Population Development, Reproductive Behavior, and Morphology of Race 4 of the Soybean Cyst Nematode, *Heterodera glycines*, on Resistant Soybeans

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ABSTRACT

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The effects of the soybean lines Toyosuzu, Essex, Cloud, PI 87637-1, Bedford, L77-994, PI 209332, and PI 88788 on numbers of females attaining maturity, reproductive behavior, and morphology of *Heterodera glycines* race 4 were determined. As the level of resistance increased, fewer females developed, and they were smaller and produced fewer eggs. The number of egg masses produced, egg mass size, and number of eggs per egg mass were also less on resistant lines. Numbers of mature females and reproduction were high on Essex and Toyosuzu and moderate on Cloud. PI 87631-1, PI 89772, and Bedford produced similar low levels of mature females, but less total reproduction occurred on Bedford. The fewest mature females occurred on PI 88788, PI 209332, and L77-994. L77-994 and Bedford both derive their race 4 resistance from PI 88788. The amount of nematode reproduction was similar but the number of females attaining maturity was greater on Bedford than on L77-994. The number of mature females on L77-994 and PI 88788 was similar, but less reproduction occurred on PI 88788 than on L77-994 and Bedford. These differing reactions between PI 88788 and its progeny support the hypothesis of residual effects of defeated resistance genes. Resistance defined on the basis of nematode reproduction is more precise than that based solely on the number of females attaining maturity in a single nematode generation.

Additional key words: cyst nematodes, *Glycine max*

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, infests soybean (*Glycine max* (L.) Merr.) production areas in 22 states. Infestations are also known in Japan, Korea, and the People's Republic of China (7). In 1979, the loss due to SCN in the United States was estimated to be 1,540,000 metric tons (1). In Illinois, the leading soybean-producing state, the loss was estimated to be 407,000 metric tons (3).

Resistance is an effective and profitable method for controlling SCN when resistant cultivars are available. In southern production areas of the United States where nematode infestations have been known for longer periods of time, there are several publicly developed resistant cultivars that can be grown in the region where soybeans in maturity groups (MG) V, VI, and VII are cultivated. In the northern states, however, where the nematode has a more recent history, there is only one publicly developed resistant MG IV cultivar, Franklin, which can be cultivated by producers in a limited area of the region.

In Illinois, race 3 occurs on approximately 75% and race 4 on 25% of the infested acreage (3). Previous experience with resistant cultivars has demonstrated that selection pressure will cause new races of *H. glycines* to predominate (8,9,12,14). As Franklin is grown on more hectares and in monoculture, race 4 will probably become the predominant race in the MG IV production area of Illinois.

The level of race 4 resistance in the soybean plant introductions (PI) used in breeding for *H. glycines* resistance has not been quantified adequately (4). While attempting to quantify resistance, we found that as the level of resistance increased, fewer mature females developed and the reproductive behavior and morphometrics of females were affected. This paper reports the level of resistance of several soybean lines, the influence of resistant soybeans on the number of mature females and the reproduction and morphology of *H. glycines* race 4.

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MATERIALS AND METHODS

A population of *H. glycines* race 4 was obtained from infested land on the West Tennessee Experiment Station, Jackson, and cultured on the soybean cultivar Forrest in a greenhouse. The reaction of this population to the following nine soybean lines was tested through one nematode generation: Essex, Toyosuzu, Cloud, PI 87631-1, PI 89772, PI 209332, PI 88788, L77-994, and Bedford. Two-day-old larvae used as inoculum were recovered from cysts placed in a mist chamber at 23–27 C. Aliquants containing 3,900 larvae were pipeted into the rhizospheres of four 10-day-old seedlings that had been transplanted into 12-cm clay pots containing 500 cm^3 of heat-treated Onarga loamy sand. Each pot was an experimental unit. Pots were arranged in a randomized complete block design with four replicates in a greenhouse where soil temperatures ranged from 23 to 30 C.

