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# Effect of the *rhg1* Gene on Population Development of *Heterodera glycines*[1]

Y. H. Li and S. Y. Chen[2]

*Abstract:* The effect of the *rhg1* gene on equilibrium population densities (*E*) and reproduction factors (*Rf*) of *Heterodera glycines* was studied by comparing the nematode population development on two near-isogenic soybean lines (NIL), differing at the *rhg1* locus. The NIL were inoculated with a series of initial egg densities (*Pi*) in the greenhouse. The relationships between final population densities (*Pf* = females per plant or eggs per plant) or *Rf* (final egg density/*Pi*) on both NIL and *Pi* were adequately described by quadratic models. The *rhg1* gene suppressed *Pf* and *Rf* at all *Pi* of a population of *H. glycines* race 3 (HG Type 0–); *E* and maximum *Rf* were higher on the NIL-S line than on the NIL-R line. After two generations of culture of the race 3 population on the NIL-R line, the population selected by the *rhg1* gene (R-eggs) had higher *Pf* and *Rf* on the NIL-R line than the population cultured on the NIL-S line (S-eggs) at all *Pi*. Both R-eggs and S-eggs produced similar egg numbers on the NIL-S line, which was higher than the egg number of either population on the NIL-R line at all *Pi*. The ratio of *E* in female numbers on the NIL-R line to *E* on the NIL-S line increased from 29% for the original race 3 population (S-eggs) to 46% for the *rhg1*-selected population (R-eggs). Regardless of different egg sources, a trend of increase in the number of eggs per female with the rise of *Pi* was observed on the NIL-S line. In contrast, female fecundity of both populations declined with the increase of *Pi* on the NIL-R line. At most inoculum densities, the highest number of eggs per female was observed on the NIL-S line inoculated with the R-eggs, whereas the lowest number of eggs per female was detected on the NIL-R line inoculated with the S-eggs. This study demonstrated that the *E* and maximum *Rf* determined by the quadratic models are useful measurements of plant resistance to nematodes.

*Key words: Glycine max, Heterodera glycines,* near-isogenic soybean, population development, resistance, resistance genes, *rhg1,* soybean cyst nematode.

Soybean cyst nematode (SCN), *Heterodera glycines,* is the most devastating pathogen of soybean, causing an estimated yield loss of $1.5 billion annually in the United States (Wrather et al., 2001). Using SCN-resistant cultivars is one of most effective tactics to reduce nematode population densities and alleviate soybean yield losses caused by the nematode (Bradley and Duffy, 1982; Chen et al., 2001; MacGuidwin et al., 1995; Noel and Sikora, 1990; Wheeler et al., 1997). The effectiveness of nematode management using resistant cultivars depends on the interaction of the cultivars and nematode populations. The resistance to *H. glycines* in soybean is controlled by multiple genes. Genetic composition of nematode populations is highly variable among fields (Niblack and Riggs, 2004). Some individuals of a field SCN population are able to reproduce on cultivars carrying resistance genes. Continuous cropping of the same SCN-resistant cultivar or cultivars from the same resistance source has been associated with increases in reproductive potentials of SCN field populations, changes in nematode races or HG types, and loss of resistance (Young, 1994; Young and Hartwig, 1992; Young et al., 1986). However, it remains undetermined whether a selected SCN population is able to reproduce to a similar population size on the cultivar that selected the population as on susceptible cultivars. It is unknown whether a host carrying SCN-resistance gene(s) and a cultivar without a corresponding SCN-resistance gene will have the same carrying capacity (equilibrium population density) for a selected SCN population that can parasitize both cultivars.

Equilibrium population density is the maximum population that a given environment can support. Nematode equilibrium population density is a function of the size of the food source and the efficiency of a nematode population using that food source in producing offspring. In the relationship between parasitic nematodes and host plants, the efficiency of use of a food source is determined by host status and external environment (Seinhorst, 1967). A good host can support a higher maximum population density than a poor host. Host status is a result of the sum of effects of resistance and other agronomic traits of the plants. Seinhorst (1967) proposed that equilibrium density *E* and maximum reproduction rate *a* (= $e^{rt}$ where *r* is intrinsic rate of population increase, *t* is time interval, and *e* is the natural logarithm base) are two parameters that can be used to quantitatively characterize plant resistance to a nematode population. A good host has high *a* and (or) *E*. To determine the effect of an SCN resistance gene on *a* and *E,* soybean near-isogenic lines that have a high percentage of their genome in common, but differ at an SCN-resistance gene locus, offer unique advantages to minimize the effects of agronomic traits.

The resistance to *H. glycines* in soybean is believed to be conditioned by a number of resistance genes (Caldwell et al., 1960; Matson and Williams, 1965; Myers and Anand, 1991). The resistance in the plant introduction line Peking, for example, was reported to be under the control of at least five genes—*rhgl, rhg 2, rhg 3, rhg 4,* and *rgh 5* (Caldwell et al., 1960; Matson and

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Williams, 1965; Myers and Anand, 1991; Rao-Arelli et al., 1992). Meksem et al. (2001) reported that the *rhg1* gene is required for the resistance in the cultivar Forrest (derived from Peking) to an SCN race 3 population. Analysis of quantitative trait loci based on restriction fragment length polymorphism (RFLP) markers revealed that the *rhg1* gene explained more than 50% of the total phenotypic variation in resistance expression to *H. glycines* in two resistance sources, Plant Introduction (PI) 209332 and PI 90763 (Concibido et al., 1996, 1997; Mudge, 1999). Further, an increasing number of simple sequence repeat (SSR) markers have been identified flanking the *rhg1* gene in close proximity (Cregan et al., 1999; Mudge et al., 1997; Mudge, 1999). The availability of high-density molecular markers targeting the *rhg1* gene, coupled with the presence of the *rhg1* gene in many resistance sources and its relatively high proportional contribution in plant resistance to *H. glycines,* has driven the development of near-isogenic lines (NIL). The NIL were released from a cross between the resistance source PI 209332 and the susceptible cultivar Evans (Mudge, 1999). Derived from individual plants still segregating at the *rhg1* gene locus in the $F_7$ generation, the NIL theoretically share 98% of their genome but differ at the locus of *rhg1* gene (Mudge, 1999). Availability of the NIL has provided opportunities to study the effect of the *rhg1* gene on SCN population development.

Equilibrium population densities can be determined by examining population dynamics over a period of time under defined food sources and environmental conditions. Alternatively, they can be estimated by comparing initial populations and final populations among different locations (spatial) with similar food sources and environmental conditions. In greenhouse or microplot studies, a series of inoculum densities can be used to generate a wide range of initial and final population densities (Seinhorst, 1967, 1970). If initial populations include densities high enough, the equilibrium population density can be determined, and the maximum reproduction rate *a* also can be determined at low initial population density. The objective of this study was to determine the effect of the *rhg1* gene from PI 209332 on the equilibrium population density and maximum reproduction rate of *H. glycines* populations by comparing nematode population development on the NIL, inoculated with a series of initial egg densities in the greenhouse.

