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Effect of Rotylenchulus reniformis on Yield and Nitrogen, Potassium, Phosphorus and Amino Acid Content of Seed of Glycine max[1]

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Abstract: Soybean cultivars varied in their response and tolerance to low initial *Rotylenchulus reniformis* populations of 10,000 nematodes/3.8 liters of soil, but a high initial population of 25,000 consistently reduced yields on resistant and susceptible cultivars by an average of 33.1%. At the 10,000 nematode inoculum level, dry seed yields of Hood decreased while those of Pickett increased significantly. Generally, total phosphorus decreased 11.1 and 11.5% and potassium increased 5.9 and 4.5% in seeds harvested from plants receiving initial inoculum levels of 5000 and 10,000 nematodes/pot, respectively. Little change in the total nitrogen content in seed was noted. Leucine content of seeds from infected plants was slightly less than from noninfected plants. *Key Words:* reniform nematode, soybean, inoculum levels, pathogenicity, resistance, susceptibility, population dynamics.

Field damage to soybean [*Glycine max* (L.) Merr.] crops associated with the presence of the reniform nematode (*Rotylenchulus reniformis*, Linford and Oliveira, 1940) in South Carolina (8), Alabama (13) and Louisiana (1) have been reported. Birchfield et al. (1) observed extensive yield reduction of soybeans caused by reniform nematode annually in commercial plantings. In 1960, Minton et al. (11) studied the effects of *R. reniformis* on 'Lee' soybean in field soil bins, but did not report any significant growth changes. Under greenhouse conditions, Rebois et al. (15) found that seed yields of 'Pickett' increased while those of 'Hood' decreased when the soil was inoculated with this nematode. However, results were not reported in detail, and inoculum levels that consistently reduced yields were not investigated.

Our work was conducted to determine the effect of reniform nematode parasitism on quality and quantity of some major elements and compounds in soybean seed, and the effect of increasing nematode populations on yields of some reniform nematode resistant and susceptible cultivars.

MATERIALS AND METHODS

Greenhouse tests were conducted in 1966, 1967 and 1968 to evaluate the effects of various initial soil population levels of *R. reniformis* on soybean seed yield and quality. Except for variations as noted below, all tests were handled in the same manner. Clay pots 20.5 cm diam were filled with approximately 3.8 liters of an autoclaved sandy clay loam from Tallassee, Alabama. Five surface-sterilized seeds of either a reniform nematode-resistant ('Pickett' or 'Dyer') or susceptible ('Hood', 'Hampton 266', 'Bragg', 'Lee' or 'Jackson') soybean cultivar (14, 15) were planted into each pot. Seeds were surface-sterilized as follows: 1 min in 95% ethanol; 15 min in commercial 5.25% sodium hypochlorite solution and water (1:4); and a rinsing for 15 min in tap water. After rinsing, the seeds were coated with a commercial mixture of *Rhizobium* spp. and planted. One week after emergence, the seedlings were thinned to two/pot. Ten days after planting, the desired number (1000 to 25,000) of surface-sterilized (6) *R. reniformis* larvae, males and preadult females, were introduced into the soil. The inoculum levels chosen were within the ranges

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of several reported field populations and considerably below those found in the greenhouse or reported from field bins (11). In 1966, one-half the desired number was introduced with a hypodermic syringe, 1.5 cm below the soil surface in a circle (2.5 cm diam) around each of the two plants in a pot. In 1967 and 1968, instead of inoculating around a germinated seedling, a one-fifth portion of the desired inoculum was placed on each seed at planting time and all but the most vigorous two seedlings were clipped at the soil line 10 days later. All tests were set up in a randomized complete block design, with each pot considered as one replicate. The 1966 and 1967 tests were replicated six times; and the 1968 test, five times. Seeds were harvested from mature plants and their dry weights recorded. From each pot, 150 ml of soil was processed and the nematodes extracted (17) and counted.

