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Fitness Components and Selection of Biotypes of *Heterodera glycines*[1]

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Abstract: Survival of biotypes of *Heterodera glycines* was studied in microplots and in the field. The field population was subjected to various cropping sequences. Viability of eggs overwintered in microplots was determined each spring by percentage hatch, percentage of hatched eggs penetrating roots, and numbers of females developing on Peking and PI 88788 soybeans. Eggs from the field were collected in the spring and fall and assayed for ability to develop on Peking and PI 88788. Hatch of isolates overwintered in the microplots averaged 13% in May 1989 and 19% in 1990. No differences in hatch were detected among the isolates in 1989. Numbers of juveniles penetrating susceptible roots averaged less than 20% of the hatched eggs each year. An isolate of a biotype parasitic on susceptible soybeans and the resistant soybean PI 88788 penetrated roots more successfully than other biotypes. A second isolate from North Carolina, parasitic on susceptible soybeans, PI 88788, and the resistant soybean Peking experienced selection against development on Peking during two winters. Only 17% of the expected numbers of females developed on Peking from this isolate. In the microplot experiment, parasitism of PI 88788 and Peking had a selective disadvantage (selection coefficient) of $s = 0.29$ and 0.62 over all isolates, respectively. In the field experiment, the relative numbers of cysts on Peking and PI 88788 increased between the spring and fall on soybean, then decreased over the winter and under corn. Selection coefficients against parasitism of PI 88788 and Peking averaged 0.19 and 0.3 in the field population. In neither experiment did juveniles lose their ability to parasitize susceptible soybeans.

Key words: fitness, genetics, *Heterodera glycines*, nematode, race, selection, soybean cyst nematode, survival.

Infestations of *Heterodera glycines* Ichinohe are difficult to eliminate; therefore, management practices must be suited to deployment over multiple growing seasons. The most successful management tactics against this nematode are crop rotation and resistant cultivars (14). Long-term deployment of resistance, however, can place strong selection pressure on a pest population to develop virulence on the resistant host. Virulent nematode biotypes may even come to predominate within a population, rendering resistant cultivars ineffective.

Most field infestations of *H. glycines* are a mixture of biotypes (15), and continual selection from resistant cultivars may change the frequency of parasitic biotypes within a population. The frequency of biotypes able to develop on a resistant host increased 25-fold after only seven generations of selection in the greenhouse (15). In an Arkansas field population, the frequency of biotypes parasitic on 'Bedford' soybean increased 20-fold after 6 years of selection under this resistant host (10). An increase in the frequency of nematode biotypes virulent on resistant cultivars has been indirectly observed in North Carolina as a decrease in the frequency of race 1 populations of *H. glycines* over the past 15 years (11). Such changes are termed "race shifts."

The race shift in North Carolina represented changes in the underlying gene frequency in the nematode population (15). Genetic frequencies were presumably altered by directional selection from resistant cultivars. Resistance in *Glycine max* (L.) Merr. to *H. glycines* is found in several accessions in the USDA soybean germplasm collection, yet is derived most frequently from Peking and (or) PI 88788 (7). Many *H. glycines*-resistant cultivars in use today have both sources in their pedigrees (7),

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and these sources are not allelic (15). Nematode parasitism of these resistant soybeans is controlled by several genes, of which at least one is inherited dominantly (15). Therefore, some research scientists are concerned that continual deployment of resistance may negate its effectiveness as a control practice.

Stabilizing selection, preventing the increase of virulence within a pathogen population (17), may be a strategy employable against race shifts and the subsequent loss of resistant cultivars. Cropping sequences involving host and nonhost plants theoretically could minimize selection of any particular nematode biotype. The nematodes that survive a crop rotation will have been selected for factors other than parasitic ability. Because virulence on a resistant host often is associated with lower pathogen fitness (5), avirulent pathogen biotypes should predominate in the absence of host resistance. The objectives of this research were to measure components of nematode fitness associated with viability and to determine if selection occurs in endemic nematode populations under different cropping sequences.

