Fertility of Three Parasitic Biotypes of *Heterodera glycines*

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ABSTRACT

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Fertility was determined for seven isolates of *Heterodera glycines* representing three biotypes (B0, B3, and B2,3) collected from North Carolina, Arkansas, and Illinois on *Glycine max* 'Lee 68.' Females produced an average of 300 progeny, 29% of which were deposited in the gelatinous matrix. The covariance between eggs deposited in the matrix and the cyst was small (0.29) and their correlation not significant; therefore, cyst egg production was used to compare fertility among isolates. B2,3 females, virulent on resistant *G. max* 'Peking' and PI88788, produced the most progeny—252 eggs per cyst. B3, virulent on PI88788, produced 217 eggs per cyst. B0, the avirulent biotype, produced 198 eggs per cyst. Females inseminated by B2,3, B0, and B3 males produced 256, 228, and 192 eggs, respectively. Nematode biotypes virulent on resistant soybeans were more fertile than the avirulent ones ($k = 100$). Isolates from Illinois produced fewer offspring than those from Arkansas or North Carolina ($k = 100$).

Additional keywords: soybean cyst nematode.

Long-term use of soybean cultivars resistant to *Heterodera glycines* Ichinohe has led to an increase in nematode biotypes virulent on resistant cultivars and, consequently, a change in the race distribution of the parasite (10). This change in race distribution has been termed a "race shift," but it represents little more than a change in the frequency of alleles for virulence within the nematode population (11).

The value of soybean cultivars resistant to *H. glycines* diminishes as the frequency of nematode virulence genes increases. Development of new resistant cultivars requires time and resources; further, breeders must contend with limited sources of resistance to *H. glycines* within the germplasm of *Glycine max* (L.) Merr. This resistance should be deployed in a manner that insures its durability. In the absence of directional selection for virulence from the host, avirulent pathogen biotypes should predominate (14,15). An experiment was designed to test this hypothesis using a cropping systems approach with susceptible hosts and nonhost rotations; however, this approach had no effect on frequency of virulence/avirulence after 5 yr within the field population (D. P. Schmitt, *unpublished data*). The inability to alter the frequency distribution of nematode biotypes through cropping system management suggests that factors other than virulence are important in the fitness of *H. glycines*.

The fitness of an individual is the contribution of offspring it makes to the next generation (2). Fitness also may be defined as the product of an individual's fertility and viability (2). These two traits are measurable in nematode populations; differences in the traits among biotypes may account for the race shifts observed throughout the soybean production areas of the United States and the inability to alter biotype frequency with different cropping systems. Fertility of *H. glycines* is measurable as the number of eggs produced per female and, as a quantitative genetic trait, should be similar among closely related individuals. The objective of this research was to determine the fertility of three biotypes of *H. glycines*.

MATERIALS AND METHODS

Seven isolates, representing three biotypes of *H. glycines,* were tested in greenhouse experiments. Biotype (B) designations are assigned following the proposal of Triantaphyllou (12) and based on the host order used by Golden et al (3). B0 is avirulent and parasitizes only susceptible soybeans, such as Lee 68. B0 isolates used in this experiment originated from Illinois (IL-B0) and North Carolina (NC-B0) and were maintained on Lee 68 in the greenhouse. B3 isolates, with virulence genes against the resistance found in PI88788, were collected from Arkansas (AR-B3), Illinois (IL-B3), and North Carolina (NC-B3) and cultured on PI88788. B2,3 has virulence genes against the resistance in Peking as well as that in PI88788. This biotype was represented by two isolates, one collected from Arkansas (AR-B2,3) and another from North Carolina (NC-B2,3). Both B2,3 isolates were cultured on Peking in the greenhouse. Four of the isolates, NC-B0, NC-B3, NC-B2,3 and IL-B0, had been maintained in greenhouse culture for several years and were homogeneous for parasitic ability. The remaining three isolates were collected from the field and maintained on the appropriate soybean cultivar for eight nematode generations to increase their parasitic homogeneity.

As a female senesces, the egg deposition into the matrix decreases and the number of eggs inside the body begins to increase (D. P. Schmitt, *unpublished data*). Because of this biological phenomenon, fertility of the nematode must be calculated in terms of eggs deposited into the gelatinous matrix and those retained within the cyst.