## Materials and Methods

*Source of near-isogenic lines:* Previously, 10 heterozygous plants segregating at the *rhg1* gene locus were selected in the $F_7$ generation to produce the NIL (Mudge, 1999). The NIL were designated as NIL7923 Resistant (NIL-R lines) and NIL7923 Susceptible (NIL-S lines) to indicate the presence of the PI 209332 *rhg1* allele in the NIL-R lines and the absence of this allele in the NIL-S lines (Mudge, 1999). Nine RFLP and SSR markers that flanked the *rhg1* gene were used to determine segregation intervals between the NIL (Mudge, 1999). All NIL-R lines and NIL-S lines share the same interval of segregation at the *rhg1* locus, although other crossover regions throughout the soybean genome may differ among the NIL-R lines and the NIL-S lines. The 10 NIL-R lines and 10 NIL-S lines were bulked separately to reduce the variation that may be caused by the regions not shared among them. Seventy seeds from each NIL were arbitrarily selected to produce seedlings. Genotyping using the SSR marker BARC-Satt309 was conducted using leaf samples from 4-day-old seedlings to detect contamination from soybean seeds of other genotypes. The contaminated plants were removed, and the seedlings with the correct genotype were fertilized and watered as needed for seed production. A subsample of purified NIL seeds was genotyped again using the same SSR marker to confirm seed purity. The purified NIL seeds were used in the following tests.

*Greenhouse setup and soybean seedling preparation:* Up to 20 plastic cone-tainers (Stuewe & Sons, Inc., Corvallis, OR) were inserted into steam-sterilized factory sand (Lakeland fine sand, Aggregate Industries, Eagan, MN) in a set of buckets (22-cm-diam. at top, 18.5-cm-diam. at bottom, 27.5 cm tall) to enable the use of a waterbath to maintain a stable temperature at 28°C. The buckets were assembled one inside of the other. The outside bucket was intact to block water entering from the waterbath. Six 1-cm-diam. holes were drilled in the bottom of the inside bucket to drain excess water provided to plants. A piece of filter paper (18.5-cm-diam., Fisher Brand) was placed on the bottom of the inside bucket. Steam-sterilized sand was used to fill the inside bucket 2/3 full before cone-tainers were inserted into the sand. The top of the cone-tainers reached the top of the inside bucket or slightly higher. The cone-tainers were filled with moist steam-sterilized sand.

Seeds of the NIL were planted with 1 seed/cone-tainer at 2 cm deep before the buckets were placed in the waterbath at 28°C. Plants were watered once every 2 days. Photoperiod was 16 hours light. No fertilizer was applied throughout the experiment.

*Effect of the rhg1 gene on population development of SCN race 3 originated from a field (Experiment 1):* An SCN race 3 population was obtained from a research plot where susceptible soybean cultivars had been grown continuously for 5 years in Waseca, Minnesota. The female indices of the population on Peking, Picket, PI 88788, and PI 90763 were 0, 0, 1.5, and 0, respectively, with 518 females/plant on the susceptible check Lee (Li et al., 2004). This population was increased on the susceptible cultivar Sturdy in a greenhouse at 20 to 32°C. A week before inoculation of 7-day-old seedlings, cysts were harvested from Sturdy plants by washing and rubbing the roots under a high-pressure water jet over a set

of nested sieves (850-µm-aperture for the top sieve, 250-µm-aperture for the bottom sieve). The cysts caught on the bottom sieve were separated from debris by centrifugation in a 76% sucrose solution for 10 minutes at 1,500 *g*. The cysts were crushed using a 40-ml glass tissue grinder (Fisher Scientific) to release the eggs. Eggs were suspended in a 37% sucrose solution, centrifuged at 1,500 *g* for 10 minutes, and collected on a 25-µm-aperture sieve. After being washed with deionized $H_2O$, the eggs were saved in a 150-ml glass beaker and the number of eggs was estimated. The eggs were stored at 4°C until the required number of eggs was obtained. Each NIL was replicated four times at each of 16 inoculum densities (50, 100, 200, 400, 800, 1,600, 3,200, 6,400, 12,800, 25,600, 51,200, 102,400, 204,800, 409,600, 819,200, and 1,638,400 eggs/plant). Eggs were re-suspended by gently stirring, and 0.8 ml of egg suspension was applied to roots through a small hole in the sand next to the stem of a seedling. The hole was closed immediately after the inoculation. The experiment was performed in February 2002 (test 1) and repeated in October 2002 (test 2).

*Comparison of an rgh1-selected nematode culture and a culture from the NIL-S line (Experiment 2):* This experiment was designed to determine whether the population selected on the NIL-R line increased in reproductive potential on the NIL as compared to the population developed on the NIL-S line. All eggs produced on the NIL-R line in test 1 of Experiment 1 were bulked after data collection was completed. The eggs were inoculated on 7-day-old seedlings of the NIL-R line. Similarly, the eggs produced on the NIL-S line in test 1 were re-inoculated on the NIL-S line. Thirty days later, females from the NIL-R line and the NIL-S line were extracted separately and eggs were collected. The eggs from the NIL-R line were named R-eggs, and the eggs from the NIL-S line were S-eggs. The R-eggs and S-eggs were increased separately on Sturdy for another 30 days to obtain sufficient inoculum. The eggs of the R-eggs population were inoculated onto 7-day-old seedlings of the NIL-R line and NIL-S line at densities of 400, 800, 3,200, 6,400, 25,600, 102,400, and 204,800 eggs/plant. The eggs of the S-eggs population were inoculated onto seedlings of the NIL at densities of 400, 800, 3,200, 6,400, 25,600, 102,400, 204,800, and 409,600 eggs/plant. Four replicates were used for each inoculation level, and the experiment was performed once.

*Data collection:* Thirty days after inoculation, numbers of females and eggs per cone-tainer were determined. To extract the females from a plant, the sand in a cone-tainer was soaked in water for 3 to 5 minutes before the cone-tainer was positioned upside down over a 5-l beaker. After the plant was gently removed from the cone-tainer, the sand in the cone-tainer was saved in the 5-l beaker. Females were captured on the bottom sieve after the plant root was rubbed and washed under a high-pressure water jet on an 850-µm-aperture sieve nested in a 250-µm-aperture sieve. This was followed by washing the sand in the beaker 3 times, and pouring the suspension through the same set of nested sieves to capture any females that were dislodged during plant handling. Females that were caught on the bottom sieve after processing both the plant and the sand in one cone-tainer were washed into 50-ml tubes with tap water. The number of females per plant was counted at a magnification of 88× using an inverted microscope. Later, the females were crushed using a 40-ml glass tissue grinder (Fisher Scientific) to release eggs, which were collected on a 25-µm-aperture sieve and then transferred into 50-ml tubes. An aliquot of 0.2 to 0.5 ml for the NIL-S line and 0.5 to 1 ml for the NIL-R line was used to count eggs at 88×, and the number of eggs per plant was determined.