One month after inoculation, soil was removed from roots and female nematodes were extracted by a gravity-sieving technique (2), using nested screens with 850- and 180-μm openings (20- and 80-mesh, respectively). Roots were placed on the upper 850-μm screen and females that still adhered to the roots were dislodged with a stream of water and caught on the second screen. The number of females that had developed was determined immediately after extraction. Female maturation on the various lines was expressed as percent of that on Essex, a common method of presenting the results of race determinations. Females then were selected at random and preserved in vials of 2.5% formalin for later determinations of morphometrics and reproduction. Because there was unequal population development among the cultivars, the following percentages of the females from each experimental unit were selected for study: Essex and Toyosuzu—2.5%; Cloud—5%; PI 87631-1, PI 89772, and Bedford—10%; and L77-994, PI 209332, and PI 88788—20%. Approximately 1,300 individual females were studied. Values used in the analysis of variance and correlations were the means of all the individuals studied from each experimental unit.

Nematodes and egg masses were measured with a camera lucida constructed using the principles outlined by Thorne (11). Female length was measured from the posterior terminus to the anterior tip of the lip region. Width was measured at the widest portion of the body perpendicular to the axis of body length. Egg mass diameter and circumference were determined similarly; however, the egg masses were seldom isodiametric and diameter values for each individual are the average of two measurements. Females were placed in 2.5% NaHClO in order to dissolve the gelatinous matrix and liberate the hatched and unhatched eggs from egg masses for counting. Females then were crushed and the number of eggs inside each nematode was determined. The number of internal eggs per female refers to those enclosed by the female body and does not include eggs deposited in the egg mass. The number of eggs per egg mass is the mean for individuals that produced an egg mass. The total number of eggs inside each female plus those deposited in the egg mass are defined as the total eggs per individual. The means for total eggs per individual and total reproduction (Table 1) were not simply additive and multiplicative, respectively, because of the variability among experimental units.

RESULTS

Levels of resistance to race 4 of *H. glycines* as indicated by the number of females that developed on the soybean lines were quite different (Table 1). Toyosuzu and Essex were highly susceptible with the largest population of females occurring on Toyosuzu. An intermediate level of resistance was found in Cloud, whereas a higher level of resistance was found in PI 87631-1, PI 89772, and Bedford. The lowest number of females developing to maturity occurred with L77-994, PI 209332, and PI 88788.

The trend in levels of resistance based on numbers of mature females was generally followed when the reproductive parameters (percentage of females producing an egg mass, eggs per egg mass, internal eggs per female, total eggs per female, and total reproduction) were analyzed (Table 1). Although the numbers of mature females and reproductive parameters were highly correlated, the means of reproductive parameters were not separable to the same extent as the numbers of females that attained maturity (Tables 1 and 2). Bedford, which has PI 88788 as one of its resistant parents, did not adhere to this trend to the same extent as did PI 87631 and PI 89772. The number of females producing an egg mass, internal eggs per female, total eggs per individual, and total reproduction was significantly lower in Bedford than in PI 87631-1 and PI 89772. The percentage of females producing an egg mass, number of internal eggs per female, total eggs per individual, and total reproduction were greater in L77-994 than in PI 88788, which is the resistant parent of L77-994, but the number of eggs per egg mass did not differ. There were no significant differences between PI 209332 and PI 88788 in any of the reproductive parameters. Total reproduction on PI 209332 was less than on Bedford or L77-994.

Females that matured on Essex and Toyosuzu were larger than those that matured on resistant lines (Fig. 1 and Table 3). Female length and width and egg mass circumference and diameter were also highly correlated with numbers of females attaining maturity, although

Table 1. Female maturation, egg mass production, and reproduction of the soybean cyst nematode, *Heterodera glycines* race 4, on nine soybean lines 1 mo after addition of 3,900 larvae

	Maturation[a]		Reproduction[a]				
Line	White females (no.)	Essex (%)	Females producing an egg mass (%)	Eggs/ egg mass[b] (no.)	Internal eggs/female (no.)	Total eggs/ individual[c]	Log_{10} total reproduction[d]
Toyosuzu	1,080	111	92	118	265	373	5.6032
Essex	973	100	93	172	241	406	5.5895
Cloud	447	46	57	53	180	220	4.9905
PI 87631-1	308	32	45	28	158	170	4.7043
PI 89772	285	29	49	35	165	188	4.7235
Bedford	252	26	26	9	99	101	4.3853
L77-994	175	18	43	23	122	132	4.3520
PI 209332	171	18	35	13	100	105	4.2191
PI 88788	160	16	25	14	81	84	4.1157
FLSD 0.05	64		13	31	26	54	0.1262
cv%	9.7		17.3	39.1	10.8	16.8	1.8

[a] Mean of four replicates.
[b] Per individual producing an egg mass.
[c] Internal eggs per female plus eggs per egg mass for each experimental unit including females without an egg mass.
[d] Total number of eggs per individual in each experimental unit × number of females.

