# The Influence of Races of Heterodera glycines on Nodulation and Nitrogen-Fixing Capacity of Soybean

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ABSTRACT

Three races of *Heterodera glycines* were compared for their effects on nodulation and nitrogen(N)-fixing capacity of Lee soybean. Alteration in N-fixing capacity was quantitatively determined, using an acetylene-ethylene gas chromatographic assay. Inoculum densities of 100, 200, and 400 crushed "cysts"/pot of race 1 of *H. glycines* plus *Rhizobium japonicum* caused a significant decrease in nodules per gram of root tissue and N-fixing capacity per plant as compared with *R. japonicum* check plants. The same inoculum densities of races 2 and 4 did not decrease nodules per gram of root tissue or the N-fixing capacity per plant. Nodule number and nodule weight were inversely correlated with increasing densities of race 1. Infection by race 1 initially increased nodular efficiency of the few nodules present; i.e., increased N-fixing capacity per milligram fresh weight of nodules. However, as race 1 multiplied, nodular efficiency and total N-fixing capacity declined. Race 1 caused severe chlorosis of soybean, whereas races 2 and 4 did not. This was correlated with the greater effectiveness of race 1 to decrease nodulation and N-fixing capacity. Phytopathology 61:1239-1244.

*Additional key words:* nitrogen metabolism, nematode, *Glycine max*.

Races of the soybean cyst nematode, *Heterodera glycines* Ichinohe, differ in symptoms caused on soybean. Differential inhibition of root nodulation has been suggested as one possible explanation (7, 19). Ichinohe & Asai (13) observed an inverse relationship between severity of infection by this nematode and nodulation on soybeans. Fumigation experiments in North Carolina (5) and in Tennessee (6) showed that a decrease in the densities of *H. glycines* resulted in increased nodulation. Other species of *Heterodera* also result in fewer nodules forming on leguminous plants; e.g., *H. goettingiana* on peas (14, 18) and *H. trifoli* on white clover (22). Taha & Raski (20) found that although *H. trifoli* reduced the number of nodules developing per plant, it did not affect the number of nodules per gram of root.

Although the number of nodules per plant has been used as an index of nitrogen(N)-fixation, it is not always a reliable measure because strains of *Rhizobium* vary in capacity to form effective symbiotic N-fixing associations with their hosts (17, 21). Nematode infection alters host nutrition (2, 4), and this in turn may affect the rate of symbiotic N-fixation. The effect of nematodes on N-fixation may be dependent upon the interaction between nematode density and host development. To fully understand the influence of plant parasitic nematodes on N-fixing capacity, it is necessary to quantitatively determine N-fixing capacity with time.

The objectives of this study were to compare races of *H. glycines* for their effects on nodulation and N-fixing capacity, and to determine the effects of nematode densities on N-fixing capacity and nodular efficiency.

Materials and Methods.—The races of *H. glycines* (SCN) used in these studies were: race 1, Wilmington, N.C., population; race 2, Holland, Va., population; race 4, Lonoke, Ark., population. Race designations are those of Golden et al. (8). Three 4-day-old Lee soybean (*Glycine max*) seedlings, germinated in vermiculite, were transplanted to each pot at the time of inoculation. Two hundred mg of a commercial preparation of *Rhizobium japonicum* (Noctin: Kalo Inoculant Co.) and nematode inoculum, consisting of a suspension of crushed yellow "cysts" (yellow adult female with egg masses) were added to each pot. Check plants (R-CK) for all experiments were inoculated only with *R. japonicum*. Plants were grown in 35-mesh sand with a mean diam of 578 μ and a range of 255 to 1,418 μ, and were watered biweekly with a modified Hoagland's nutrient solution minus N (11). Supplementary light was used to provide a 16-hr day.

Replicates from each treatment were harvested at various time intervals after inoculation to determine the effects of *H. glycines* on nodule number, nodule fresh weight, N-fixing capacity, nodular efficiency, and root dry weight. Reproductive rates of *H. glycines* were determined at each harvest. Cysts were removed from roots with a high-pressure water rinse and caught on a 60-mesh sieve. Cysts were separated from the sand by repeated flotation and sieving. The total volume of the cysts and water from each replicate was adjusted to 200 ml; this was stirred mechanically while two 10-ml aliquots were removed by pipette for counting.