Matrix eggs. Eggs of the seven isolates were separately inoculated onto roots of 5-day-old Lee 68 seedlings (1,000 eggs per plant) growing in 8-cm-diameter clay pots filled with soil (84% sand, 12% silt, and 4% clay). Pots were maintained in a greenhouse with air temperatures ranging from 18–30 C. Thirty days after inoculation, plants were removed from the pots and rinsed in water. Yellow females were dislodged from the roots with a high-pressure stream of water. The gelatinous matrices were gently separated from 21 females of each isolate and transferred individually into glass watch dishes containing 0.5 ml of water. A drop of 5.25% NaOCl was added to each dish to dissolve the matrix and release the eggs for counting. The experiment was repeated five times.

Cyst eggs and isolate crosses. Eggs of each isolate were collected from 35-day-old cultures, placed in hatching chambers, aerated, and incubated at 27 C for 72 h to collect second-stage juveniles. Lee 68 seedlings with 1.5- to 2.0-cm-long radicles were inoculated with 200–250 of the freshly hatched second-stage juveniles (three seedlings per isolate) in 8-cm-diameter pots. Seedlings were removed from the infested soil 3 days later, rinsed with tap water, and transferred into 8-L grey containers filled with 0.5-strength Hoagland's nutrient solution (5). The hydroponic containers were placed in the greenhouse and aerated, and the nutrient solution was changed every other day.

Males began to emerge from the roots 10 days after transfer to the hydroponic solution and were collected on a 25-μm-mesh screen during each nutrient solution change. Males were stored at 4 C until needed for mating. Soybeans with virgin females still attached to the roots 22 days after placement into the hydroponic system were separately replanted into soil-filled pots. Males of the desired isolate were added to two of the three infected plants at this time. Two weeks were allowed for copulation and female senescence. In the remaining plant, to which no males were added, the numbers of females with eggs and those without eggs were counted to provide an estimate of the amount of sibmating that occurred in each run.

Plants were removed from the pot and the soil was rinsed off the roots. Senescent females (light brown cysts) were dislodged from the soybean roots with a high-pressure stream of water and collected on a 250-μm sieve. Cysts were individually placed into glass watch dishes containing 0.5 ml of water. If eggs were still present in matrices, they were removed and discarded, and the cyst was crushed. A drop of 5.25% NaOCl was added to each dish to assist in dispersing the eggs throughout the dish. Eggs were transferred to a counting dish and enumerated. Each cross was repeated four times with two plants per cross, from which up to 21 females were collected per plant.

Numbers of matrix and cyst eggs were analyzed separately for differences among isolates, and least significant difference was calculated. Individual runs of the cyst egg experiment were analyzed and tested for homogeneity of variance. This chi-square value was not significant; thus, the data were combined for further analysis. Isolate data were combined and matrix and cyst egg production were analyzed according to biotype or origin. Differences in mean number of progeny were detected using a Waller-Duncan k-ratio t test. Correlation and covariance between these traits were calculated. Analysis was made for number of progeny sired by males and for female fertility. Heritability (h^2) was calculated for matrix and cyst eggs according to the following model: fertility $= \mu + T_i + R_j + TR_{ij} + E$, where μ is the mean fertility, T is the cross, R is the run, and E is the sampling error. Only data from isolates NC-B0, NC-B3, NC-B2,3, and IL-B0 were used to estimate h^2 of cyst egg production.

RESULTS

Fertility (based on over 1,800 females) did not differ between replicate plants in a run, so plant was disregarded as a factor in the analysis. Females of *H. glycines* deposited an average of 81 eggs into their gelatinous matrices and retained an average of 219 eggs within the cyst, as calculated from the matrix and cyst egg experiments. The coefficient of variation was similar between the two experiments (69 and 68 for matrix and cyst eggs, respectively). In both matrix eggs and cyst eggs, run and run $\times$ isolate interactions were significant ($P = 0.01$). The h^2 of matrix and cyst egg production was 16.26 and 27.25, respectively. Covariance between number of matrix eggs and cyst egg production was 0.29. The correlation between the traits was not significant ($P = 0.52$).

Sibmating averaged 11.7% over all runs and isolates. In one run 46% of the females were sibmated on one plant, but in all other runs sibmating ranged from 0 to 10% per plant.