Materials and Methods

Experiment 1: Fitness components associated with viability

Three parasitic biotypes of *H. glycines* were selected for overwintering studies in microplots. The biotypes corresponded to races 1, 2, and 3 of *H. glycines* and are designated B3; B2,3; and B0, respectively (16). B3 was parasitic on susceptible cultivars and the resistant soybean PI 88788. B2,3 parasitized susceptible soybeans, as well as the resistant soybeans Peking and PI 88788. B2,3 and B3 were represented by two isolates each, one originating in North Carolina and a second from Arkansas. B0 parasitized only susceptible soybeans and was represented by a single isolate from North Carolina. Parental populations of these five isolates had been maintained in the greenhouse for many generations and were homogeneous for parasitic abilities (i.e., fixed for or against virulence on Peking and [or] PI 88788). Nematode viability was determined through percentage egg hatch, percentage penetration of Lee 68 soybean roots, and development of females on Peking and PI 88788.

To preclude contamination from previous experiments, microplots were treated with 1,3-dichloropropene (1,3-D) at 7 ml/microplot (0.29 m^2) in mid June 1988. Seven holes were evenly placed in a microplot to a depth of 30 cm, and 1 ml of 1,3-D was added to each hole. The holes were immediately covered with soil and the surface was sealed with water. Two weeks after treatment, the soil in each microplot was turned with a shovel, and sorghum was planted. The sorghum cover crop allowed reestablishment of some normal biological activity in the microplots and abated residual 1,3-D toxicity. The sorghum was removed from the microplots in mid-August, and the soil was prepared for soybean planting. The microplots were treated and prepared similarly in 1989.

Two-day-old Lee 68 soybean seedlings were planted into 8-cm-d (235 cm^3) clay pots filled with soil (84% sand, 12% silt, 4% clay) and inoculated with 1,000 eggs of either B3-NC, B2,3-NC, B0-NC (North Carolina isolates of B3, B2,3, and B0, respectively) or combinations of two biotypes (B3XB2,3-NC; B3XB0-NC; B2,3XB0-NC). The seedlings were maintained in the greenhouse for 1 week to allow nematode infection and then transplanted into microplots on 29 August 1988. The shoots of the soybean plants were clipped at ground level 30 days after transplanting to induce plant senescence and cyst formation. These first-generation offspring were left in the microplots to overwinter. Each biotype or mixture of biotypes was replicated six times in 1988. In 1989, the soybean seedlings were inoculated similarly, except that eggs of B3-AR and B2,3-AR (Arkansas isolates of B3 and B2,3, respectively) and their mixture (B3XB2,3-AR) were in-

cluded. Plants were transplanted into microplots on 8 September 1989. Each isolate or isolate mixture was replicated four times in 1989.

Thermograph soil probes (Weathertronics, Sacramento, CA) were placed 20 cm below the soil surface in two of the microplots at soybean transplanting. A rain gauge and recorder (Belfort Instrument Co., Baltimore, MD) were situated near the microplots. Degree days ($DD_{5/32}$) were calculated based on the average daily high and low soil temperature between 5 C and 32 C (1). Rainfall data were compared on a cumulative monthly basis between years.

Soil samples were collected on 15 May 1989 and 1990 to a depth of 15 cm with a 4-cm-d soil probe placed directly over the center of the dead soybean plant. Cysts were extracted from samples by elutriation (3) and centrifugation (9). Ten cysts from each microplot were placed into individual glass watch dishes filled with 0.5 ml of water and crushed, a drop of 5.25% NaOCl was added, and numbers of eggs were determined. The remainder of the cysts were placed in a glass tissue grinder and crushed to free the eggs. These eggs were suspended in 100 ml of water and subdivided to test percentage hatch, percentage penetration, and ability to develop on Peking and PI 88788. The latter assay was used to determine biotype survival.

Hatch: Ten milliliters of the egg suspension were placed in small hatching screens placed in 5-cm-d petri dishes (2). Ten milliliters of soybean root leachate were added to each petri dish to enhance egg hatch. The leachate was collected by passing 1 liter of deionized water through a pot of 30-day-old Forrest soybean plants. The hatching chambers were placed in an incubator and maintained at 27 C for 4 days. On the fourth day, eggs and vermiform second-stage juveniles (J2) were enumerated. Percentage hatch was calculated by dividing the number of J2 by the sum of eggs and J2 and multiplying by 100. The data were analyzed for variance, and a least significant difference (LSD) was calculated for the data.

