Effect of Soil Temperature on Infectivity and Development of Rotylenchulus reniformis on Resistant and Susceptible Soybeans, Glycine max[1]

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Abstract: The effect of soil temperature on parasitism and development of *Rotylenchulus reniformis* on resistant ('Peking' and 'Custer') and susceptible ('Hood' and 'Lee') soybean (*Glycine max*) cultivars was studied. Soil temperatures of 15, 21.5, 25, 29.5 and 36 C ± 1 C were maintained in temperature tanks in a greenhouse. *R. reniformis* developed best at 25 and 29.5 C. The female life cycle can be completed within 19 days after inoculation under favorable conditions at 29.5 C. Plant root growth was best at 21.5 C. During a 27-day period, no egg masses were present on nematodes feeding on roots grown at 15 and 36 C. Egg masses developed on Hood but not on Lee when nematodes were introduced into soil and maintained at 29.5 C for 2 days before raising the temperature to 36 C. *Key words:* reniform nematode, parasitism, temperature effects.

Jones (6) and Wallace (14) reviewed the effects and importance of soil environment on phytonematode movement, development and distribution.

Little is known of the effects of soil temperature on *Rotylenchulus reniformis* Linford and Oliveira, 1940 (8, 13). According to Sivakumar and Seshadri (12), fecundity and population development of *R. reniformis* are influenced by the host plant, and the female can complete its life cycle in 24-29 days and the male in 16-20 days.

This study was undertaken to determine the effects of soil temperature on the parasitism and development of the reniform nematode on resistant and susceptible soybean [*Glycine max* (L.) Merr.] cultivars.

MATERIALS AND METHODS

Seed from *R. reniformis*-resistant ('Peking' and 'Custer') and -susceptible ('Hood' and 'Lee') cultivars of soybean (9) were treated for 15 min with a commercial 5.25% sodium hypochlorite solution and water (1:4) and germinated on sterile potato-dextrose agar. Axenic seedlings were transferred to 150 g (dry wt) of an autoclaved (15 min at 15 psi) sandy clay loam in plastic containers (165-ml capacity). *Rhizobium* spp. were not included in these tests. The soil contained approximately 63% sand, 21% clay and 16% silt as determined by the hydrometer method (3). Soil pH was 6.4, and cation exchange capacity was 10-11 meq/100 g (5). Tops of the containers were covered with aluminum foil containing a small hole through which the plant shoot protrudes. This provided sufficient insulation so temperature in the top centimeter of soil approximated that in the center of the container as determined by small temperature probes. Greenhouse air temperatures ranged from 20 to 35 C. Soil moisture was maintained near field capacity between -1/10 and -1/3 bars (approximately 100 to 350 cm of water suction) (10). Nematodes used for the inoculations were cultured on cotton and extracted from the soil by elutriation (11). Before inoculation, nematodes were placed in 0.002% quinolinol sulfate for 30 min (4), rinsed four times in sterile water, and separated from the rinse water by centrifugation for 2 min at 270 *g*. Each container was placed in the appropriate temperature tank preset at either 15, 21.5, 29.5 or 36 C ± 1 C for 2 days prior to infesting the soil with 300 washed reniform nematodes (males, larvae and infective females). A 1-ml aliquot of an aqueous nematode suspension was introduced into a 1-cm deep hole made near the center of each container with a small sterile glass rod. The ratio of males to females in the inoculum was approximately 1:1. This sex ratio was also obtained from larvae hatched over a 24-hr period and maintained at a constant 29 C for 10 days. Each treatment was replicated 12 times. Three replicates were rated at 6, 12, 19 and 27 days after inoculation. Ratings were based on: (i) nematodes elutriated from the

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soil; (ii) mature females (females with egg masses) attached to roots; (iii) immature females (larvae and females without egg masses); and (iv) eggs per egg mass. Fresh root weights and dry top weights were recorded. Roots were stained with acid-fuchsin in lactophenol and cleared in lactophenol (7) to facilitate counting of nematodes and eggs.

In a second experiment, soil temperatures were 25, 29.5 and 36 C ± 1 C, and sampling was performed on the 7th and 18th day after inoculation. One series which included Lee, Hood and Peking was inoculated at 29.5 C and after 2 days transferred to a 36-C temperature tank to determine whether the higher temperature affected nematode development and/or root penetration. Lee tests were replicated four times and the other cultivars three times.