The seven isolates of *H. glycines* deposited different numbers of eggs into the gelatinous matrix (Table 1). The number of eggs deposited into the gelatinous matrix at 30 days ranged from 1 to 289 and was normally distributed ($W = 0.94$, skewness $= 0.75$). AR-B3 females deposited the most eggs into the matrix (101 eggs) and IL-B0 females the fewest (41 eggs). Isolates could be divided into three groups on the basis of number of matrix eggs. These statistical groupings correspond to the parasitic biotype of the nematode except for NC-B0. B3 deposited the most eggs into the matrix, followed by B2,3, and then B0 (Table 2).

The number of cyst eggs also differed among the isolates and biotypes. The number of eggs inside the cyst at female senescence ranged from 1 to 693, also following a normal distribution ($W = 0.94$, skewness $= 0.49$). Overall, B2,3 females produced more progeny per cyst than the other two biotypes (Table 2). B3 males sired more progeny than males from B2,3 or B0 (Table 2). NC-B2,3 females produced the greatest number of eggs per cyst, although it was not statistically different ($k = 100$) from the numbers produced by AR-B2,3 or AR-B3 females (Table 1). Females from Arkansas and North Carolina did not produce statistically different numbers of progeny (239 and 224 eggs per cyst, respectively), but both groups were more fertile than females of the Illinois isolates (194 eggs per cyst) ($P = 0.01$). NC-B2,3 males sired the greatest number of progeny, but it was not statistically greater ($k = 100$) than those of NC-B0 or AR-B3 males (Table 1). Arkansas males sired more progeny (241 eggs per cyst) than males from North Carolina (228 eggs per cyst) or Illinois (188 eggs per cyst) ($P = 0.01$).

Both the male and female isolates determined the number of cyst eggs resulting from a mating (Table 3). NC-B2,3 females mated with NC-B2,3 males produced the most progeny, averaging

TABLE 1. Average number of progeny deposited into the matrix and retained within the cyst of seven isolates of *Heterodera glycines* grown on Lee 68 soybean

State of origin	Biotype	Matrix eggs	Cyst eggs: Female parent[x]	Cyst eggs: Male parent[y]
Arkansas	B3	101 a[z]	240 a	245 ab
	B2,3	66 b	237 ab	237 b
Illinois	B3	93 a	203 c	185 c
	B0	41 c	185 c	190 c
North Carolina	B3	93 a	210 bc	171 c
	B2,3	74 b	260 a	265 a
	B0	63 b	206 c	264 ab

[x]Number of eggs for each female biotype averaged over all male biotypes mated to them.

[y]Number of eggs sired by each male biotype averaged over all female biotypes used.

[z]Numbers with the same letter within columns do not differ ($k = 100$) among biotypes on the basis of a Waller-Duncan k-ratio t test. Number of progeny deposited into the matrix or cyst (conducted in separate experiments) is a pooled average for each parent over all crosses involving that parental isolate.

TABLE 2. Average number of eggs deposited in the gelatinous matrix or retained within the cyst of *Heterodera glycines* grouped according to parasitic biotypes

Biotype	Matrix eggs	Cyst eggs: Female parent[x]	Cyst eggs: Male parent[y]
B3	96 a[z]	217 b	192 c
B2,3	69 b	252 a	256 a
B0	55 c	198 c	228 b

[x]Number of eggs for each female biotype averaged over all male biotypes mated to them.

[y]Number of eggs sired by each male biotype averaged over all female biotypes used.

[z]Numbers with the same letter within columns do not differ ($k = 100$) among biotypes on the basis of a Waller-Duncan k-ratio t test. Number of progeny deposited into the matrix or cyst (conducted in separate experiments) is a pooled average for each parent over all crosses involving that parental isolate.

343 eggs per cyst (Table 3). The cross of NC-B3 females with NC-B3 males resulted in the fewest average number of progeny per cyst, yet these NC-B3 females produced 2.25 times more progeny when copulating with NC-B0 males (Table 3). Males of AR-B3, IL-B3, and NC-B3 were never able to maximize the number of progeny produced by a female (Table 3). The male isolate that maximized female fertility varied among the female isolates. Males of a given isolate could not maximize fertility in all females to which they were mated.