Penetration: Twenty milliliters of the egg suspension were inoculated onto roots of 2-day-old Lee 68 soybean seedlings transplanted into 8-cm-d clay pots filled with soil. The plants were maintained in the greenhouse for 7 days and then removed from the infested soil. Roots were stained using a NaOCl-acid fuschin staining technique (4) and the number of infecting J2 determined. Percentage penetration was derived by dividing the number of J2 recorded in the roots by the number of hatched eggs (as determined in the previous experiment) and multiplied by 100. Percentage penetration was analyzed for variance and statistical differences were separated with a LSD.

Biotype survival: Twenty milliliters of the egg suspension were pipetted onto each of the roots of 5-day-old Lee 68, PI 88788, and Peking seedlings transplanted into 8-cm-d clay pots. Plants were grown in the greenhouse for 30 days to allow females to mature. Plants were then removed from the pots and the soil gently washed away from the roots. The females were dislodged from the roots with a high-pressure stream of water and collected on a 250-μm sieve. The number of females recovered from each soybean plant was recorded. A chi-square analysis was conducted using the observed data and expected numbers of females. Numbers of females expected to develop on Peking and PI 88788 were derived from the gene frequencies of the parental nematode populations and the number of females recorded on Lee 68. When significant differences were found between observed and expected values, selection coefficients (s) were derived by subtracting the proportion of observed:expected females from 1 (8).

Experiment 2: Selection under different cropping sequences

Six cropping sequences (Table 1) were established in a Washington County, North Carolina, field (a coarse–loamy, mixed nonacid, thermic, Typic, Humaquepts) infested with *H. glycines* B2,3 (race 2). Plots within the field were 8 rows wide

by 9 m long with rows on 1-m centers. Corn, *Zea mays* L. DeKalb 636, was planted in mid-April each year (1988–1990). Sorghum, *Sorghum bicolor* (L.) Moench DeKalb 38, and soybean 'Deltapine 105' were planted in late May of those same years. Deltapine 105 was also planted in late June following harvest of November-planted wheat, *Triticum aestivum* T. Coker 916. Each cropping sequence was replicated four times and in all possible sequences. Weeds were controlled with cultivation and appropriate herbicides.

Soil samples were collected in late May of 1988, 1989, and 1990. Soybean plots were sampled again at the end of August in all 3 years. One liter of soil was collected during each sampling, except during 1988, when only 500 cm^3 of soil was collected. Cysts were collected from the soil by roiling and sieving. The cysts were further separated from soil debris by centrifugation and sugar flotation (8). Eggs were released by crushing the cysts with a glass tissue grinder and then used to inoculate four seedlings each of Lee 68, Peking, and PI 88788. The seedlings were planted into 8-cm-d clay pots filled with soil (84% sand, 12% silt, 4% clay) and maintained in the greenhouse for 30 days. After the 30 days, soil was gently removed from the soybean roots and the cysts dislodged from the roots with a high-pressure stream of water. The cysts were collected on a 250-μm screen and transferred to counting dishes for enumeration.

TABLE 1. Cropping sequences used in Washington County, North Carolina, between 1988 and 1990.

Environment	1988	1989	1990
1	S_e†	S_e	S_e
2	S_lW	S_lW	S_lW
3	C	S_e	C
4	S_e	C	S_e
5	CW	S_l	CW
6	S_l	CW	S_l
7	S_e	CW	M
8	CW	M	S_e
9	M	S_e	CW
10	S_l	CW	MW
11	CW	MW	S_l
12	MW	S_l	CW

Corn 'DeKalb 636' was planted in mid-April of each year. Sorghum 'DeKalb 38' was planted in early June. Soybean 'Deltapine 105' was planted during the third week of May or a month later following wheat harvest. Wheat 'Coker 916' was planted in early November of each year.

† C = corn, M = sorghum, S_e = soybean early, S_l = soybean late, and W = wheat.