RESULTS AND DISCUSSION

Reniform nematode-susceptible cultivars (Lee and Hood) responded similarly to treatments in the first test, and the data are combined in Table 1. *R. reniformis*-resistant cultivars (Peking and Custer) also had like responses to treatments in the first test, and these results are presented in Table 2. Table 3 summarizes results for both resistant and susceptible cultivars from the second test.

Egg production: One Lee soybean root system maintained at 29.5 C had four egg-bearing females that averaged 2.7 eggs each after 6 days incubation. Apparently this seedling favored more rapid development of *R. reniformis* females compared to other seedlings. No eggs were produced at the other temperatures by 7 days after inoculation. The number of eggs/egg mass from the 27-day samples maintained at 21.5 C was comparable to that obtained from the 12-day sampling of roots maintained at 29.5 C. No eggs were produced 27 days after inoculation on roots maintained at 15 or 36 C.

Among the high-temperature tests, eggs were produced only on Hood roots when the soil was infested at 29.5 C 2 days prior to increasing the temperature to 36 C. Further studies should be conducted to determine if nematode development at the higher temperature is restricted by the hosts' inability to provide suitable nutrients for *R. reniformis* development or whether there was a direct effect of temperature on the nematode.

Egg production at the various temperatures on resistant cultivars was generally similar to

TABLE 1. Effects of constant soil temperatures on parasitism and development of *Rotylenchulus reniformis* on susceptible ('Hood' and 'Lee') soybean cultivars.

Days after inoc.	Temp (C)	No. nemas in soil	No. nemas/root system: Females with egg matrix	Immature females	Total nemas	No. eggs/ female	Avg wt (g): Root (fresh)	Top (dry)
6	15.0	51 ab[a]	0 a	5 a	5 a[b]	0 a	0.38 a	0.11 a
6	21.5	67 a	0 a	15 a	15 a	0 a	0.55 a	0.10 a
6	29.5	25 b	1 a	11 a	12 a	1 a	0.62 a	0.10 a
6	36.0	39 ab	0 a	5 a	5 a	0 a	0.37 a	0.13 a
12	15.0	36 a	0 c	2 a	2 c	0 b	0.92 b	0.12 ab
12	21.5	21 b	16 a	25 a	41 b	1 b	1.18 a	0.11 b
12	29.5	15 b	89 a	11 a	100 a	33 a	1.09 ab	0.13 a
12	36.0	23 ab	0 c	9 a	9 bc	0 b	0.39 c	0.13 a
19	15.0	40 b	0 c	5 b	5 c	0 c	1.52 b	0.19 a
19	21.5	16 b	44 b	10 ab	54 b	17 b	1.99 a	0.22 a
19	29.5	447 a	236 a	26 a	262 a	42 a	1.84 ab	0.20 a
19	36.0	11 b	0 c	5 b	5 c	0 c	0.62 c	0.17 a
27	15.0	7 c	2 c	9 b	11 c	0 c	2.08 ab	0.19 b
27	21.5	50 b	32 b	9 b	41 b	31 b	2.49 a	0.32 a
27	29.5	756 a	215 a	62 a	277 a	59 a	1.71 b	0.30 a
27	36.0	7 c	0 c	3 c	3 d	0 c	0.72 c	0.23 b

[a]Grouped means for a sampling date followed by uncommon letters are significantly different at the 5% level t-test. Figures represent the mean of six replications from the first test.
[b]Inoculum level 300 nematodes, male to female sex ratio approximately 1:1.

TABLE 2. Effects of constant soil temperatures on the parasitism and development of *Rotylenchulus reniformis* on resistant ('Peking' and 'Custer') soybean cultivars.

Days after inoculation	Temp (C)	No. nemas in soil	No. nemas/root system: Females with egg matrix	No. nemas/root system: Immature females	No. nemas/root system: Total nemas	No. eggs/ female	Avg wt (g): Root (fresh)	Avg wt (g): Top (dry)
6	15.0	50 ab[a]	0 a	4 b	4 b[b]	0 a	0.22 a	0.04 a
6	21.5	58 a	0 a	9 ab	9 ab	0 a	0.32 a	0.06 a
6	29.5	29 b	0 a	19 a	19 a	0 a	0.34 a	0.05 a
6	36.0	44 ab	0 a	7 ab	7 ab	0 a	0.18 a	0.05 a
12	15.0	37 a	0 c	3 b	3 c	0 b	0.65 ab	0.07 a
12	21.5	32 ab	11 b	11 ab	22 b	1 b	1.08 a	0.07 a
12	29.5	21 b	37 a	22 a	59 a	5 a	0.57 b	0.07 a
12	36.0	17 b	0 c	6 b	6 c	0 b	0.21 c	0.08 a
19	15.0	30 bc	0 b	3 b	3 b	0 b	1.31 a	0.13 a
19	21.5	5 d	28 a	22 a	50 a	5 a	1.73 a	0.13 a
19	29.5	43 a	30 a	23 a	53 a	6 a	1.57 a	0.12 a
19	36.0	14 cd	0 b	2 b	2 b	0 b	0.65 b	0.13 a
27	15.0	19 ab	0 b	17 a	17 b	0 b	1.73 ab	0.17 a
27	21.5	13 b	15 a	22 a	37 ab	7 a	2.09 a	0.21 a
27	29.5	35 a	25 a	26 a	51 a	5 a	1.47 b	0.20 a
27	36.0	6 b	0 b	1 b	1 c	0 b	0.68 c	0.18 a