For chemical analysis, dry seeds were ground through a 40-mesh screen in a Wiley Mill and desiccated for 48 hr. Total nitrogen content in 0.5 g of seed was determined for each sample pot by the Kjeldahl method (5). Total crude protein was computed as follows: total crude protein = % nitrogen in sample × 6.25 × dry weight of seed from sample pot ÷ 100 (10).

Phosphorus and potassium determinations were based on 0.5 g of ground dry seed from each replication of the 1967 test. Samples were dry ashed at 450 C for 5 hr, cooled, and mixed with 10 ml of 1 N nitric acid before evaporation to dryness in a sand bath. When the sample reached dryness it was removed immediately from the bath to prevent overcooking or burning. The residue was dissolved in 10 ml of 1 N hydrochloric acid and subsequently heated to near boiling. The dissolved residue was diluted to 100 ml and passed through filter paper. The filtrate was used for analysis. Potassium was determined by the flame photometry method and phosphorus by the vanadate method (12).

Total seed amino acid content from a sample was determined by hydrolysis of 0.5 g of dry-ground seed with 6 N hydrochloric acid

TABLE 1. Effect of different *Rotylenchulus reniformis* inoculum levels on soybean seed yield, expressed as dry weight and total crude protein, 1966-1967 tests. Means of 12 replications.

Cultivar	Initial no. of nematodes/ 3.8 liters of soil	Final no. nematodes/ 3.8 liters of soil[a]	Mean dry wt. of seed/pot (g)[b]	Mean crude protein/pot (g)[b]
'Lee'	0	0	14.82 a	5.77 a
'Lee'	1000	14,230	15.70 a	6.15 a
'Lee'	5000	58,090 | A	15.78 a	6.14 a
'Lee'	10,000	63,560 |	14.39 a	5.67 a
'Bragg'	0	0	18.47 a	7.57 a
'Bragg'	5000	84,000 A	17.00 a	6.90 a
'Hood'	0	0	16.24 A	6.47 A
'Hood'	1000	24,250	15.63 A	6.07 A
'Hood'	5000	68,430 | A	15.14 A	6.09 A
'Hood'	10,000	73,430 |	12.09 B	4.98 B
'Hampton 266'	0	0	19.10 a	6.98 a
'Hampton 266'	5000	48,150 A	16.72 a	6.28 a
'Jackson'	0	0	15.39 a	5.98 a
'Jackson'	5000	64,980 A	14.36 a	5.44 a
'Dyer'	0	0	15.52 a	5.85 a
'Dyer'	5000	580 | B	15.20 a	5.84 a
'Dyer'	10,000	430 |	15.68 a	5.88 a
'Pickett'	0	0	14.89 B	5.88 b B
'Pickett'	5000	330 | B	17.10 A	6.75 a AB
'Pickett'	10,000	530 |	17.59 A	7.01 a A

[a]Figures followed by different letters are significantly different at the 1% level (t-test). Only the common 5000 initial population treatments were tested for significant differences between cultivars. Final population figures followed by common bar are not significantly different for any one cultivar.

[b]Means within cultivars followed by different letters are significantly different (Duncan's multiple range test). Significance: Upper case letters = 1% level. Lower case letters = 5% level.

TABLE 2. Effect of different *Rotylenchulus reniformis* inoculum levels on soybean seed yield, expressed as dry weight and total crude protein, 1968 test.

Cultivar	Initial no. nematodes/ pot	Mean dry wt. of seed/pot (g)[a]	Mean crude protein/pot (g)[a]
'Lee'	0	10.5 a	4.05 a
'Lee'	5000	11.8 a	4.52 a
'Lee'	25,000	6.8 a	2.52 a
'Bragg'	0	10.8 a	4.02 a
'Bragg'	25,000	8.5 a	3.16 a
'Hood'	0	10.1 A	3.67 A
'Hood'	5000	5.5 B	2.01 B
'Hood'	25,000	7.1 B	2.73 B
'Hampton 266'	0	13.4 a	4.90 a
'Hampton 266'	25,000	9.2 b	2.42 b
'Jackson'	0	13.9 a	5.12 a
'Jackson'	25,000	7.4 a	2.71 a
'Dyer'	0	8.4 a	3.28 a
'Dyer'	25,000	4.5 a	1.66 a
'Pickett'	0	12.8 a	4.85 a
'Pickett'	5000	13.2 a	5.05 a
'Pickett'	25,000	9.9 a	3.64 a
All cultivars	0	11.41 A	4.27 A
All cultivars	25,000	7.63 B	2.69 B