*Data analysis:* Theoretically, population growth of a sedentary endoparasitic nematode follows Seinhorst logistic model $Pf = aPi/(1 + (a - 1)Pi/E)$ (Seinhorst, 1966, 1967). Ferris (1985) found that field data fit an exponential model better than the logistic model. Both Seinhorst and Ferris models are based on the assumption that the size of food sources and the population's associated equilibrium density are stable. Thus, these two models can be applied only at the range of nematode population densities that cause undetectable damage to host plants. In this study, however, a wide range of nematode population densities was used, and because the SCN is highly pathogenic to soybean, *E* may vary with inoculum densities. Seinhorst (1970) postulated a model in which *E* in the logistic model is estimated at different population densities by multiplying *E* at low population densities with relative yield $y = m + (1 - m)z^{Pi-T}$, where *m* is minimum yield (in proportion to the yield in the absence of the nematode) at extremely high population densities, Z is a constant that represents the proportion of root system not damaged when parasitized by a single nematode, *Pi* is the initial population density, and *T* is nematode population density below which there is no detectable damage to plants. In this model, a number of parameters need to be estimated and it is difficult to compare statistically population development curves for various nematode populations on different host plants. Alternatively, quadratic models have been used to describe nematode population development where *E* was leveled off by high initial population densities in field studies (McSorley and Dickson, 1989; McSorley and Gallaher, 1993). A quadratic model is similar to the curves described by the Seinhorst model (Seinhorst, 1967). In addition, quadratic models are easier to compare statistically. For these reasons, a quadratic model was used to describe the population growth and estimate the *E* values and maximum reproduction factor *Rf*(= *Pf*/*Pi*) of nematode populations on the NIL:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_2 + \beta_4 X_1 X_2 + \beta_5 X_1^2 X_2$$

Where $Y = \ln Pf$ (*Pf* = number of eggs per plant, number of females per plant, or number of eggs per female) or $\ln Rf$, $X_1 = \ln Pi$, $X_2$ = the resistance in the NIL to SCN ($X_2 = 0$ if NIL-S, $X_2 = 1$ if NIL-R). For the comparison of the two tests in Experiment 1 or egg sources (R-eggs and S-eggs) in Experiment 2, a third independent variable was added into the model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_2 + \beta_4 X_1 X_2 + \beta_5 X_1^2 X_2 + \beta_6 X_3 + \beta_7 X_1 X_3 + \beta_8 X_1^2 X_3 + \beta_9 X_2 X_3 + \beta_{10} X_1 X_2 X_3 + \beta_{11} S_1^2 X_2 X_3$$

Where $X_3$ = tests ($X_3 = 1$ for test 1, and $X_3 = 2$ for test 2) or egg sources ($X_3 = 0$ for S-eggs, and $X_3 = 1$ for R-eggs). The data for females per plant and eggs per plant used in the regression were averages of four replicates. *Rf* was calculated by dividing the average final egg density by *Pi,* and the number of eggs per female was calculated by dividing the average number of eggs per plant by the average number of females per plant. Multiple linear regression was performed to determine the significance (at $P = 0.05$) of the factors (Ln*Pi,* resistance in the NIL, egg sources, and tests) and their interactions (Weisberg, 1985). The model of population development on each NIL was determined by substituting $X_2$ with the number designated for the NIL and $X_3$ by the number designated for the tests in Experiment 1 or the SCN egg source in Experiment 2. With the resulting equation on each line for each nematode population, the maximum *Y* was calculated and the maximum $Pf = e^Y$ is considered as the approximate estimation of *E* values. The same procedure was used to determine the maximum *Rf.*

## Results

*Effect of the rgh1 gene on population development of SCN race 3 originated from a field (Experiment 1):* The regression analyses of *Pf* in relation to *Pi* of an *H. glycines* race 3 on the NIL are summarized in Table 1 and Figures 1–3. Overall, *Pi* and host resistance (*rhg1* gene) both affected *Pf* ($P < 0.05$). Quadratic models fit the data quite well, with coefficients of determination ($r^2$) greater than 0.73 (Table 1). The *rhg1* gene suppressed ($P < 0.05$) final population densities at all initial population densities.

*Egg populations:* Similar quadratic models for egg population development were observed on the two NIL

Table 1. Relationship between final population and initial population density of *Heterodera glycines* race 3 on two near-isogenic soybean lines (Experiment 1).[a]

| Final population (*Pf*) or *Rf*) | Test | Host | Model | $r^2$ | *Pi* at predicted maximum *Pf* or *Rf* | Predicted maximum *Pf* or *Rf* | Figure |
|---|---|---|---|---|---|---|---|
| Eggs per plant | Test 1 and Test 2 | NIL-R and NIL-S | $Y = 2930X_1 - 0.130X_1^2 - 1.658X_2 + 4.667X_3 - 0.886X_1X_3 + 0.042X_1^2X_3 - 5.295$ | 0.92 | | | |
| | Test 1 | NIL-R and NIL-S | $Y = 2.045X_1 - 0.088X_1^2 - 1.658X_2 - 0.629$ | | | | Fig. 1A |
| | | NIL-R | $Y = 2.0447X_1 - 0.088X_1^2 - 2.287$ | | 108,453 | 14,277 | |
| | | NIL-S | $Y = 2.0447X_1 - 0.088X_1^2 - 0.629$ | | 108,453 | 74,958 | |
| | Test 2 | NIL-R and NIL-S | $Y = 1.159X_1 - 0.047X_1^2 - 1.658X_2 + 4.038$ | | | | Fig. 1B |
| | | NIL-R | $Y = 1.159X_1 - 0.047X_1^2 - 2.38$ | | 246,459 | 14,396 | |
| | | NIL-S | $Y = 1.159X_1 - 0.047X_1^2 + 4.0382$ | | 246,459 | 75,579 | |
| Females per plant | Test 1 and Test 2 | NIL-R and NIL-S | $Y = 3.017X_1 - 0.132X_1^2 - 1.065X_2 + 5.044X_3 - 0.951X_1X_3 + 0.044X_1^2X_3 - 11.105$ | 0.93 | | | |
| | Test 1 | NIL-R and NIL-S | $Y = 2.066X_1 - 0.088X_1^2 - 1.065X_2 - 6.061$ | | | | Fig. 1C |
| | | NIL-R | $Y = 2.0658X_1 - 0.088X_1^2 - 7.126$ | | 123,536 | 146 | |
| | | NIL-S | $Y = 2.066X_1 - 0.088X_1^2 - 6.061$ | | 123,536 | 424 | |
| | Test 2 | NIL-R and NIL-S | $Y = 1.115X_1 - 0.044X_1^2 - 1.065X_2 - 1.017$ | | | | Fig. 1D |
| | | NIL-R | $Y = 1.115X_1 - 0.044X_1^2 - 2.082$ | | 334,633 | 150 | |
| | | NIL-S | $Y = 1.115X_1 - 0.044X_1^2 - 1.017$ | | 334,633 | 434 | |
| Rf | Tests 1 and 2 | NIL-R and NIL-S | $Y = 1.93X_1 - 0.13X_1^2 - 1.568X_2 - 4.667X_3 - 0.886X_1X_3 + 0.042X_1^2X_3 - 5.295$ | 0.96 | | | |
| | Test 1 | NIL-R and NIL-S | $Y = 1.045X_1 - 0.088X_1^2 - 1.568X_2 - 0.629$ | | | | Fig. 2A |
| | | NIL-R | $Y = 1.045X_1 - 0.088X_1^2 - 2.197$ | | 374 | 2.5 | |
| | | NIL-S | $Y = 1.045X_1 - 0.088X_1^2 - 0.629$ | | 374 | 11.8 | |
| | Test 2 | NIL-R and NIL-S | $Y = 1.59X_1 - 0.047X_1^2 - 1.568X_2 - 4.038$ | | 5 | | Fig. 2B |
| | | NIL-R | $Y = 1.59X_1 - 0.047X_1^2 - 2.470$ | | 5 | | |
| | | NIL-S | $Y = 1.59X_1 - 0.047X_1^2 - 4.038$ | | | | |
| Eggs per female | Tests 1 and 2 | NIL-R and NIL-S | $Y = 5.074 + 0.015X_1 - 0.041X_2 - 0.061X_1X_2$ | 0.73 | | | Fig. 3 |
| | | NIL-R | $Y = 5.033 - 0.045X_1$ | | | | |
| | | NIL-S | Not significant at $P = 0.05$ | | | | |