[a]Grouped means for a sampling date followed by uncommon letters are significantly different at the 5% level t-test. Figures represent the mean of six replications from the first test.
[b]Inoculum level 300 nematodes, male to female sex ratio approximately 1:1.

TABLE 3. Effects of soil temperature on the parasitism and development of *Rotylenchulus reniformis* on resistant ('Peking') and susceptible ('Hood' and 'Lee') cultivars.

Cultivar	Days after inoculation	Temp (C)	No. nemas/root system: Females with egg matrix	No. nemas/root system: Immature females and larvae	No. nemas/root system: Total nemas	No. eggs/ egg mass	Avg wt (g): Root (fresh)	Avg wt (g): Top (dry)
Lee	18	25.0	88 a[a]	5 ab	93 a	33 a	1.00 a	0.28 a
Lee	18	29.5	117 a	6 a	123 a	32 a	1.50 a	0.31 a
Lee	18	36.0	0 b	1 b	1 c	0 b	0.55 b	0.28 a
Lee	18	29/36[b]	0 b	7 a	7 b	0 b	0.63 b	0.24 a
Hood	18	36.0	0 b	3 a	3 b	0 a	0.60 a	0.19 a
Hood	18	29/36[b]	7 a	8 a	15 a	16 b	0.70 a	0.20 a
Peking	18	36.0	0 a	3 a	3 a	0 a	1.00 a	0.21 a
Peking	18	29/36[b]	0 a	10 a	10 a	0 a	0.70 a	0.19 a

[a]Column means for any one cultivar followed by uncommon letters are significantly different at the 5% level t-test. Figures represent the mean of four replications Lee and three for Hood and Peking in second test.
[b]29/36 = soil inoculated and maintained at 29.5 C for 2 days before elevating the temperature to 36 C.

that on susceptible soybeans, but at a greatly reduced level.

Soil and root populations: Population development and fecundity of *R. reniformis* were highest on soybean roots maintained at 29.5 C. Root growth was significantly better at 21.5 C, which is similar to that reported for *M. javanica* on some tomatoes (15) and *H. rostochiensis* on certain potato varieties (2). At the 12-day sampling (Table 1), the number of mature females and the total number of nematodes per root were significantly higher at 29.5 C than at the other temperatures. Thus temperature not only affected nematode development, but the invasion rate as well. Significant increases in the soil and root populations of the nematode on susceptible cultivars occurred at the 19-day sampling in the

29.5-C treatment. Root populations observed at that time were too high (>150) and well developed to be composed entirely of fifth stage females from the original inoculum. Only fifth-stage *R. reniformis* females have been reported to parasitize roots (12). Therefore under these conditions the female life cycle of the reniform nematode can be completed within 19 days at 29.5 C, but it takes much longer at 21.5 C under the same conditions. A comparably short life cycle measured from egg to egg has been reported on cotton by Birchfield (1). More nematodes were recorded on Lee roots maintained at 29.5 C than at 25 C (Table 3), but the differences were not significant.

CONCLUSION

Temperature has a significant effect on the rate of infectivity and development of *R. reniformis* on soybean cultivars. At 29.5 C, the female life cycle of the reniform nematode can be completed within 19 days on Lee and Hood soybeans. A soil temperature of 21.5 C favored soybean root growth over nematode development. Twenty-seven days after inoculation, no egg masses were found on roots of plants grown at a constant temperature of 15 or 36 C. Eggs were produced on Hood but not on Lee when roots were inoculated and held at 29.5 C for 2 days prior to elevating the soil temperature to 36 C. At the higher temperature it was not clearly established whether nematode development was affected directly or indirectly by reduced root growth and/or interference with giant cell development in soybeans (9).

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