Correlations were computed between numbers of cysts developing on Peking and PI 88788. Chi-square values were calculated between numbers of cysts observed and expected on Peking or PI 88788 for all sampling date combinations beginning in fall 1988 and continuing through fall 1990. Expected numbers of cysts were obtained by taking the proportion of cysts on the resistant cultivar to Lee 68 from a previous sampling and multiplying this proportion by the number of cysts recorded on Lee 68 of the current sampling date. When chi-square values were significant, selection coefficients (s_1 against parasitism on Peking and s_2 against parasitism on PI 88788) were computed by subtracting the observed proportions of cysts on the resistant:susceptible soybean from 1. An analysis of variance was then conducted on both selection coefficients, s_1 and s_2, to test for differences from $H_0 = 0$. The relative numbers of cysts developing on Peking and PI 88788 were computed by dividing the number of cysts on the resistant soybean by the number on Lee 68. Relative numbers of cysts on Peking and PI 88788 were subjected to an analysis of variance to test for differences among cropping sequences and years.

RESULTS

Experiment 1: Fitness components associated with viability

The average numbers of eggs per cyst were lower in spring 1990 than in spring 1989 (183 ± 13 vs. 243 ± 5 egg/cyst, respectively) (Fig. 1). The lowest (B2,3XB0-NC) and highest (B0-NC) fertility were recorded in spring 1990. The average number of eggs per cyst did not differ among the isolates or isolate mixture in spring 1989. In spring 1990, the B3XB0-NC and B2,3XB0-NC isolate mixtures contained

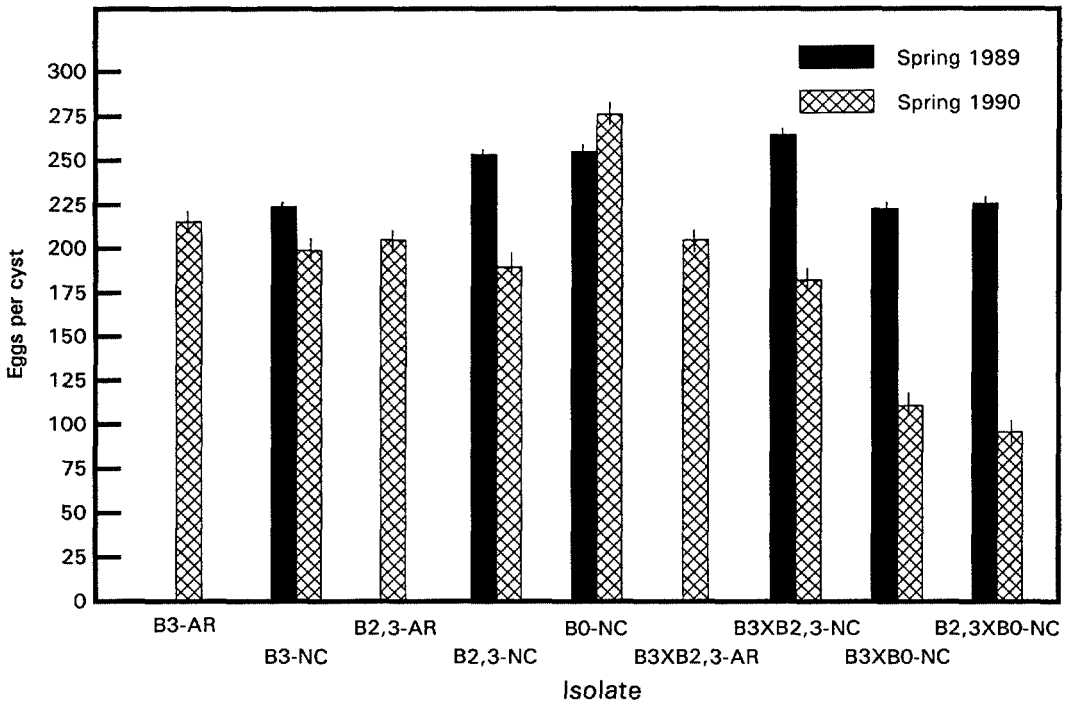

FIG. 1. The fertility of nine isolates and isolate mixtures of *Heterodera glycines* measured in eggs per cyst recorded in spring 1989 and spring 1990. Lines represent standard errors of the means. B3-AR and B3-NC are Arkansas and North Carolina isolates of B3 (a biotype parasitic on PI 88788 and susceptible soybeans). B2,3-AR and B2,3-NC are Arkansas and North Carolina isolates of B2,3 (a biotype parasitic on Peking, PI 88788, and susceptible soybeans). B0-NC is a North Carolina isolate of B0 (a biotype parasitic on susceptible soybeans only). B3XB2,3-AR, B3XB2,3-NC, B3XB0-NC, and B2,3XB0-NC are mixtures of the previously mentioned isolates.

fewer eggs than the B0-NC isolate. B3 isolates (B3-AR and B3-NC) averaged the fewest eggs per cyst over all sampling dates (144 ± 18 vs. 233 ± 12 and 260 ± 8 eggs/cyst for B3, B2,3, and B0, respectively). The number of eggs recorded in a preliminary fall 1989 sample to assay for onset of diapause gave egg numbers similar to those recorded in the greenhouse for these isolates.