[a] NIL-R = Near-isogenic line containing *rhg1* gene; NIL-S = Near-isogenic line without *rhg1.* In the model, $Y = \ln Pf$ (*Pf* = number of eggs per plant, number of females per plant, or number of eggs per female 30 days after inoculation) or $\ln Rf$ ($Pf/Pi$ where *Pi* is initial number of eggs per plant), $X_1 = \ln Pi$, $X_2$ = the resistance to SCN ($X_2 = 0$ if NIL-S, $X_2 = 1$ if NIL-R). $X_3$ = test ($X_3 = 1$ for test 1, and $X_3 = 2$ for test 2). $r^2$ = coefficient of determination; all $r^2$ values are significant at $P < 0.001$.

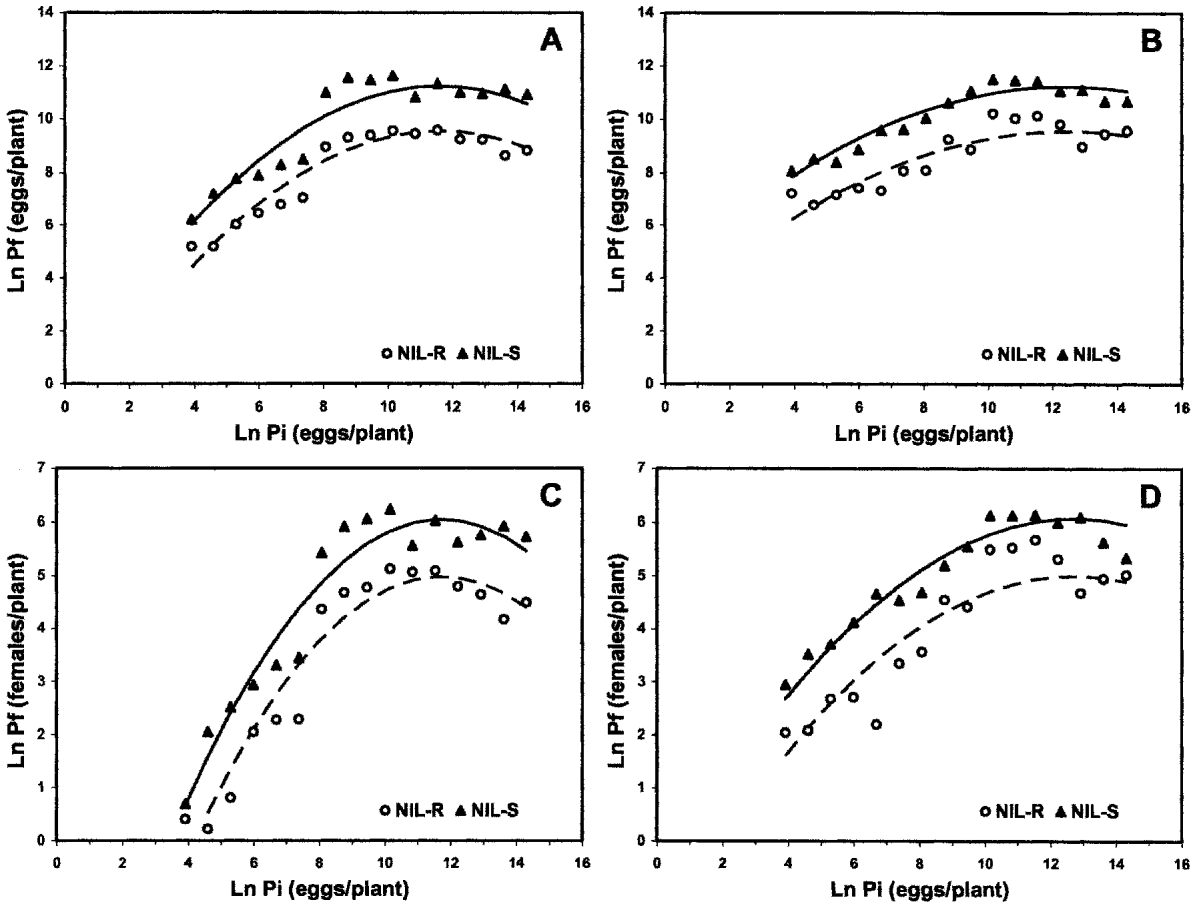


FIG. 1. Relationship between final population densities (*Pf* = eggs per plant or females per plant 30 days after inoculation) and initial population densities of *Heterodera glycines* race 3 on two near-isogenic lines (NIL) (Experiment 1). NIL-R = the near-isogenic line carrying *rhg1;* NIL-S = the near-isogenic line without *rhg1*. A and C) Test 1. B and D) Test 2. The regression analysis is presented in Table 1.

within a test (Fig. 1A,B). However, there was an interaction ($P < 0.001$) between test and *Pi* that influenced *Pf* (Table 1). The *Pi* at which the *E* was reached was the same for both NIL within a test, but it was higher in test 2 than in test 1 ($P < 0.001$). In test 1, the *E* of egg populations was 14,277 and 74,958 eggs/plant on the NIL-R and NIL-S lines, respectively, when the *Pi* was 108,453 eggs/plant (Table 1; Fig. 1A). In test 2, the *E* of egg populations on both NIL lines (14,396 and 75,579 eggs/plant on NIL-R and NIL-S, respectively) was similar to that in test 1, but a *Pi* of 246,495 eggs/plant was needed to obtain the *E* (Table 1 and Fig. 1B).

