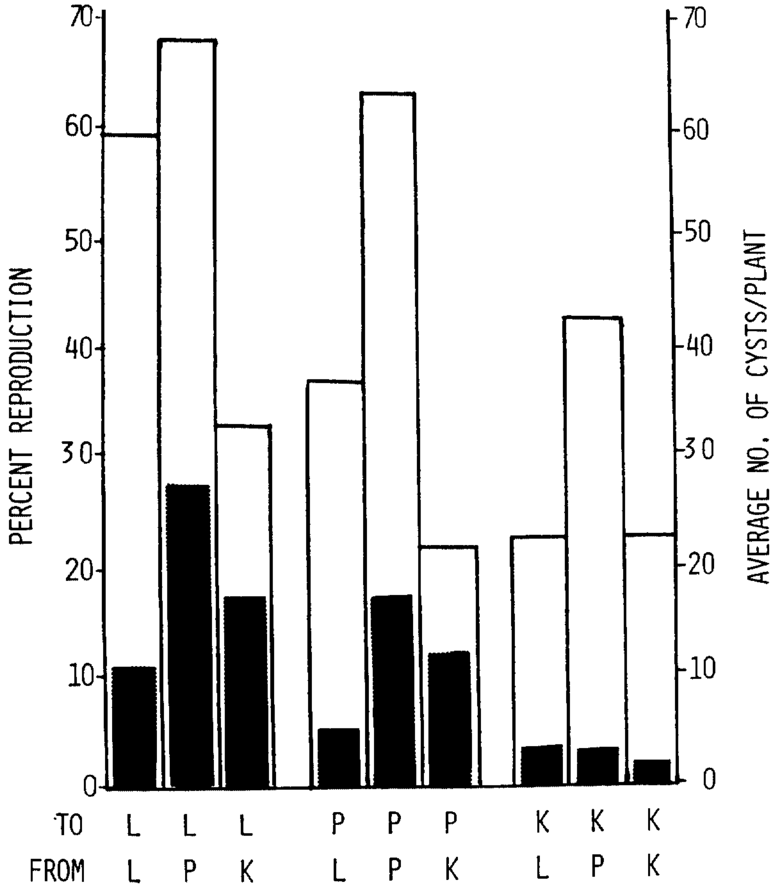

FIG. 2. Reproduction of Race 3 of *Heterodera glycines* when single cysts were placed on Lee (L), Picket (P), or Peking (K) soybeans, expressed in terms of percentage of the total inoculation and the average number of mature females recovered from each plant. White bar refers to % of inoculations that reproduced. Black bar refers to average number of cysts/plant where reproduction occurred.

Reproduction of Race 4 of *H. glycines* on Lee, Pickett, and Peking remained relatively constant when the nematodes were maintained on Lee soybeans (Table 1). The reproductive indices on P and LLLLP were about the same. However, the index on Peking in sequence LLLLK was slightly lower than on K, though not significantly. After 1 or 2 generations on Pickett, the index on Pickett increased noticeably (PP, 74%; LLP, 70%; LPLLP, 70%). Three or more generations on Pickett increased the index on Pickett even more in some cases (PPP, 101%; LPPPP, 94%), whereas in others there was no change (PLPPP, 16%; PPPPP, 42%; KPPPP, 38%). The highest indices on Pickett or Peking generally were produced when Pickett and Peking had both occurred in the inoculation sequence (LLKKP, 98%; LPKKP, 158%; LKPPP, 121%; LKKKP, 115%; PLKKP, 100%), but these sequences did not always produce high indices on Pickett and Peking (PKK, 3%; KPK, 6%; PLPPK, 2%; PPPPK, 2%; KPPPK, 2%). Increase in index on Peking did not generally occur except when Peking was included in the inoculation sequence (LLKKK, 27%; LKKKK, 24%; PLKKK, 24%).

Comparison of four races: Two tests comparing the reproduction of four races of *H. glycines* on Lee, Pickett, Peking, PI 90763 & PI 88788 were conducted. In one test, there were two inoculum levels (200 & 1,000 eggs & larvae/pot) and the cyst indices obtained at the two levels were similar. The results reported are from another test in which 1,000-2,000 eggs and larvae/pot were used. The cyst indices for Races 1 and 3 were similar on all cultivars and for Races

TABLE 1. Relative cyst indices of *Heterodera glycines* Races 3 and 4 on three soybean cultivars for the first and fifth of five serial transfers.