Egg hatch differed between the two overwintering periods. In 1989, egg hatch averaged 12.8%, whereas in 1990 hatch averaged 18.6%. All isolates, except the B3XB0-NC isolate, had higher egg hatch in 1990 than in 1989 (Table 2). Hatching of B3-NC increased 82% between 1989 and 1990. B2,3-AR and B3XB2,3-AR behaved similarly to the North Carolina biotype isolates and mixtures. Eggs from the biotype mixtures generally had lower levels of hatch than eggs from unmixed parental biotype isolates (Table 2).

Penetration of Lee 68 roots by J2 was greater in 1990 than in 1989 (Table 2). Penetration averaged over all isolates was 7.3% in 1989 and 17.5% in 1990, and ranged from 1.5% to 21.5% over both years. All isolates and mixtures had increased percentage of penetration in 1990. Penetration was only different ($P = 0.05$) between the B3-NC and B2,3-NC isolates in 1989. No differences in percentage of penetration were detectable among any of the nine isolates or mixtures in 1990 (Table 2).

Selection against parasitic ability on Peking and (or) PI 88788 was stronger during the first winter than the second. Selection coefficients averaged 0.45 against biotypes parasitic on PI 88788 in 1989 and 0.19 in 1990. Several isolates experienced

TABLE 2. Percentage egg hatch and juvenile penetration of nine isolates of *Heterodera glycines* overwintered in microplots with selection coefficients against parasitism on the resistant soybeans PI-88788 and Peking.

					Selection coefficients			
	1989		1990		1989		1990	
Isolate	% Hatch	% Penetration	% Hatch	% Penetration	PI 88788	Peking	PI 88788	Peking
B3-AR	—†	—	16.1	20.7	—	—	0.63	—
B3-NC	12.8	18.7	23.4	21.5	0.68	—	0.27	—
B2,3-AR	—	—	23.2	13.0	—	—	0	0
B2,3-NC	14.3	1.5	21.0	14.5	0.81	0.81	0.59	0.92
B0-NC	11.9	9.1	20.9	16.9	—	—	—	—
B3XB2,3-AR	—	—	18.0	20.1	—	—	0	0
B3XB2,3-NC	12.8	4.7	15.7	17.3	0	0.76	0	0.86
B3XB0-NC	13.0	8.2	11.4	17.7	0	—	0	—
B2,3XB0-NC	11.9	5.4	19.5	15.3	0.76	0.97	0	0.67
lsd	ns	14.0	6.4	ns				
CV	58	121	15	146				
STD					0.43	0.23	0.44	0.36

† Signifies not appropriate to derive.

no selection during either winter. The number of females developing on PI 88788 was not different from the expected number ($P = 0.05$) in two of five instances in 1989, and in five of eight instances in 1990 (Table 2). Isolates B2,3-AR, B3XB2,3-AR, B3XB2,3-NC, B3XB0-NC, and B2,3XB0-NC experienced no selection against parasitism on PI 88788 in 1990. Several times, the number of females developing on PI 88788 was greater than the number observed on the susceptible host Lee 68. Isolates parasitic on Peking experienced strong selection during both winters (average selection coefficients of 0.85 and 0.49 in 1989 and 1990, respectively). Selection coefficients ranged from 0 to 0.97 against parasitism on Peking (Table 2). Selection was not detectable in the B2,3-AR isolate or B3XB2,3-AR combination. All nematode populations derived from the B2,3-NC isolate resulted in fewer females developing on Peking than were expected. None of the isolates produced more females on Peking than on the susceptible host.