*Female populations:* The response of female populations to the *rhg1* gene was similar to that of the egg

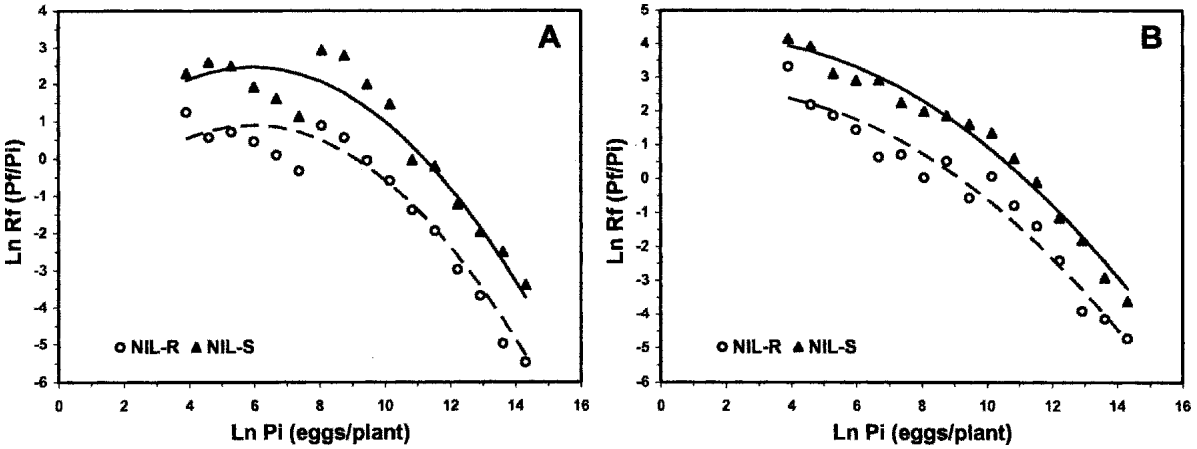


FIG. 2. Relationship between reproduction factor *Rf* (*Rf* = *Pf*/*Pi* where *Pf* = eggs per plant 30 days after inoculation) and initial population densities (*Pi*) of *Heterodera glycines* races 3 on two near-isogenic lines (NIL) (Experiment 1). NIL-R = the near-isogenic line carrying *rhg1;* NIL-S = the near-isogenic line without *rhg1*. A) Test 1. B) Test 2. The regression analysis is presented in Table 1.

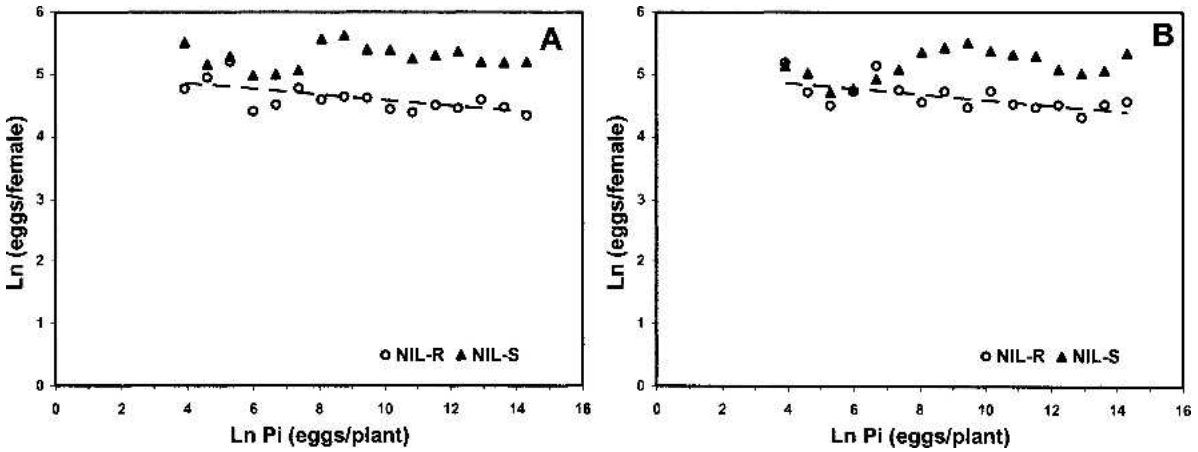


FIG. 3. Relationship between female fecundity (number of eggs produced per female 30 days after inoculation) and initial population densities (*Pi*) of *Heterodera glycines* race 3 on two near-isogenic lines (NIL) (Experiment 1). NIL-R = the near-isogenic line carrying *rhg1;* NIL-S = the near-isogenic line without *rhg1*. A) Test 1. B) Test 2. The regression analysis is presented in Table 1.

populations. Similar quadratic models for female population development were observed on the two NIL within a test (Fig. 1C,D). In test 1, the *E* of female populations was 146 and 424 females/plant on the NIL-R and NIL-S lines, respectively, when the *Pi* was 123,536 eggs/plant (Table 1; Fig. 1C). In test 2, the *E* of female populations on both NIL lines (150 and 434 females/plant on NIL-R and NIL-S, respectively) was similar to that in test 1, but a higher *Pi* (334,633 eggs/plant) was needed to obtain the *E* (Table 1; Fig. 1D).

*Reproduction factor (Rf = Pf/Pi):* The *Rf* increased with increasing *Pi* at low inoculum levels and decreased with further increases in *Pi*. The predicted maximum *Rf* and the *Pi* at which the maximum *Rf* was obtained differed between the two tests. In test 1, the predicted maximum *Rf* was 2.5 and 11.8 on the NIL-R and NIL-S lines, respectively, at *Pi* = 374 eggs/plant (Table 1; Fig. 2A). In test 2, the maximum *Rf* was predicted at *Pi* = 5 eggs/plant, which was out of the inoculation range (Table 1; Fig. 2B).

*Female fecundity:* An interaction of *Pi* and the NIL on the number of eggs produced per female was observed ($P < 0.001$). The curves in tests 1 and 2 showed a similar pattern (Fig. 3). Overall, fewer eggs were produced by a female on the NIL-R line than on the NIL-S line. On the NIL-R line, number of eggs per female decreased with increasing *Pi* (Table 1; Fig. 3). On the NIL-S line, the relationship between number of eggs per female and *Pi* was not described by either a quadratic or linear model (Fig. 3).