[a]Means within cultivars followed by different letters are significantly different. There was no significant difference in the way each cultivar responded to the 25,000 inoculum level. Analysis of Variance F test. Significance: Upper case letters = 1% level. Lower case letters = 5% level.

for 20 hr under vacuum (2). Amino acids were quantitatively separated by column chromatography on an automatic amino acid analyzer (18). Amino acid analyses were made on seed from the 0 and 25,000 nematode inoculum levels in the 1968 test only.

RESULTS

Results of the 1966 and 1967 tests are combined in Table 1. The final nematode soil population about any one susceptible cultivar consistently increased with increasing inoculum levels in all tests, but the differences were not significant when the initial population was 5000 or more nematodes/pot. The final population was significantly lower with an initial population of 1000 than for 5000 or above. When the experiments were terminated, the soil populations of *R. reniformis* were significantly lower in soil planted to reniform nematode-resistant cultivars ('Dyer' and 'Pickett') than in soil planted to susceptible cultivars. Only two cultivars, 'Hood' and 'Pickett', had significantly different dry seed yields associated with the presence of the reniform nematode. 'Hood' soybean yields decreased whereas 'Pickett' yields increased with increasing inoculum levels up to 10,000 nematodes/pot.

The 1968 results are presented in Table 2. Some insecticide spray injury occurred to three of the five replications about 2 months after planting. The spray damage resulted in an overall 37% seed yield reduction in spray-damaged plants which was significant for 'Pickett' and 'Hood'. However, the

FIG. 1. 'Hood' soybean plants inoculated with 10,000 *Rotylenchulus reniformis*/pot on the right and uninoculated controls on the left, photographed 1 month prior to termination of the 1966 tests. The inoculated plants produced 19.3% less seed by weight.

TABLE 3. Effect of different *Rotylenchulus reniformis* inoculum levels on the phosphorus, potassium and nitrogen content of soybean seed obtained from the 1967 test.

Cultivar	Initial no. nemas/pot	% Seed phosphorus[a]	% Seed potassium[a]	% Seed nitrogen[a]
'Lee'	0	.51 bB	1.30 b	6.48 a
'Lee'	1000	.60 aA	1.36 ab	6.31 a
'Lee'	5000	.47 bBC	1.49 a	6.15 a
'Lee'	10,000	.41 cC	1.36 ab	6.38 a
'Bragg'	0	.52 a	1.28 a	6.83 a
'Bragg'	5000	.46 a	1.42 a	6.63 a
'Hood'	0	.51 a	1.26 cC	6.46 aA
'Hood'	1000	.55 a	1.50 abAB	5.96 bAB
'Hood'	5000	.53 a	1.55 aA	6.23 abA
'Hood'	10,000	.53 a	1.43 bAB	5.90 bB
'Hampton 266'	0	.43 a	1.47 a	6.05 a
'Hampton 266'	5000	.42 a	1.42 a	6.15 a
'Jackson'	0	.54 a	1.46 a	6.38 A
'Jackson'	5000	.42 b	1.48 a	6.02 B
'Dyer'	0	.58 A	1.49 a	5.93 a
'Dyer'	5000	.48 B	1.42 a	6.00 a
'Dyer'	10,000	.47 B	1.52 a	5.95 a
'Pickett'	0	.50 a	1.42 a	6.24 a
'Pickett'	5000	.44 b	1.43 a	6.25 a
'Pickett'	10,000	.45 b	1.41 a	6.06 a
All cultivars	0	.51 A	1.38 B	6.34 a
All cultivars	5000	.46 B	1.46 A	6.21 b

[a]Means within cultivars followed by uncommon letters are significantly different (Duncan's multiple range test). Significance: Upper case letters = 1% level. Lower case letters = 5% level.