Environmental data collected from the microplots are presented in Figure 2. Thirty-three percent more precipitation was recorded in the first winter than the second winter (41.3 cm vs. 31.1 cm). December and January were drier in the first winter than in the second (Fig. 2A). However, spring 1989 was much wetter than spring 1990 (Fig. 2A). Ninety-six more $DD_{5/32}$ were accumulated in 1989–90 than in 1988–89 (2,279 and 2,183 $DD_{5/32}$, respectively). The second spring turned warmer in March and accumulated $DD_{5/32}$ faster than during the first spring (Fig. 2B).

Experiment 2: Selection under different cropping sequences

Numbers of cysts developing on the resistant soybeans were positively correlated with those recorded on Lee 68 ($r = 0.60$ and 0.67 for Lee 68:Peking and Lee 68:PI 88788, respectively, $P = 0.01$). Additionally, number of cysts on Peking was positively correlated to number of cysts on PI 88788 ($r = 0.65$). This correlation indicates that the population consisted of individuals with alleles for parasitism on both resistant soybeans.

The relative number of cysts on Peking and PI 88788 (number recorded on the resistant host divided by the number recorded on Lee 68) fluctuated among sampling dates (Fig. 3). Typically the relative number decreased when soybean was absent and increased after soybean had been

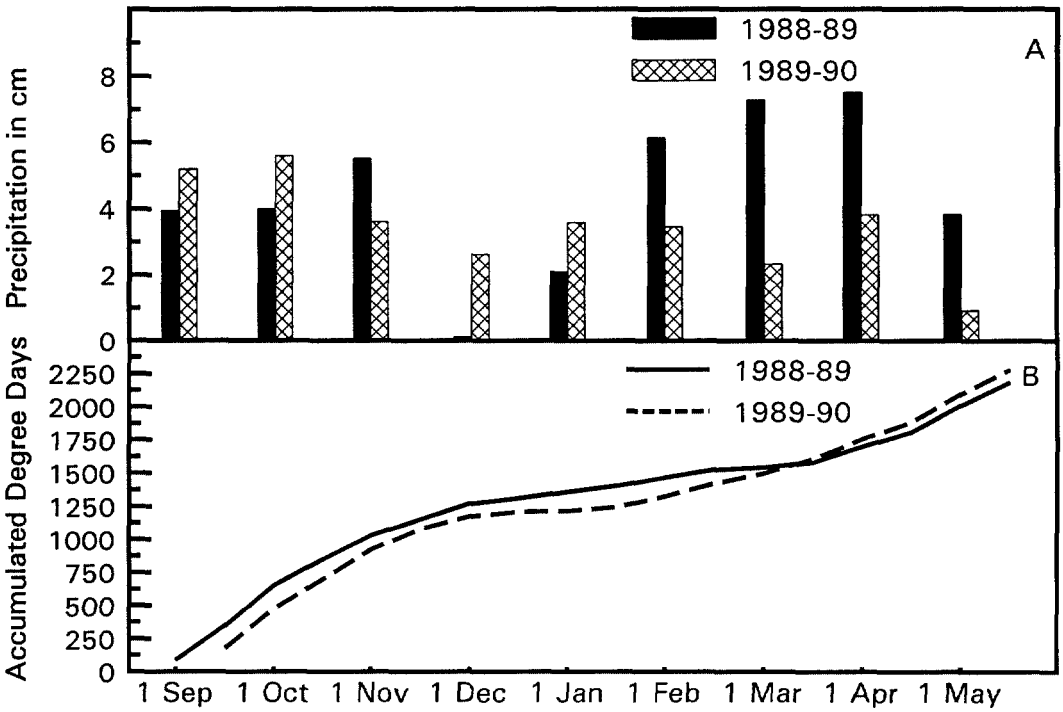

FIG. 2. Degree days ($DD_{5/32}$) accumulated and precipitation recorded during the winters 1988–89 and 1989–90. A) Precipitation (cm). B) $DD_{5/32}$ accumulated.

grown. The number of cysts on the resistant cultivars occasionally was greater than the number on the susceptible host, especially on PI 88788. The relative numbers of cysts on Peking and PI 88788 were not different among cropping sequences or years; however, the cropping sequence × year interaction was significant ($P = 0.05$).