Nitrogen-fixing capacity was determined using the acetylene-ethylene assay for $N_2$-fixation (10) utilizing an F and M Model 700 gas chromatograph with an $H_2$-flame ionization detector. Temperatures used were: injection port, 90 C; column, 60 C; detector, 280 C. Helium was used as carrier gas at a flow rate of 40 cc/min through a 318-mm × 180-cm column containing 120- to 150-mesh Porapak-Gel T (Waters Associate,

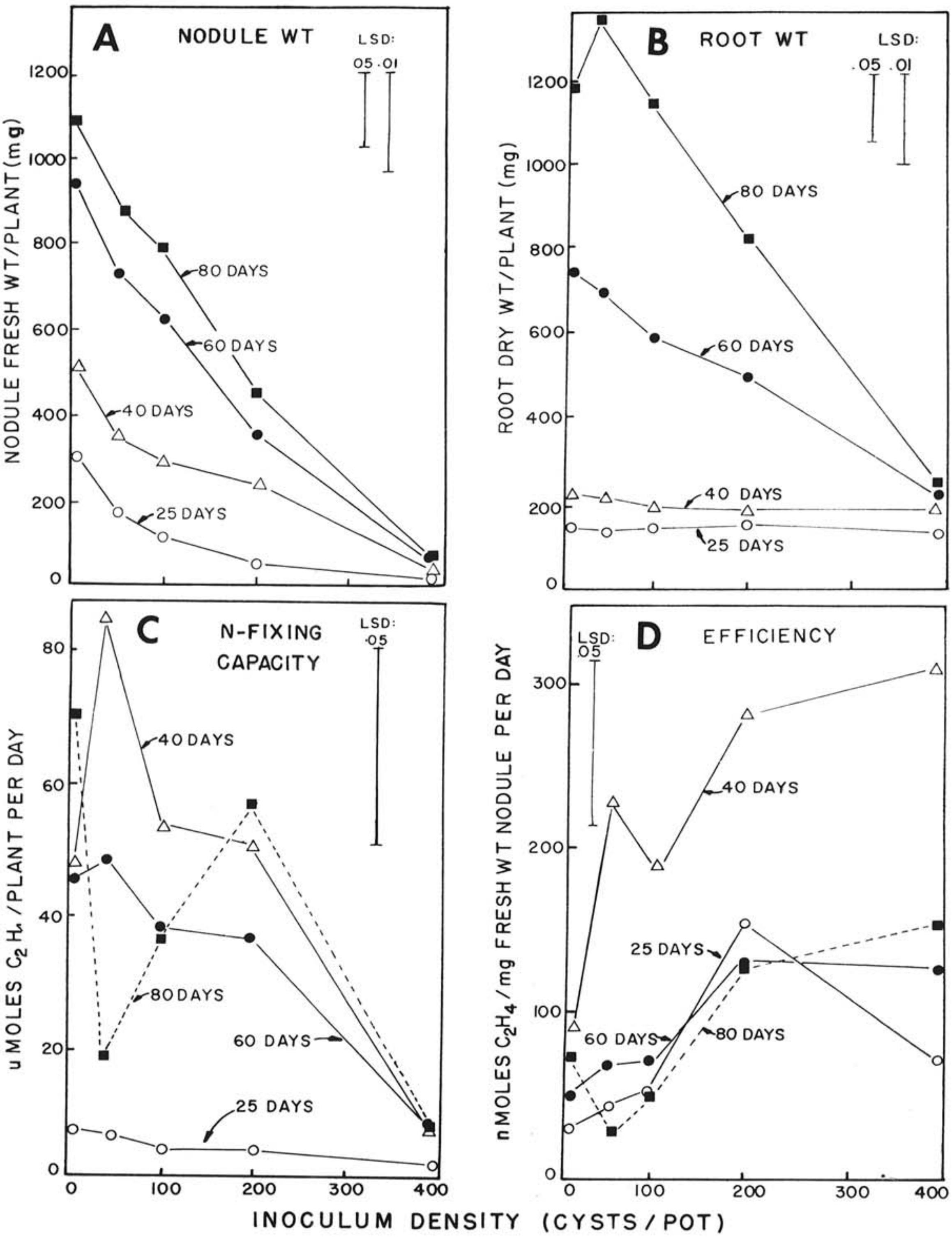


**Fig. 1.** Effects of inoculum density of race 1 of *Heterodera glycines* at different time intervals after inoculation on nodulation, root weight, and N-fixing capacity of soybean: **A)** mg nodules per plant; **B)** mg roots per plant; **C)** µmoles of $C_2H_4$ per plant per day; **D)** efficiency = nmoles of $C_2H_4$ per milligram fresh weight of nodules per plant per day (LSD values for a given variable can be used to compare any treatment with the check for a given harvest time).

Inc.) (12). Samples of 25 μliters were injected with complete resolution of the acetylene and ethylene peaks. Representative retention time for ethylene was 1.1 min, and for acetylene, 2.0 min.

RESULTS.—*Influence of H. glycines on nodule development and $N_2$-fixation.—Effects of different densities of race 1.*—Inoculum densities of 0, 50, 100, 200, 400 crushed cysts of race 1/15-cm pot were tested, and four replicates of each treatment were harvested 25, 40, 60, and 80 days after inoculation. Inoculum densities of 100, 200, and 400 cysts/pot caused a significant reduction in nodule weight per plant in all harvests (Fig. 1-A). The correlation (r = .99) between nodule number and nodule weight over all harvest times was highly significant. Nodule number and weight were inversely correlated with initial inoculum densities of race 