	Race 3		Race 4 Starting With					
				Pickett		Peking		
Transfer	Inoculation sequence[a]	Cyst index[b]	Lee Cyst index	Inoculation sequence	Cyst index	Inoculation sequence	Cyst index
1	L	100	100	L	100	L	100
	P	3	32	P	2	P	32
	K	0.1	4	K	4	K	4
5	LLLLL	100	100	PLLLL	100	KLLLL	100
	LLLLP	0.3	31	PLLLP	69	KLLLP	17
	LLLLK	0.01	1	PLLLK	1	KLLLK	2
	LLPPL	100	100	PLPPL	100	KLPPL	100
	LLPPP	41	86	PLPPP	16	KLPPP	33
	LLPPK	14	2	PLPPK	2	KLPPK	3
	LLKKL	100	100	PLKKL	100	KLKKL	100
	LLKKP	11	98	PLKKP	100	KLKKP	93
	LLKKK	2	27	PLKKK	28	KLKKK	17
	LPLLL	100	100	PPLLL	100	KPLLL	100
	LPLLP	1	70	PPLLP	47	KPLLP	82
	LPLLK	0.1	2	PPLLK	2	KPLLK	1
	LPPPL	100	100	PPPPL	100	KPPPL	100
	LPPPP	44	94	PPPPP	42	KPPPP	38
	LPPPK	4	4	PPPPK	2	KPPPK	2
	LPKKL	100	100	PPKKL	100	KPKKL	100
	LPKKP	68	158	PPKKP	53	KPKKP	69
	LPKKK	52	12	PPKKK	6	KPKKK	14
	LKLLL	100	100	PKLLL	100	KKLLL	100
	LKLLP	2	13	PKLLP	88	KKLLP	79
	LKLLK	0.1	2	PKLLK	2	KKLLK	1
	LKPPL	100	100	PKPPL	100	KKPPL	100
	LKPPP	18	121	PKPPP	100	KKPPP	5
	LKPPK	14	7	PKPPK	3	KKPPK	3
	LKKKL	*no cysts in*	100	PKKKL	100	KKKKL	100
	LKKKP	transfer 4	115	PKKKP	76	KKKKP	78
	LKKKK	to transfer	24	PKKKK	11	KKKKK	31

[a]Inoculation sequence of cultivars L, Lee; P, Pickett; K, Peking.

[b]Cyst Index = $\frac{\text{no. females from cultivar}}{\text{ave. no. females from Lee}} \times 100.$

2 and 4 they were also similar on all cultivars (Table 2). Race 1 did not reproduce as well on PI 88788 as expected. Reproduction of Races 1 and 3 on Pickett appeared to increase in the second inoculation (Table 3). However, the reproduction levels were very low on all cultivars. Race 4 reproduced better on Peking and PI 90763 in the second inoculation than in the first (Table 3). Continuous culture of all races on the cultivars on which reproduction had occurred resulted in substantial populations of all except Race 1 on PI 88788 (Table 4). Even Races 2 and 4 did not produce as high populations on PI 88788 as on the other cultivars. Race 3 reached high populations on Pickett, Peking, and PI 88788 (Table 4).

Mature female size and the number of eggs produced were directly related to the suitability of the host in most cases (Tables 2, 4). On suitable hosts, cyst length was usually 750 μm or more in the initial inoculation, whereas the width was 500 μm or more. However, Race 2 cysts on PI 88788 were the largest of the groups even though PI 88788 was not a very good host. The final measurements were very similar for all races on all cultivars (Table 4). Race 1 cysts from PI 88788 were not much smaller than those from Lee but contained very few eggs. Cysts of Races 2 and 4 from PI 88788 were the only groups which had appreciably smaller cysts than average (Table 4).

TABLE 2. Differential cyst indices, cyst length, cyst width, and eggs/cyst for four races of *Heterodera glycines* on five soybean cultivars.

Parameter	Cultivar	RACE 1	2	3	4
Cyst index [a]	L[b]	100	100	100	100
		(117)[c]	(213)	(712)	(148)
	P	1	123	1	98
		(1)	(263)	(5)	(145)
	K	0	25	0.1	23
		(0)	(54)	(1)	(34)
	9	0	11	0	8
		(0)	(23)	(0)	(12)
	8	0.3	39	0.1	30
		(0.4)	(83)	(1)	(45)
Cyst length (μm)	L	764[d]	749	778	776
	P	652	786	703	774
	K	—	732	731	772
	9	—	701	—	708
	8	664	791	657	745
		LSD: P @ 0.05 = 45[e]			
		P @ 0.01 = 59			
Cyst width (μm)	L	537	552	566	581
	P	427	598	519	565
	K	—	544	498	556
	9	—	498	—	556
	8	363	583	488	575
		LSD: P @ 0.05 = 33			
		P @ 0.01 = 43			
Eggs/cyst	L	180	208	189	173
	P	106	231	156	186
	K	—	198	110	159
	9	—	121	—	124
	8	100	219	123	156
		LSD: P @ 0.05 = 59			
		P @ 0.01 = 77			

[a]Cyst Index $= \frac{\text{no. cysts on cultivar}}{\text{ave. no. cysts on Lee}} \times 100.$

[b]L = Lee, P = Pickett, K = Peking, 9 = PI 90763, 8 = PI 88788.

[c]Numbers in parenthesis are actual cyst counts, average of five replicates inoculated with 1,000-2,000 eggs and larvae/replicate.

[d]Length, width, and egg counts are averages of 10 cysts or as many as were available.

[e]LSD's are used between cultivars.