Apparent selection against biotypes able to parasitize PI 88788 and Peking was rarely detected in this field population. Selection against PI 88788, s_1, occurred on three occasions; twice in the soybean–wheat cropping sequence ($s_1 = 0.13$ and 0.17, spring 1989/spring 1990 and spring 1990/fall 1990, respectively) and once in the continuous soybean plots ($s_1 = 0.27$, spring 1989/fall 1990). Selection against parasitism on Peking, s_2, was detected more frequently. In continuous soybean, $s_2 = 0.26$ between spring 1989/fall 1990 and 0.32 between spring 1990/fall 1990. The soybean–wheat sequence had an $s_2 = 0.29$ between spring 1989/fall 1989. Selection coefficients were also significant in the corn–soybean sequence, where $s_2 = 0.34$ between spring 1989/fall 1990. Coefficients of variation ranged from 277 to 1,386 in the comparisons among sampling periods.

Discussion

Comparing the numbers of eggs from greenhouse grown cysts and cysts overwintered in microplots suggests that eggs of B2,3 and B3 may not survive as well as those of B0. Numbers of eggs per cyst of *H. glycines* in the microplot experiment were lower than that recorded in greenhouse experiments (13). This difference may have arisen because eggs in the microplot were subject to parasitism and predation by soil flora and fauna. Some eggs probably died due to low soil temperatures (1), and some may have hatched. Lower temperatures during the winter of 1989–90 may be reflected in the lower number of