Table 2. Correlation coefficients between *Heterodera glycines* race 4 developmental and reproductive parameters

	No. of mature females	Internal eggs/female	Eggs/ egg mass	Total eggs	Total reproduction	Female length	Female width	Egg mass production	Egg mass circumference	Egg mass diameter
No. of mature females	...	...	...	...	...	...	...	...	...	...
Internal eggs/female	0.91[a]	...	...	...	...	...	...	...	...	...
Eggs/egg mass	0.88	0.87	...	...	...	...	...	...	...	...
Total eggs	0.93	0.96	0.96	...	...	...	...	...	...	...
Total reproduction	0.98	0.90	0.94	0.96	...	...	...	...	...	...
Female length	0.89	0.95	0.88	0.95	0.89	...	...	...	...	...
Female width	0.87	0.95	0.84	0.93	0.86	0.98	...	...	...	...
Egg mass production	0.91	0.93	0.86	0.94	0.91	0.94	0.92	...	...	...
Egg mass circumference	0.86	0.84	0.88	0.89	0.87	0.90	0.86	0.83	...	...
Egg mass diameter	0.82	0.79	0.88	0.87	0.85	0.85	0.82	0.80	0.97	...

[a] All correlations significant at $P<0.01$.

Bedford and L77-994 did not follow this trend (Table 3). Females that matured on Bedford were significantly shorter than those on PI 87631-1, PI 89772, and L77-994 (Table 2). Mature females from PI 88788 were smaller than females that matured on PI 209332 and L77-994, although the population development was the same. Width of females did not differ between PI 88788 and PI 209332 and between Bedford and PI 88788; however, mature females from L77-994 were significantly wider than those on PI 88788. The largest female was recovered from Essex and measured 1,000 × 550 μm. This nematode contained 386 eggs in its body and 346 eggs in the egg mass. The smallest female was recovered from PI 88788 and measured 410 × 170 μm. It contained 10 eggs inside the body and did not produce an egg mass.

DISCUSSION

This study indicates that PI 88788 and PI 209332 are sources of resistant germ plasm that should be used in breeding for resistance to race 4 of SCN. On noninfested land, soybean cultivars resistant to the nematode will seldom yield as well as their susceptible parents or other high-yielding susceptible cultivars (13). In addition, the full level of resistance of the plant introduction is rarely obtained in the resistant cultivar developed from the PI. For these reasons Cloud, PI 87631-1, and PI 89772 should be abandoned as sources of germ plasm for resistance to race 4. Nematode reproduction on these lines is at such a level that nematode damage would occur even if the full level of resistance was incorporated into an agronomically acceptable cultivar. These lines, however, may be useful against other races of SCN and early generation selections from these lines, which already have been developed, should be preserved for future use.

In terms of numbers of mature females, the highly susceptible Essex was not as susceptible as Toyosuzu. Although root weights were not determined in this study, the difference in numbers of females may be due to differences in root mass, which could influence the number of infection sites. Toyosuzu, originally obtained from Japan and maintained in the soybean germ plasm collection at Urbana as PI 423871, was included in this study because it was believed to have SCN resistance (5). The large number of mature females indicates that this line probably does not have resistance to other races.

L77-994, a recent MG III germ plasm release, and Bedford both have PI 88788 as a source of resistance to race 4. The differences in numbers of mature females, egg mass production, internal eggs per female, total eggs per individual, total reproduction, and female morphology found in PI 88788, L77-994, and Bedford support the hypothesis that resistance is conditioned by several genes (10) and indicates that the three lines carry different levels of resistance to race 4. It is also possible that the Peking resistance for races 1 and 3 that was incorporated into Bedford influenced the reaction of Bedford to the population of nematodes used in this study. These results support the hypothesis of Nass et al (6) that "defeated" resistance genes can exert a residual influence on host reaction to a pathogen to which the host is susceptible.

The percentage of females producing an egg mass on Essex and Toyosuzu was probably close to 100. Several egg masses free of a female were found during counting. No dislodged egg masses were detected in extracts from resistant lines. The mean number of eggs in each female from Essex was considerably lower than the 600/cyst reported in some popular articles concerning SCN. Unrealistically high estimates of cyst contents lead to exaggerated potentials for population increases and numbers of overwintering progeny.