TABLE 4. A comparison of the percent soybean seed amino acids from *Rotylenchulus reniformis*-infected and uninfected plants. Each amino acid is expressed as the total amino acid plus ammonia obtained from acid hydrolyzed seed. Figures represent the mean of 14 replications.

Amino acid	Uninfected	Infected	LSD.05
Alanine[a]	5.62	5.79	.44
Ammonia	.18	.17	.03
Arginine	8.25	7.81	.71
Aspartic	11.63	12.17	1.05
Cystine	1.15	1.18	.25
Glutamic[a]	18.58	18.36	.93
Glycine[a]	4.51	4.75	.40
Histidine	4.23	4.44	.56
Isoleucine	4.34	4.13	.51
Leucine	7.25	6.55[b]	.50
Lysine	8.57	8.91	.77
Methionine	.70	.64	.20
Phenylalanine	.78	1.04	.52
Proline[a]	7.12	7.28	.91
Serine	6.42	6.11	.65
Threonine[a]	5.14	4.96	.46
Tyrosine	.40	.46	.22
Valine	5.12	5.23	.29

[a]Amino acid varied significantly between some cultivars but not in relation to nematode infection or nematode resistance in plants.

[b]Leucine significantly lower in seed from nematode inoculated plants at the 5% level (Duncan's multiple range test).

spray-damaged and undamaged replications responded similarly to the nematode inoculum levels. Analysis of variance indicated that all cultivars responded similarly to the 25,000 nematode inoculum level. When average yields from controls and the high inoculum levels were combined into their respective groups, the result was a highly significant 33% loss in seed dry weight and a 37% loss in total crude protein yield associated with parasitism.

The effect of the 10,000 nematode inoculum level compared to the uninoculated control of 'Hood' is shown in Fig. 1. The picture was taken several days before harvesting and illustrates the difficulty in visual detection of a 19.3% reduction in yield due to nematode infection. No unusual aboveground symptoms were associated with *R. reniformis* parasitism.

In Table 3 are listed the average percent phosphorus, potassium and nitrogen content in dry seed of the various cultivars subjected to different nematode inoculum levels in the 1967 test. Under conditions of this test, significant differences in the N, P or K content of the dry seed resulted from nematode infection. When all the cultivars were considered together at the 0 and 5000 nematode inoculum levels, the phosphorus and nitrogen percentages in the seed decreased while potassium increased in seed harvested from nematode-infected plants.

Only seed from the 0 and 25,000 (Table 2) nematode inoculum levels, and not spray-damaged, were analyzed for amino acid content. Seventeen amino acids (aspartic, threonine, serine, glutamic, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine) plus ammonia were detected in the acid hydrolysate (AH) of the dry seed from each cultivar. Amino acid (AA) percentages (Table 4) were calculated as follows:

$$\frac{\text{mg of individual AA in AH}}{\text{Total mg of 17 AA's} + NH_4 \text{ in AH}} \times 100 = \% \text{ of the individual AA}$$

The divisor in the above equation will be hereafter referred to as the total amino acids. Statistical analyses revealed that the percentages of threonine, glutamic, proline, glycine and alanine varied significantly between cultivars with respect to the total amino acids, but the differences were not related to reniform nematode susceptibility or resistance in soybeans. Only leucine showed a significant ($P<.05$) response to the presence of *R. reniformis* (Table 4). All cultivars (14 infected and 14 control replications) responded to nematode infection with a reduction in leucine (0.71% average) content with respect to the total amino acids in the seed.