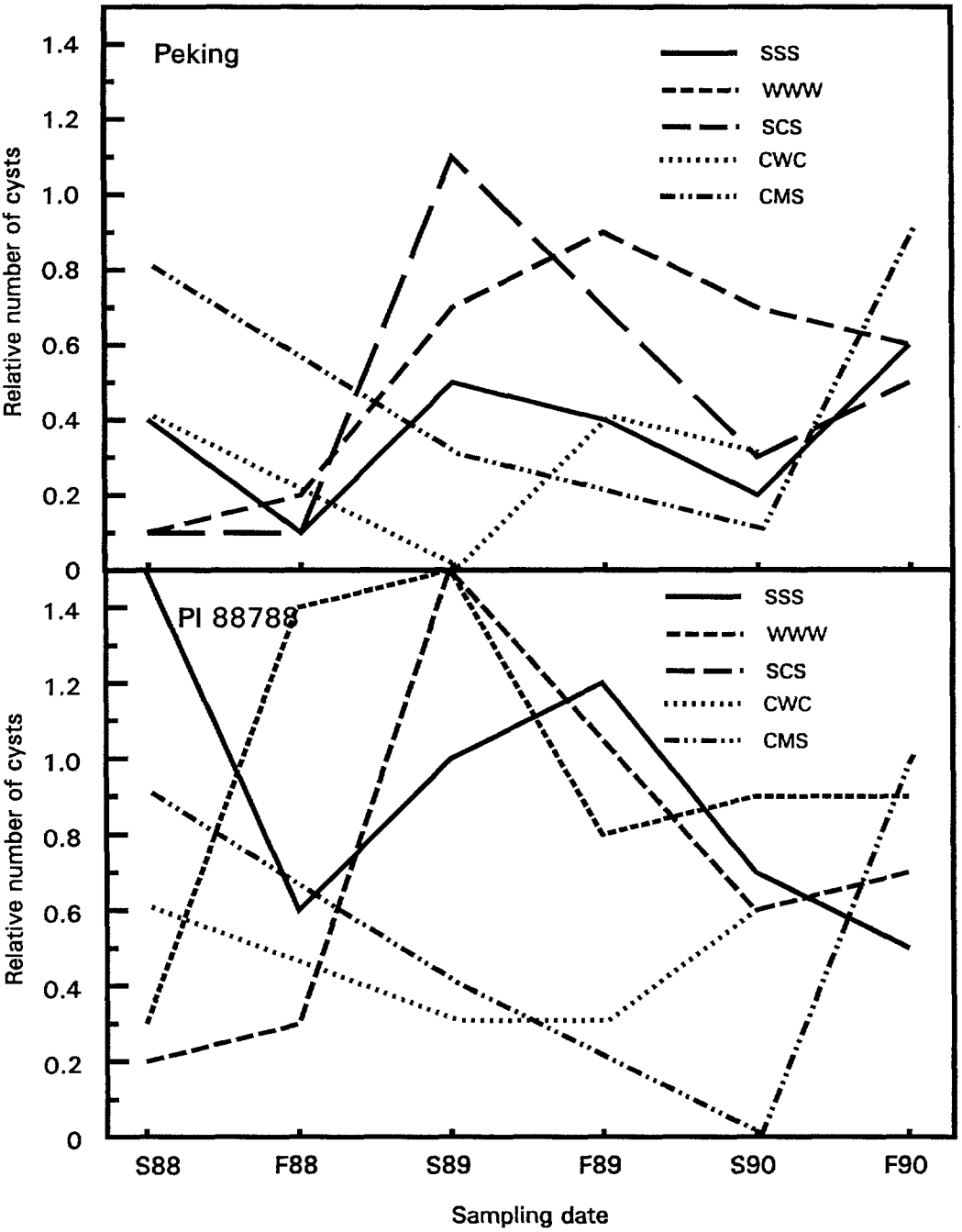

FIG. 3. Relative numbers of *Heterodera glycines* cysts recorded on the resistant soybeans Peking and PI 88788 (numbers recorded on the resistant cultivar divided by the number on Lee 68) between spring 1988 (S88) and fall 1990 (F90) under selected cropping sequences in Washington County, North Carolina. Crops are shown in order of year present (S = soybean, W = soybean–wheat, C = corn, M = sorghum).

eggs recorded per cyst in spring 1990 as compared to spring 1989.

Egg survival, as measured by hatch and J2 penetration of soybean roots, showed only small differences among the biotypes. Percentage egg hatch was less than 20% averaged over all isolates each year, with no isolate demonstrating greater levels of

hatch than any other. The unhatched eggs were assumed to be in diapause. They may, however, have contained dead J2. A test to ascertain if eggs still respired could have served as an indication of eggs in diapause. Penetration of host roots by J2 was also low, even when adjusted for unhatched eggs. Isolates of B3 penetrated roots slightly more successfully than isolates of the other biotypes. The time allowed for penetration probably was not a limiting factor, as most J2 penetrate within 48 hours after inoculation (6). Sampling was timed to coincide with the spring flush in nematode activity to maximize egg hatch and J2 penetration of host roots (12). It is conceivable that each isolate could have had a flush of activity at different times, which a single sampling would not have detected.

The nematode isolates used in the microplot experiment experienced greater selection against virulence on resistant soybeans than the endemic population did under different cropping sequences. B2,3-NC survived the winter the poorest. Interestingly, juveniles of all isolates and from the field were able to develop on the susceptible host even if they failed to develop on the resistant hosts. We used homogeneous isolates and know that virulence is inherited dominately in *H. glycines* (15). Consequently, if a nematode sample originates from a virulent isolate and develops on a susceptible host, another sample from the same isolate should develop on the resistant host. This suggests that gene products expressed in J2, which allow successful parasitism of resistant hosts, are either not expressed or the gene product no longer functions after overwintering.

Although the microplot and field experiments may appear contradictory in terms of the amount of selection against different biotypes, the differences between the histories of the nematodes must be considered. The North Carolina isolates used in the microplot experiment may have adapted to the greenhouse environment over the period of their indoor culturing and have become ill-suited to winter temperatures. Additionally, crop rotations reduced the population densities; consequently, the sample size may not have been large enough to detect small amounts of selection against specific biotypes.

Selection against alleles for parasitism on resistant soybeans appears to be small, and the alleles may even be nearly neutral. Relative fitness of the biotypes studied seems to be equal. Fertility was higher in B2,3 and B3, yet B0 survived the winter better. Other researchers also report finding little stabilizing selection in field populations of *H. glycines* (18,19). The frequency of parasitism on resistant hosts seldom increases to 100% in a nematode population, nor is it entirely lost from populations (18,19). However, even after 10 years of a susceptible soybean, alleles for parasitism on Peking were not entirely lost from a nematode field population (18).

Because there appeared to be little selection against parasitism on Peking or PI 88788 in the endemic field population and in several of the microplot isolates, alleles for parasitism on resistant hosts are probably never completely lost from a population. Consequently, only one season of resistant soybeans in the field may maintain the alleles for parasitism on resistant soybeans for long periods of time.

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