1. Nematode-inoculated plants not only had lower nodule numbers and weight per plant than the check plants inoculated only with *Rhizobium* (R-CK), but nodule numbers and weight were also lower than the R-CK per g of root tissue at 20 and 40 days. Root weight generally increased with time, but this increase was less for SCN-inoculated plants (Fig. 1-B). At 60 days, initial densities of 200 and 400 cysts reduced nodule weight per g of root, but by 80 days, only 400 cysts/plant showed a reduction as compared with the R-CK.

Nitrogen-fixing capacity per plant varied with inoculum density and with time (Fig. 1-C). After 25 days, plants initially inoculated with 400 cysts/pot had lower N-fixing capacity than did R-CK plants. At the 40-day harvest, nematode-infected plants exhibited increased nodular efficiency regardless of initial density (Fig. 1-D). At the 60- and 80-day harvests, however, the N-fixing capacity of inoculated plants declined; the 50-cyst initial density evidenced the greatest decline. In spite of some variability, the trends were evident and significant.

*Comparison of races of H. glycines.*—Races 1, 2, and 4 of *H. glycines* were tested at densities of 0, 100, 200, or 400 cysts/10-cm pot. Nitrogen-fixing capacity was measured 55 days after inoculation. Race 1 caused a significant decrease in nodule number, nodule weight, and N-fixing capacity at all inoculum densities. Races 2 and 4 resulted in decreased nodule number and weight only at 400 cysts/pot (Fig. 2), and did not significantly reduce N-fixing capacity.

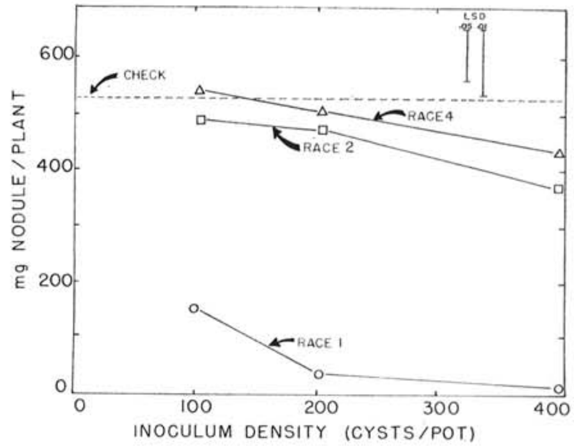


**Fig. 2.** Comparative effects of races 1, 2, and 4 of *Heterodera glycines* on nodule weight 55 days after inoculation (LSD can be used to compare any treatment with the *Rhizobium* only check).