*Comparison of an rhg1-selected nematode culture and a culture from the NIL-S line (Experiment 2):* Regression analysis of nematode final populations on the NIL in relation to *Pi* of two different egg populations (R-eggs and S-eggs) that had been separately cultured on the NIL-R line and the NIL-S line are summarized in Table 2 and Figures 4–6. Overall, the egg population selected on the NIL-R line (R-eggs) had increased reproductive potential on the NIL-R line as compared with the population developed on the NIL-S line (S-eggs). In all combinations of nematode populations and the NIL, the relationship between *Pf* (eggs per plant and females per plant) and *Pi* was described by quadratic models with $r^2$ greater than 0.86 (Table 2).

*Egg populations:* An interaction between egg sources and the NIL was observed ($P < 0.01$), indicating that the effect of egg source on nematode population development differed between the two NIL. On the NIL-R line, the R-eggs had a greater reproductive potential than the S-eggs (Fig. 4A). The predicted *E* of egg populations on the NIL-R line was 33,066 eggs/plant for the R-eggs and 16,339 eggs/plant for the S-eggs (Table 2; Fig. 4A). On the NIL-S line, both R-eggs and S-eggs had a similar reproductive potential based on predicted *E,* which was higher than the *E* for egg populations on the NIL-R line (Table 2; Fig. 4A).

*Female populations:* The effect of nematode source on female population growth was similar to the effect on egg populations (Fig. 4). On the NIL-R line, the R-eggs produced a greater ($P < 0.001$) number of females than did the S-eggs (Fig. 4B). The predicted *E* of female populations on the NIL-R line was 159 females/plant for the S-eggs and 249 females/plant for the R-eggs. On the NIL-S line, similar numbers of females formed for R-eggs and S-eggs with predicted *E* of 540 females/plant, which was higher ($P < 0.001$) than the *E* populations on the NIL-R line (Fig. 4B).

*Reproduction factor:* The effect of nematode source on *Rf* differed between the two NIL (Table 2; Fig. 5). The R-eggs had a higher ($P < 0.001$) *Rf* than the S-eggs when they were inoculated separately on the NIL-R line. On the NIL-S line, no difference in *Rf* was detected between the two egg sources. The predicted maximum *Rf* of the R-eggs on NIL-R line was 4.6 at *Pi* = 114 eggs/plant, whereas the maximum *Rf* of the S-eggs was only 2.3 on the NIL-R line at the same *Pi* level. The maximum *Rf* of both R-eggs and S-eggs was 14.7 on the NIL-S line at the same *Pi* level (Table 2).

TABLE 2. Relationship between final population and initial population density of two *Heterodera glycines* populations that were separately cultured on resistant and susceptible near-isogenic soybean lines (NIL) and each of them was re-inoculated onto the NIL (Experiment 2).[a]

| Final population (*Pf*) or *Rf* | Egg source | Host | Model | $r^2$ | *Pi* at predicted maximum *Pf* or *Rf* | Predicted maximum *Pf* or *Rf* | Figure |
|---|---|---|---|---|---|---|---|
| Eggs per plant | R-eggs and S-eggs | NIL-R and NIL-S | $Y = 1.571X_1 - 0.06X_1^2 - 1.858\ X_2 + 0.004X_3 - + 0.701X_2X_3 + 1.333$ | 0.95 | | | Fig. 4A |
| | R-eggs | NIL-R and NIL-S | $Y = 1.571X_1 - 0.06X_1^2 - 1.158X_2 + 1.333$ | | | | |
| | | NIL-R | $Y = 1.571X_1 - 0.06X_1^2 + 0.18$ | | 449,636 | 33,066 | |
| | | NIL-S | $Y = 1.571X_1 - 0.06X_1^2 + 1.337$ | | 449,636 | 105,221 | |
| | S-eggs | NIL-R and NIL-S | $Y = 1.571X_1 - 0.06X_1^2 - 1.858X_2 + 1.333$ | | | | |
| | | NIL-R | $Y = 1.571X_1 - 0.06X_1^2 - 0.525$ | | 449,636 | 16,339 | |
| | | NIL-S | $Y = 1.571X_1 - 0.06X_1^2 - 1.333$ | | 449,636 | 104,792 | |
| Females per plant | R-eggs and S-eggs | NIL-R and NIL-S | $Y = 1.441X_1 - 0.054X_1^2 - 1.225X_2 + 0.451X_2X_3 - 3.402$ | 0.96 | | | Fig. 4B |
| | R-eggs | NIL-R and NIL-S | $Y = 1.441X_1 - 0.054X_1^2 - 0.773X_2 - 3.402$ | | | | |
| | | NIL-R | $Y = 1.441X_1 - 0.054X_1^2 - 4.176$ | | 698,609 | 249 | |
| | | NIL-S | $Y = 1.441X_1 - 0.054X_1^2 - 3.402$ | | 698,609 | 540 | |
| | S-eggs | NIL-R and NIL-S | $Y = 1.441X_1 - 0.054X_1^2 - 1.225X_2 - 3.402$ | | | | |
| | | NIL-R | $Y = 1.441X_1 - 0.054X_1^2 - 4.627$ | | 698,609 | 159 | |
| | | NIL-S | $Y = 1.441X_1 - 0.054X_1^2 - 3.402$ | | 698,609 | 540 | |
| Rf | R-eggs and S-eggs | NIL-R and NIL-S | $Y = 0.571X_1 - 0.06X_1^2 - 1.858X_2 + 0.004X_3 - + 0.701X_2X_3 + 1.333$ | 0.97 | | | Fig. 5 |
| | R-eggs | NIL-R and NIL-S | $Y = 0.571X_1 - 0.06X_1^2 - 1.158X_2 + 1.337$ | | | | |
| | | NIL-R | $Y = 0.571X_1 - 0.06X_1^2 + 0.18$ | | 114 | 4.6 | |
| | | NIL-S | $Y = 0.571X_1 - 0.06X_1^2 + 1.337$ | | 114 | 14.7 | |
| | S-eggs | NIL-R and NIL-S | $Y = 0.571X_1 - 0.06X_1^2 - 1.858X_2 + 1.333$ | | | | |
| | | NIL-R | $Y = 0.571X_1 - 0.06X_1^2 - 0.525$ | | 114 | 2.3 | |
| | | NIL-S | $Y = 0.571X_1 - 0.06X_1^2 - 1.333$ | | 114 | 14.7 | |
| Eggs/female | R-eggs and S-eggs | NIL-R and NIL-S | $Y = 0.049X_1 + 0.317X_2 + 0.198X_3 - 0.09X_1X_2 + 4.773$ | 0.86 | | | Fig. 6 |
| | R-eggs | NIL-R and NIL-S | $Y = 0.049X_1 + 0.317X_2 + 0.09X_1X_2 + 4.972$ | | | | |
| | | NIL-R | $Y = -0.004X_1 + 5.288$ | | | | |
| | | NIL-S | $Y = 0.049X_1 + 4.972$ | | | | |
| | S-eggs | NIL-R and NIL-S | $Y = 0.049X_1 + 0.317X_2 + 0.09X_1X_2 + 4.773$ | | | | |
| | | NIL-R | $Y = -0.04X_1 + 5.09$ | | | | |
| | | NIL-S | $Y = 0.049X_1 + 4.773$ | | | | |