The smaller nematode size associated with resistance to SCN may have been overlooked by other workers because sieves with 250-μm openings rather than 180-μm openings are commonly used for cyst extractions. Riggs et al (8) did not observe an effect on female size or eggs per cyst during one generation of race 4 development on PI 88788, but development on this line through several generations appeared to have resulted in decreased female size and fecundity. The decreases were not as pronounced as observed in this study. Small females with few eggs may be infertile or virgin females. Larger females associated with low levels of resistance do not appear to be smaller than females that are recovered from a susceptible line unless critical measurements are made. Resistance also does not seem to be manifested by increasing the time required for female development. Small females that developed on resistant lines appeared to be as mature as their counterparts from Essex and Toyosuzu because some hatched eggs were found inside egg

Table 3. Effect of nine soybean lines on female and egg mass morphometrics of the soybean cyst nematode, *Heterodera glycines* race 4

	Female[a]		Egg mass[a]	
Line	Length (μm)	Width (μm)	Circumference (μm)	Diameter (μm)
Toyosuzu	800	490	1,540	480
Essex	830	490	1,810	570
Cloud	700	420	1,120	360
PI 87631-1	650	370	1,100	350
PI 89772	690	400	1,320	410
Bedford	570	310	1,020	330
L77-994	620	340	940	330
PI 209332	590	310	910	290
PI 88788	540	280	970	340
FLSD 0.05	40	40	160	60
cv%	3.8	6.3	8.7	10.7

[a] Mean of four replicates.

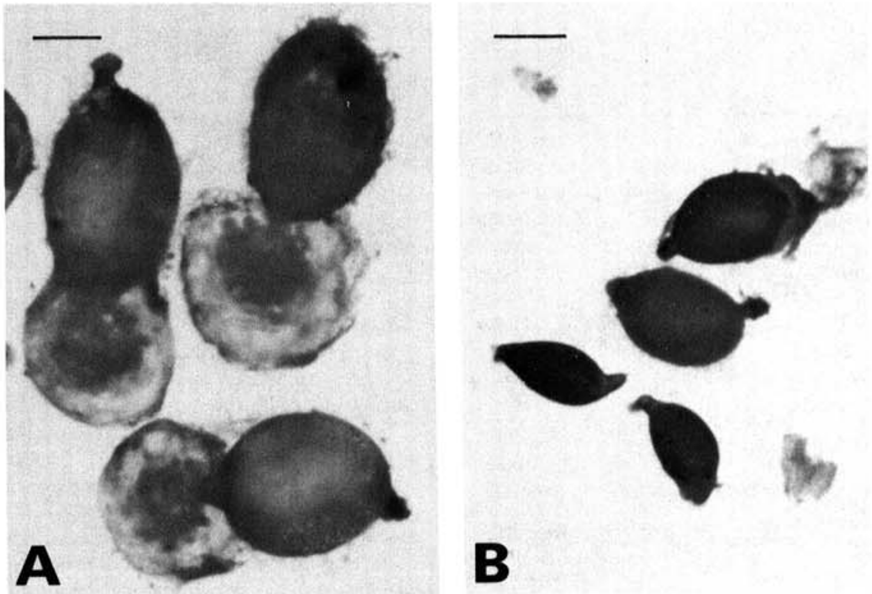

Fig. 1. Females of *Heterodera glycines* race 4 recovered from roots of soybeans 1 mo after infection: (**A**) from susceptible Essex and (**B**) from resistant PI 209332. Note the lack of egg masses on females recovered from PI 209332. Bars represent 250 μm.

masses and females that developed on all lines, regardless of resistance level. This indicated that the females were of the same physiological age.

Defining resistance solely on the basis of numbers of mature females can lead to erroneous conclusions. Significantly fewer mature females occurred on L77-994 than on Bedford, yet the total reproduction on the two lines was nearly identical. Similar final populations could be expected to develop on these two lines during the course of a growing season. Preliminary greenhouse evaluation of large numbers of plants for SCN resistance should rely on numbers of mature females because it is rapid and not labor intensive. However, more critical evaluations of resistance using total reproduction should be conducted when more precise comparisons are required. Total reproduction would be particularly useful when deciding which resistant line to release: one in which more females attain maturity but with superior agronomic traits or an agronomically inferior line in which fewer females attain maturity.

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