In a subsequent series of experiments, one soybean plant was used/10-cm pot, and seven replicates of each treatment were harvested at 40, 60, and 80 days after inoculation to compare the effects of races 1 and 4 on N-fixing capacity.

Race 1 differed from the other races of *H. glycines* in symptoms caused on soybean. An inoculum density of 400 cysts of race 1 caused chlorosis and stunting by 40 days, whereas 800 cysts of race 4 caused no chlorosis and only a slight reduction in growth as compared with the R-CK. These differences were correlated with a differential reduction in nodulation. Race 1 caused a highly significant decrease in nodule number at all inoculum densities (Table 1). Although both races 1 and 4 decreased total nodule weight as compared to the R-CK, race 1 caused significantly greater reduction. Race 1 decreased root weight as compared with R-CK, and decreased nodule numbers and nodule

TABLE 1. Nodule number, nodule weight, and root weight of soybean inoculated with races 1 and 4 of *Heterodera glycines*[a]

| Treatment | No. nodules | mg Nodule fresh wt | Root dry wt, g | Nodules/g roots | mg nodules/g roots |
|---|---|---|---|---|---|
| Races 1 (N.C.) | | | | | |
| 25 cysts | 45 | 347 | 0.87 | 63 | 479 |
| 100 cysts | 33 | 214 | 0.58 | 71 | 408 |
| 400 cysts | 16 | 103 | 0.34 | 53 | 268 |
| Race 4 (Ark.) | | | | | |
| 100 cysts | 109 | 822 | 1.23 | 105 | 717 |
| 800 cysts | 97 | 734 | 0.92 | 127 | 845 |
| R-Check | | | | | |
| 0 cysts | 97 | 988 | 1.36 | 91 | 818 |
| LSD[b] .05 | 10 | 98 | 0.15 | 21 | 122 |
| .01 | 13 | 129 | 0.19 | 28 | 161 |

[a] Data expressed as means of 40, 60, and 80-day harvest.
[b] LSD for a given variable can be used to compare any treatment with the check.

weight per gram of root tissue (Table 1). In contrast, although the highest density of race 4 decreased root weight, the nodule weight per gram of root tissue for this treatment was higher than the R-CK.

Race 1 was more effective than race 4 in decreasing the N-fixing capacity per plant. At 40, 60, and 80 days, plants inoculated with 100 cysts of race 1 had significantly lower $C_2H_4$ production per plant per day than the R-CK, whereas 100 cysts of race 4 did not significantly reduce the N-fixing capacity (Table 2). However, the effect of density of race 1 on N-fixing capacity interacted with harvest time, especially at the lower inoculum densities. For example, at 40 days, 25 cysts of race 1 did not reduce the N-fixing capacity per plant, but as nematode population increased with time, reduction in N-fixing capacity occurred. Although inoculation with 800 cysts of race 4 significantly decreased N-fixing capacity, this reduction was still less than the reduction found with an inoculum density of 25 cysts of race 1 (Table 2).

At 40 days, plants inoculated with race 1 had higher nodular efficiencies than the R-CK; i.e., the few nodules that were present were more effective in reducing $C_2H_2$ to $C_2H_4$, but at 60 and 80 days, nodular efficiencies were much lower than the R-CK. Inoculation with race 4 did not significantly alter nodular efficiency as compared to the R-Check (Table 2).

*Relation of nematode activity to N-fixing capacity.*—An experiment was conducted to determine whether the different effects of the races of *H. glycines* on N-fixing capacity were due to differential rates of nematode hatching, penetration, and/or development. Since previous experiments indicated that race 1 multiplied at a greater rate on Lee soybean than races 2 and 4, higher inoculum densities were used for race 4 than for race 1 as follows: race 1, 25, 100, and 400 crushed cysts; race 4, 100 and 800 crushed cysts.