[a] NIL-R = Near-isogenic line containing *rhg1* gene; NIL-S = Near-isogenic line without *rhg1*. R-eggs = egg population of race 3 cultured for two generations on NIL-R plants; S-eggs = egg population of race 3 cultured only on the NIL-S plants. In the model, $Y = \ln Pf$ ($Pf$ = number of eggs per plant, number of females per plant, or number of eggs per female 30 days after inoculation) or $\ln rf$ ($Pf/Pi$ where $Pi$ is initial number of eggs per plant); $X_1 = \ln Pi$; $X_2$ = the resistance to SCN ($X_2 = 0$ if NIL-S, $X_2 = 1$ if NIL-R); $X_3$ = egg sources ($X_3 = 1$ for R-eggs, and $X_3 = 0$ for S-eggs). $r^2$ = coefficient of determination; all $r^2$ = coefficient of determination; all $r^2$ values are significant at $P < 0.001$.

*Female fecundity:* Selection of SCN by the *rhg1* gene on the NIL-R line increased the number of eggs per female in two subsequent generations on either the NIL-R or NIL-S line (Table 2; Fig. 6). An interaction of the NIL and *Pi* on number of eggs per female was observed ($P < 0.001$). Regardless of the egg source, numbers of eggs per female increased with increasing *Pi* on the NIL-S line, while it declined with increasing *Pi* on the NIL-R line (Fig. 6).

## DISCUSSION

The resistance of a soybean cultivar to *H. glycines* is measured by comparison of the number of females developed on the resistant cultivar with the number of females on a susceptible cultivar. This study showed that numbers of females that developed on the NIL were influenced by initial population density. Further, it appeared that the ratio of females developed from the NIL-R line to females from the NIL-S line was positively related to the initial population density. Because female development was also affected by environment, this work confirms that, for a relative measurement of SCN resistance based on numbers of females, standardized procedures including initial population densities and constant environmental conditions are important (Riggs and Schmitt, 1991; Wang et al., 1998).

The effect of a resistance gene on female development may be only one aspect of resistance. Seinhorst (1967) theorized that the maximum multiplication rate *a* and equilibrium population density *E* should be used in the evaluation of resistance. On a good host, both *a* and *E* are large, and on very poor hosts both are small. In intermediate cases one of them may be fairly large and the other may be small or both intermediate. In logistic models, $a = Rf$ when $Pi \rightarrow 0$. In this study, predicted *E* and maximum *Rf* of SCN populations were consistently lower on the NIL-R line than on the NIL-S line (Tables 1, 2). This suggests that *E* and maximum *Rf* described or predicted with quadratic models are also

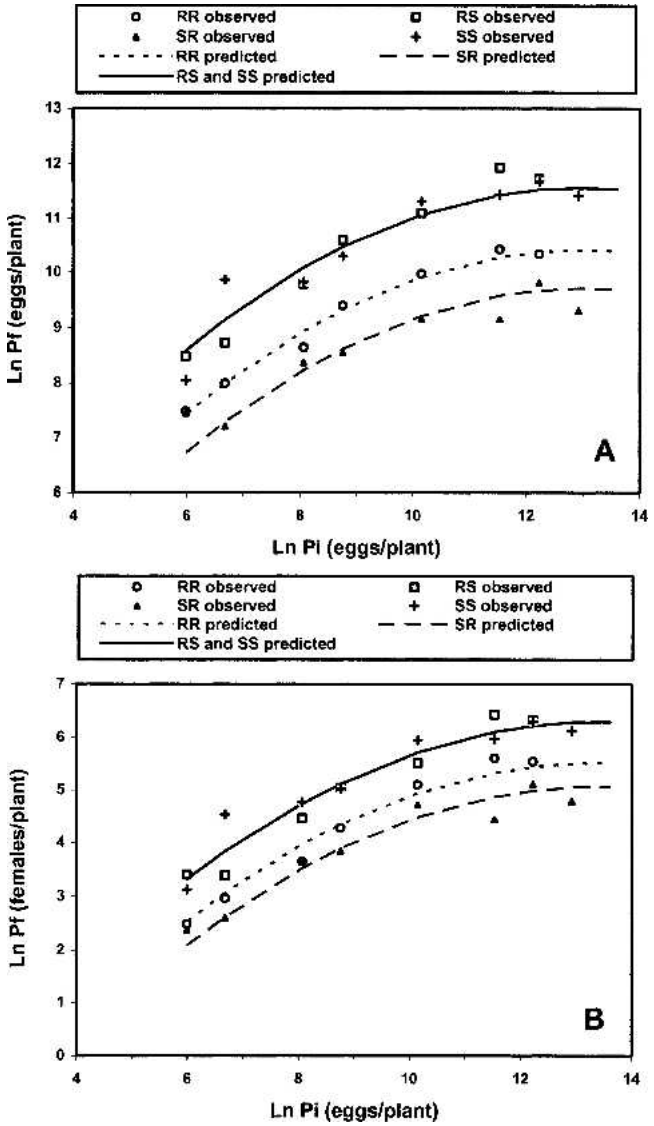


FIG. 4. Relationship between final population densities (*Pf*) as eggs per plant (A) or females per plant (B) 30 days after inoculation and initial population densities (*Pi*) of two *Heterodera glycines* populations that were cultured separately on the resistant and susceptible near-isogenic lines (NIL) (Experiment 2). NIL-R = the near-isogenic line carrying *rhg1;* NIL-S = the near-isogenic line without *rhg1*. R-eggs = the egg population of a field race 3 cultured on the NIL-R line for two generations; S-eggs = the egg population of the same field race 3 developed on the NIL-S line for two generations. RR = R-eggs inoculated on the NIL-R line; RS = R-eggs inoculated on the NIL-S line; SR = S-eggs inoculated on the NIL-R line; SS = S-eggs inoculated on the NIL-S line. The regression analyses are presented in Table 2.