DISCUSSION

Much variability exists between cysts of *Heterodera* in their capacity to reproduce. This reproductive capacity is influenced by the host on which it was produced and the host to which its progeny are subjected. Mass reproduction on the Lee cultivar tended to maintain the parasitic capabilities of a given population or race, whereas reproduction on a less susceptible host, such as the cultivars Peking, PI 90763, and PI 88788, tended to restrict the reproductive capacity and change the parasitic capabilities to a greater extent. In all instances where reproduction could occur, the population eventually reached a high reproduction level on the host, except for Race 1 on PI 88788. Triantaphyllou (9) found that continuous culture of *H. glycines* populations on resistant cultivars increased the ability of the populations to mature on the resistant cultivar.

The infection level and reproductive rates of single cyst inoculations were not high enough to provide information on the

TABLE 3. Differential reproduction of *Heterodera glycines* races on five soybean cultivars as measured by cyst index.[a]

Initial Cultivar	Final Cultivar	Cyst indices for race[b]			
		1	2	3	4
Lee	Lee	100	100	100	100
	Pickett	2	57	1	67
	Peking	0.3	26	0.1	16
	PI 90763	0.3	29	0	11
	PI 88788	0.3	42	0	12
Pickett	Lee	100	100	100	100
	Pickett	30	55	67	78
	Peking	30	22	2	30
	PI 90763	0	13	6	24
	PI 88788	0	22	0	19
Peking	Lee	—	100	100	100
	Pickett	—	60	100	64
	Peking	—	20	20	41
	PI 90763	—	21	20	28
	PI 88788	—	25	0	28
PI 90763	Lee	—	100	—	100
	Pickett	—	84	—	42
	Peking	—	14	—	13
	PI 90763	—	10	—	30
	PI 88788	—	10	—	27
PI 88788	Lee	100	100	0	100
	Pickett	0	110	0	76
	Peking	0	31	0	32
	PI 90763	0	10	0	12
	PI 88788	0	20	0	35

[a]Cyst Index $= \frac{\text{no. cysts on cultivar}}{\text{ave. no. cysts on Lee}} \times 100.$

[b]Average of three replicates.

percentage level of a type in the populations. Kort (4) reported that aggressive biotypes of *H. rostochiensis* demonstrated a higher rate of reproduction, up to 129-fold, when kept singly in a pot. He also indicated that, in a mixture of biotypes where the infestation is low, the individual biotypes tended to build at a faster rate. Conversely, under high population levels the mixture of types tended to keep the lesser members of the mixture at a lower level. Anderson (1) showed considerable variation in *Heterodera avenae* cysts of different appearance. Part of the variability in the single cyst inoculations is in the fact that, in many cases, all cysts from Peking had to be used and some had few eggs. Another possible factor is that cysts from Pickett may have contained segregating mixtures of races, all of which would reproduce on the Lee cultivar but only part of them on Pickett and fewer on Peking. This variability could be even further magnified with cysts from Peking.

In the mass inoculation studies, the higher the inoculation level on poor hosts, the greater the number of mature females formed, but the number relative to the number on a good host did not increase as much. This relationship may be due to the available food supply. Miller (7) indicated that, on good hosts, more mature females were recovered from 70 and 244 cysts/pot inoculum levels than from 857 or 3,000 cysts/pot inoculation levels. In contrast, he found that, on poor hosts, the number of mature females recovered was directly related to inoculum level.

These studies should help to interpret field populations and predict the relative

TABLE 4. Cyst counts, cyst measurements, and egg counts of *Heterodera glycines* on five soybean cultivars after three sequential inoculations.

Race	Cultivar sequence	Final cyst count[a]	Cyst length (μm)[b]	Cyst width (μm)[b]	Eggs/cyst[b]
1	L-L-L-L[c]	5,260	737	475	176
	L-P-P-P	4,060	739	502	226
	L-8-8-8	4	700	418	57
2	P-L-L-L	608	749	537	214
	P-P-P-P	364	726	492	162
	P-K-K-K	3,880	716	500	145
	P-9-9-9	4,160	720	492	199
	P-8-8-8	264	664	459	118
3	L-L-L-L	4,420	778	554	210
	L-P-P-P	4,080	759	504	141
	L-K-K-K	6,280	778	521	236
	L-8-8-8	3,668	753	517	156
4	P-L-L-L	8,440	745	502	221
	P-P-P-P	4,060	730	500	126
	P-K-K-K	2,920	706	504	202
	P-9-9-9	1,420	710	496	263
	P-8-8-8	555	668	459	157

[a]Average of two 15-cm pots.
[b]These data are averages of 10 cysts, except Race 1 on PI 88788 which had only 8 cysts. First host in sequence was source host and last host was used to maintain population for three months after two intermediate inoculations of one generation each. L = Lee, P = Pickett, K = Peking, 9 = PI 90763 and 8 = PI 88788.

amount of damage which can be expected at certain population levels. This information will also aid in advising on crop sequence when determinations of population levels of specific races of *H. glycines* are made.

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