Races 1 and 4 were also compared for their rate of hatching and penetration of plants in sand culture. At 6 and 12 days after inoculation, five replicates of each treatment were harvested and the larvae extracted from the sand (3). Roots were stained with acid-fuchsin lactophenol, and the number of larvae that penetrated the roots was counted. The penetration pattern was recorded; i.e., the number of larvae that were scattered versus the number in aggregates. An aggregate was arbitrarily defined as 20 or more larvae/linear cm of root.

No significant differences in the percentage hatching occurred for the two races at 6 days, but at 12 days, race 1 had a higher percentage hatching than did race 4. With a density of 100 cysts/pot, no difference in total penetration between races 1 and 4 at 6 days was observed (Table 1). However, as nematode density increased, or as it increased with time due to hatching, greater numbers of larvae of race 1 than of race 4 penetrated the roots. A significantly greater number of larvae of race 4 than of race 1 penetrated in an aggregated pattern (Table 3). Based on cyst counts at 40, 60, and 80 days, race 1 reproduced at a higher rate than race 4 (Table 2).

DISCUSSION.—The results establish that the races of the soybean cyst nematode differ quantitatively not only in their influence on nodulation but also on nitrogen fixation and nodular efficiency. Variation in virulence of the races of *H. glycines* has previously been associated with a differential reduction of nodulation of soybeans grown in the field (6).

The data also indicate that the number of nodules is not always a reliable index of nematode damage, because the effect of *H. glycines* on the N-fixing capacity and nodular efficiency per plant varied with time, depending on nematode race and nematode population density. An understanding of the interaction of time with population density and host nutrition is important in interpreting the effects of this nematode on nitrogen fixation.

Plants infected with race 1 initially had higher nodular efficiencies than R-CK plants or plants inoculated with race 4. Thus, although 25 cysts of race 1 at 40 days reduced the number of nodules per plant 33% below the R-CK, the μmoles of $C_2H_4$ produced/plant

TABLE 2. Effect of races 1 and 4 of *Heterodera glycines* on the nitrogen-fixing capacity of soybean, nodular efficiency, and nematode population density at 40, 60, 80 days after inoculation

| Treatment | Nitrogen-fixing capacity (μmoles $C_2H_4$/plant/day) | | | Nodular efficiency (nmoles $C_2H_4$/mg nodule/day) | | | Final nematode population density (cysts/plant at harvest) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 40 days | 60 days | 80 days | 40 days | 60 days | 80 days | 40 days | 60 days | 80 days |
| Race 1 (N.C.) | | | | | | | | | |
| 25 cysts | 95 | 2 | 2 | 243 | 4 | 4 | 431 | 6,337 | 8,086 |
| 100 cysts | 37 | 2 | 2 | 238 | 16 | 6 | 1,251 | 4,120 | 6,660 |
| 400 cysts | 14 | 4 | 1 | 314 | 73 | 33 | 1,720 | 3,200 | 4,448 |
| Race 4 (Ark) | | | | | | | | | |
| 100 cysts | 81 | 59 | 69 | 178 | 80 | 63 | 219 | 2,185 | 2,508 |
| 800 cysts | 49 | 59 | 57 | 134 | 78 | 55 | 480 | 984 | 1,825 |
| R-Check | | | | | | | | | |
| 0 cysts | 92 | 72 | 93 | 143 | 80 | 66 | 0 | 0 | 0 |
| | LSD[a] | .05 = 32<br>.01 = 42 | | LSD[a] | .05 = 63<br>.01 = 84 | | LSD[b] | .05 = 1,148<br>.01 = 1,524 | |

[a] LSD for a given variable can be used to compare any treatment with the check for a given harvest time.

[b] LSD can be used to compare any two treatments at a given harvest time.