TABLE 3. Number of progeny resulting from matings between females and males of seven isolates of *Heterodera glycines* of data pooled over all crosses[y]

	Biotype[z]						
	Male						
Female	AR-B3	AR-B2,3	IL-B3	IL-B0	NC-B3	NC-B2,3	NC-B0
AR-B3	255	297	200	...	195	...	...
AR-B2,3	242	200	...	...	...	266	...
IL-B3	263	...	174	264	163	...	...
IL-B0	...	...	170	194	...	...	219
NC-B3	205	...	192	...	144	267	321
NC-B2,3	...	206	...	...	237	343	329
NC-B0	...	...	...	184	163	208	233

[y] Least significant difference ($P = 0.05$) = 71.17.

[z] AR-B3 = Arkansas isolate virulent on PI88788; AR-B2,3 = Arkansas isolate virulent on PI88788 and Peking; IL-B3 = Illinois isolate virulent on PI88788; IL-B0 = an avirulent Illinois isolate; NC-B3 = North Carolina isolate virulent on PI88788; NC-B2,3 = North Carolina isolate virulent PI88788 and Peking; NC-B0 = an avirulent North Carolina isolate.

DISCUSSION

Fertility of *H. glycines* is a quantitative genetic trait with a low h^2. Life history traits, such as fertility, typically have low h^2 (8). The environment in which the nematode develops is particularly important because the organism is a poikilotherm and an obligate parasite. The rate of nematode development depends on temperature, and extremes can slow development or even incite death (1,9). A female cyst nematode depends intimately on the status of her host because she continues to feed after reaching maturity. A poor host plant can cause female death and/or limit her size (6). This environmental impact is probably the reason that the run and run × isolate interactions were significant in our experiments and is a contributing factor in the small h^2. Although the experiments were conducted under controlled greenhouse conditions, some differences were likely to exist between runs. Runs were conducted sequentially and therefore included time as a contributor to environmental and sampling error.

Genotype also is an important factor in determining fertility of *H. glycines*. The female genotype controls fertility through oocyte production, cyst size, and rate of senescence. The correlation between female size and fertility is probably high because egg size is relatively uniform within a species (4,16) and, thus, egg production is limited in smaller females. Matrix eggs are not correlated to cyst eggs; therefore, it does not appear that females deposit eggs into the matrix at the expense of retaining eggs within their bodies. Egg deposition appears to be mostly dependent on time. Matrix eggs are easily dislodged and separated from a female, resulting in a temporal nature. Because the covariance between matrix and cyst eggs is not significant and matrix eggs are temporal in nature, an estimate of fertility in *H. glycines* can be derived from cyst egg production alone.

The genotype of the male parent also affects fertility. A specific, yet inconsistent, interaction between the male and female isolates determined the number of progeny resulting from a mating. No male isolate, however, consistently sired the most progeny in all females. The differences in the number of progeny sired by males may be due to sperm viability or efficacy of copulation. It is, however, unlikely that the amount of sperm produced by males is a limiting factor in the number of progeny sired (13). The efficacy of copulation could reflect slight morphological aberrations, under genetic control, in the spicules or gubernaculum of particular isolates. Yet these effects were not uniform over all crosses. Inability of a single male isolate to maximize fertility across female isolates may indicate genetic incompatibilities between the different isolates. An incompatibility may exist in *H. glycines* among alleles not associated with virulence; however, it does not appear to be self-incompatibility, as females of several isolates produced the most progeny when mated with males of their own isolate. Other authors have also reported incompatibilities among isolates and races of this nematode (7,11).

The greater fertility of biotypes virulent on resistant soybean cultivars may help to explain the declining frequency of certain biotypes of *H. glycines,* especially B3 (race 1), in North Carolina (10) and other states. The inability of rotations with nonhost and susceptible crops to alter the effects of directional selection on *H. glycines* virulence can be attributed in part to the lower fertility of the avirulent B0 (race 3) nematodes. The avirulent biotype must depend on other factors, i.e., viability, to increase its fitness relative to virulent biotypes. Investigations into the viability of biotypes may show that B0 nematodes survive better than biotypes virulent on resistant cultivars. Traits that favor B0 nematode survival will be useful in cultural manipulations to insure the long-term durability of *H. glycines* resistance in soybeans.

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