DISCUSSION

It is evident from the 1966, 1967 and 1968 data that each cultivar has a level of tolerance to increasing initial nematode populations beyond which detrimental effects occurred. 'Lee', 'Bragg', 'Hampton 266', 'Jackson' and 'Dyer' seed yields were not significantly affected when subjected to low initial nematode inoculum levels. Significant seed yield reductions or increases were encountered in 'Hood' and 'Pickett', respectively, at inoculum levels of 5000 and 10,000 nematodes/pot. However, the highest inoculum level had a detrimental effect on seed yields from all cultivars tested. The overall reduction in seed yield was 33.1% at the 25,000 nematode inoculum level. Reasons for the increased seed yields from 'Pickett' plants exposed to low initial nematode inoculum levels were not determined. We know from unreported observations on axenically grown and inoculated 'Peking' roots in water and nutrient agars that lateral roots often develop adjacent to the point of reniform nematode attack. Therefore, we presume that an increase in the numbers of lateral roots and possibly an increase in the total root weight may occur to varying extents on cultivars following low reniform nematode infestation. At high inoculum levels, the increased parasitic burden may damage new and old roots to such an extent that reduced seed yields result.

The overall phosphorus content of the seed dropped 11.1 and 11.5% at the 5000 and 10,000 nematode inoculum levels, respectively. The 1000 nematode inoculum level increased the total seed phosphorus of 'Lee' and showed a trend towards the same for 'Hood', but these were the only two cultivars analyzed at this level (Table 3). These data indicate that very low inoculum levels may increase seed phosphorus levels, whereas the 5000 and 10,000 inoculum levels generally decrease the seed phosphorus levels when compared to the controls. These results indicated that nematode inoculum levels and individual cultivar interactions should be considered when determining effects of nematodes on seed phosphorus.

When the cultivars were grouped by nematode inoculum levels, there was an overall increase over the control in seed potassium of 5.9% at the 5000 level for all cultivars, and 4.5% at the 10,000 level for 'Lee', 'Hood', 'Pickett' and 'Dyer'. Only 'Hood' and 'Lee' (Table 3) showed significant differences in seed potassium which could be related to nematode inoculum levels. As with seed yields and phosphorus content, a relationship between initial nematode populations and final potassium content of the seed is indicated. Low initial populations tend to increase the seed potassium content; but the trend appears to have limits, at least in 'Hood' and 'Lee', beyond which the potassium level tends to decrease.

Total (Kjeldahl) nitrogen content of seed was not affected significantly by *R. reniformis* parasitism in the 1966 and 1968 tests. Some small, but significant, differences in seed nitrogen were observed in the 1967 test. The reasons for these differences are not understood. Reduced nodulation in soybean roots associated with *Heterodera glycines* has been reported (7). Nitrogen fixation in soybeans is complicated by the many interrelationships that exist between *Rhizobium* strains and the various cultivars (3, 19). These interactions may be further affected by planting date (4, 9) and other environmental factors. What effect this may have on seed yield and quality depends to a large extent on the nitrogen-fixing cycle during the various stages of plant growth and parasitism. Under conditions of this test, total nitrogen content and total crude protein per seed did not change significantly from year to year as a result of *R. reniformis* parasitism.

Insufficient replications were available to draw conclusions or correlations between seed amino acid content and parasitism of individual cultivars. A significant net decrease in the leucine content of the seed averaged for all cultivars was associated with *R. reniformis* parasitism (Table 4). A nematode requirement for leucine (16) does not seem to be the cause for a lower content of this amino acid in the seed, since the largest decreases occurred with the resistant cultivars, 'Peking' and 'Dyer'. Also, the leucine content in seeds was approximately the same for all cutlivars. Amino acid studies were made on plants which were inoculated with 0 and 25,000 nematodes/pot. Reduced yields were associated with all inoculated plants under these conditions. Consequently, the drop in seed leucine in the infected plants appears to be related more to reduced growth or increased stress on the plant. This drop in leucine in the seed averaged only 0.7% and probably is not an important factor in limiting the protein quality for human nutrition.

The percent nonproten seed-nitrogen, which would include some free amino acids, is generally very small in mature soybeans (10). Under drought or stress conditions the nonprotein nitrogen may increase. Since free and bound amino acids were not examined separately it is not known if there was a change in the free amino acid level in the seed as a result of nematode infection.

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