TABLE 3. Hatching and penetration of races 1 and 4 of *Heterodera glycines* at 6 and 12 days after inoculation[a]

| Treatment | Hatching | | Penetration | | |
|---|---|---|---|---|---|
| | Total/pot | % | Total/plant | % | % Aggregated |
| | | | *6 days* | | |
| Race 1 (N.C.) | | | | | |
| 100 cysts | 1,291 | 9 | 191 | 13 | 48 |
| 400 cysts | 5,437 | 10 | 1,257 | 23 | 22 |
| Race 4 (N.C.) | | | | | |
| 100 cysts | 796 | 6 | 114 | 14 | 83 |
| 800 cysts | 9,064 | 8 | 315 | 4 | 80 |
| LSD | | | | | |
| .05 | 1,535 | NS[b] | 562 | 12 | 32 |
| .01 | 2,152 | NS | NS | NS | 45 |
| | | | *12 days* | | |
| Race 1 (N.C.) | | | | | |
| 100 cysts | 2,593 | 19 | 1,269 | 51 | 28 |
| 400 cysts | 10,122 | 18 | 3,563 | 23 | 36 |
| Race 4 (N.C.) | | | | | |
| 100 cysts | 1,199 | 9 | 315 | 27 | 72 |
| 800 cysts | 13,283 | 12 | 875 | 7 | 86 |
| LSD | | | | | |
| .05 | 1,886 | 5 | 672 | 10 | 19 |
| .01 | 2,645 | 7 | 942 | 13 | 27 |

[a] Inoculations with crushed cysts. Races 1 and 4 both had an average of 140 eggs/cyst.
[b] NS = no significant difference.

were as great as the R-CK, due to the higher efficiency of the fewer nodules present. As nematode density increased at later harvests, nodular efficiency and the total N-fixing capacity per plant greatly declined for plants infected with race 1 compared to the R-CK, or compared with plants infected with race 2 or race 4. Since race 1 of *H. glycines* reduces nodule number per plant, this may increase the carbohydrate available per nodule, which may temporarily increase the efficiency of those fewer nodules present. At very low nematode densities, this apparently compensated for a slight reduction in nodulation, but at higher nematode densities, the increase in efficiency was not sufficient to compensate for the severe reduction in the number of nodules per plant caused by race 1.

The influence of race 1 of *H. glycines* on soybean nodulation differs from that described for *Meloidogyne javanica* and *H. trifoli* on white clover (20). The latter do not reduce the number of nodules per gram root on clover. The influence of races 2 and 4 of this nematode on nodulation is similar to that of *M. javanica* and *H. trifoli* in that these races of *H. glycines* do not alter nodule weight per gram of root and that any reduction in nodulation is correlated with an over-all reduction in the root system.

Races 2 and 4 had lower rates of population increase than did race 1. This suggested that the differential inhibition of nodulation might be correlated with rates of reproduction. Using very high initial inoculum densities of race 4 and low densities of race 1, however, we proved this was not the case.

The aggregate penetration pattern characteristic of race 4 may limit development of this race, and may also reduce its potential to inhibit nodulation. Race 4 may only be effective in inhibiting nodulation at these localized areas of aggregation, whereas the scattered penetration pattern of race 1 may more effectively inhibit nodulation throughout the entire root system.

Our data provide quantitative evidence of the capacity of race 1 to inhibit the N-fixing capacity of the host. Studies on the role of inorganic ions indicate that nitrogenous compounds inhibit larval emergence of race 1 (15). The effects of nitrogen on the development of other races of *H. glycines* are not known. Barker & Huisingh (1) presented evidence for a hypersensitive interaction between *R. japonicum* and race 1 of *H. glycines*. Ammonia and phenylalanine ammonia lyase are implicated in certain hypersensitive reactions (9, 16). Thus a delicate balance apparently exists among nematode, host, and *Rhizobium*. Some aspect of the physiology of race 1 alters this balance more drastically than is the case with races 2 and 4. Race 1 is particularly capable of decreasing symbiotic N-fixation, and differences in virulence of the races of *H. glycines* are primarily due to differential effects on N-fixing capacity.

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