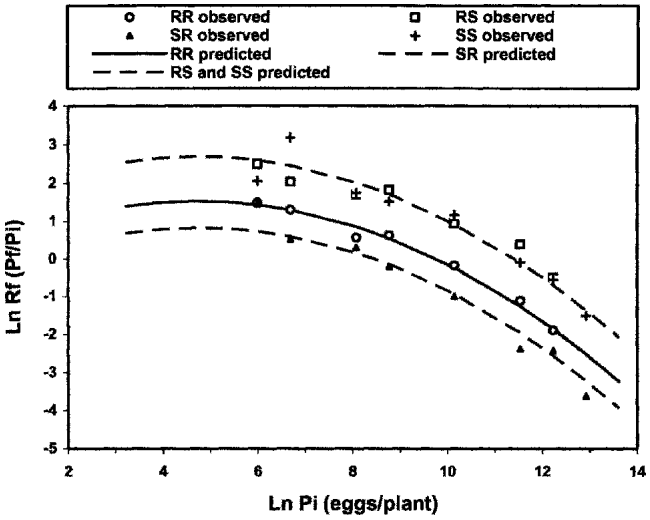


FIG. 5. Relationship between reproduction factor *Rf* (*Rf* = *Pf*/*Pi* where *Pf* = eggs per plant 30 days after inoculation) and initial population densities (*Pi*) of two *Heterodera glycines* populations that were cultured separately on the resistant and susceptible near-isogenic lines (Experiment 2). NIL-R = the near-isogenic line carrying *rhg1;* NIL-S = the near-isogenic line without *rhg1*. R-eggs = the egg population of a field race 3 cultured on the NIL-R line for two generations; S-eggs = the egg population of the same field race 3 developed on the NIL-S line for two generations. RR = R-eggs inoculated on the NIL-R line; RS = R-eggs inoculated on the NIL-S line; SR = S-eggs inoculated on the NIL-R line; SS = S-eggs inoculated on the NIL-S line. The regression analyses are presented in Table 2.

useful measurements in examining soybean resistance to *H. glycines*.

It remains unclear why the maximum *Rf* of SCN predicted with the quadratic models were at low $Pi > 0$ rather than at $Pi \rightarrow 0$. It could be a phenomenon of underpopulation (Kort, 1962; Seinhorst, 1968). The soybean cyst nematode is an amphimictic species, with mating between females and males required for production (Triantaphyllou and Hirschmann, 1962). Mating probabilities between males and females may be reduced when nematode populations are low. This may result in a decrease in population growth, compared with a logistic growth where the mating probability is not assumed to influence reproduction. Further study is needed to confirm the underpopulation phenomenon of SCN and at which *Pi* it may occur. Because of the underpopulation effect, the actual maximum multiplication rate *a* should be higher than that predicted using quadratic models.

We observed an increase in reproduction potential of the nematode population after being cultured on the NIL-R line for two generations. This suggests that the resistant line selected compatible individuals. However, the selected individuals may not be genetically homogenous; segregation is expected in the first few generations where both compatible and incompatible offspring are produced. If we consider that the compatible individuals are those that are able to develop to females on the NIL-R line regardless of the number of eggs per female, 29% of the individuals in the original race 3 population were compatible (maximum number of females developed on the NIL-R line per maximum number of females developed on the NIL-S line). However, after selection on the NIL-R line for two generations, the compatible individuals in the selected nematode population increased to 46%. This appears to be a rapid change in genetic composition of a nematode population. In the field, it may take several years of planting a resistant cultivar for an SCN population to

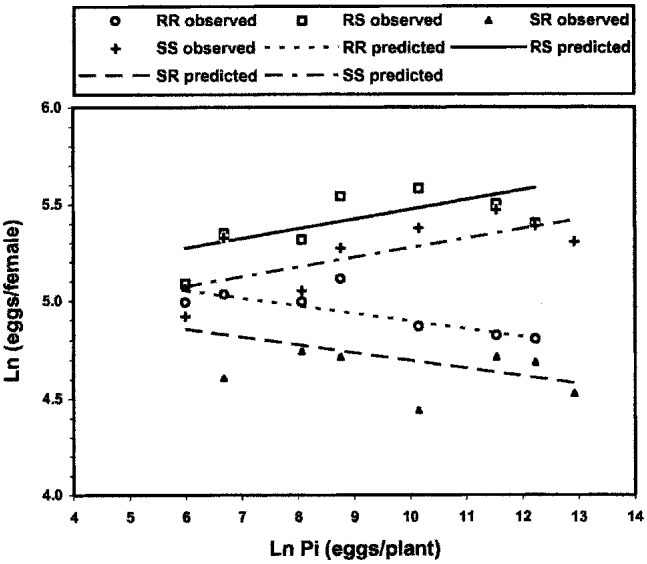


FIG. 6. Relationship between female fecundity (number of eggs produced per female 30 days after inoculation) and initial population densities (*Pi*) of two *Heterodera glycines* populations that were cultured separately on the resistant and susceptible near-isogenic lines (Experiment 2). NIL-R = the near-isogenic line carrying *rhg1;* NIL-S = the near-isogenic line without *rhg1*. R-eggs = the egg population of a field race 3 cultured on the NIL-R line for two generations; S-eggs = the egg population of the same field race 3 developed on the NIL-S line for two generations. RR = R-eggs inoculated on the NIL-R line; RS = R-eggs inoculated on the NIL-S line; SR = S-eggs inoculated on the NIL-R line; SS = S-eggs inoculated on the NIL-S line. The regression analyses are presented in Table 2.

change its race status (Young, 1994). This is because both the individuals produced on a resistant cultivar and the surviving individuals from previous susceptible cultivars represent initial inocula in the following seasons. The incompatible individuals from previous seasons may compete with the compatible individuals because both compatible and incompatible individuals are able to penetrate soybean roots (Li et al., 2004) and may slow the rate of genetic shift. Males from early generations can mate with recently selected compatible females, maintaining genetic diversity for incompatibility (Jones and Parrott, 1965). The variation in environmental conditions may be another possible reason for the slower change of nematode populations in the field.

In this study, the highest *Pi* was 1,638,400 eggs/plant. At this level, an estimate of more than 475,000 eggs/plant ($Pi \times 29\%$) may be able to reproduce on the NIL-R line, far above the *Pi* level at which the predicted *E* in the number of eggs per plant was obtained on the NIL-S line. If population growth is regulated only by these compatible individuals, the *E* of a population of 100% compatible individuals is expected to occur before reaching this *Pi*. Because the predicted *E* of the original race 3 population on the NIL-R line was lower than that of the R-eggs on the NIL-R line, it could be expected that a 100% compatible nematode population would have a much higher *E* than what was observed with the original race 3 population on the NIL-R line. These results suggest that the incompatible individuals also regulated nematode population development, which may be mainly through the ability of these nematodes to penetrate and develop to third and fourth stages of juveniles in the roots of the NIL-R line (Li et al., 2004). The penetration and development of the incompatible nematodes to later juvenile stages will certainly compete with compatible individuals for space and possibly for nutrients.

Although both *Rf* and *E* increased after a nematode population was cultured on the NIL-R line for two successive generations, it remains unknown whether it is possible to obtain a selected nematode population in which every individual is compatible with the *rhg1* gene and whether this population will have similar *a* and *E* as the original population on the NIL-S line. Knowledge of *a* and *E* for a compatible population on a resistant cultivar is important for the development of SCN management programs. In the meantime, further research is needed to determine the effect of the *rhg1* gene on SCN population dynamics under field conditions.

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