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Reproduction of the Reniform Nematode on Thirty Soybean Cultivars[1]

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Abstract: In greenhouse experiments conducted in 1991 and 1992, the 30 soybean (*Glycine max*) cultivars most commonly grown in Arkansas in 1990 were tested for resistance to the reniform nematode, *Rotylenchulus reniformis*. 'Forrest' was the most resistant cultivar, whereas 'Braxton' was the most susceptible to *R. reniformis*. Cultivars Coker 485, Centennial, Stonewall, and Sharkey did not differ from Forrest ($P = 0.01$). Cultivars Lee 74, Coker 6955, Walters, Davis, Pioneer 9442, and Narow did not differ from Braxton ($P = 0.01$). Cultivar Lloyd had the second highest reproductive index (Pf/Pi) in 1992 and for the combined test, but was significantly different from Braxton in 1991. The remaining cultivars were inconsistent in their reproductive indices. Two cultivars, Leflore and Lloyd, exhibited large variation in Pf/Pi. This may be due to multiple resistance genes and (or) segregation for resistance among individual seedlings. Segregation is possible because these varieties were not selected or tested for reniform nematode resistance during the cultivar development process.

Key words: Glycine max, nematode, reniform nematode, reproductive index, resistance, *Rotylenchulus reniformis,* soybean, susceptibility.

The reniform nematode, *Rotylenchulus reniformis,* was first observed on cowpea roots in Hawaii (8). The nematode is found primarily in tropical regions of the world and has been reported from 38 countries (4). *Rotylenchulus reniformis* causes yield losses to soybean (*Glycines max*) in the southern United States (4), and was first reported on soybean in Arkansas in 1982 (14).

From 1965 through 1974, several reports were made on resistance of soybean to *R. reniformis* (1,2,10,11). Resistance to soybean cyst nematode, *Heterodera glycines,* and *R. reniformis* are closely linked (10) such that *H. glycines*-resistant cultivars are also resistant to *R. reniformis* (2,6,10–13). The cultivar Mack has resistance to *H. glycines* but not to the reniform nematode (2). Resistance to *R. reniformis* in soybean is regulated by multiple genes (2,3,17). Apparently, resistance to *Meloidogyne* spp. is not linked to *R. reniformis* resistance (6,10–12). Screenings have been made both in the greenhouse (16) and in the field (9).

A list of the 30 soybean cultivars most commonly grown in Arkansas in 1990 (L. Ashlock, pers. comm.) was the basis for experiments in 1991 and 1992 on host suitability for *R. reniformis.* All of the cultivars had been tested previously for resistance to *Meloidogyne* spp. (6,12) and (or) *H. glycines* (6,12,13). Our objective was to determine which cultivars had resistance to reniform nematode in order to develop recommendations for growers whose fields were infested with the nematode.

Materials and Methods

Soybean seeds of 30 soybean cultivars obtained from the Arkansas soybean variety testing program, were germinated in vermiculite in a greenhouse. In the cotyledonary stage, individual seedlings were transplanted into 10-cm-d clay pots containing 500 cm^3 fine sandy loam (ca. 91% sand, 5% silt, 4% clay, <1% O.M.). Ten pots of each cultivar were used in 1991; in 1992, 10 fallow pots were added. One-half of the pots of each cultivar and fallow treatment were infested with ca. 1,000 vermiform reniform nematodes. Pots were arranged in a randomized complete block design with five replications per treatment.

Vermiform reniform nematodes were obtained from greenhouse-grown soybean plants (cv. Braxton). Soil was washed from

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the roots, suspended in water, and poured through nested 841- (20 mesh) and 38- (400 mesh) μm-pore sieves. The material on the 38-μm-pore sieve was placed on a tissue in a Baermann funnel. All vermiform stages of *R. reniformis* were collected after 16 hours and injected with an autopipette into three 1-inch deep holes made in the soil with a dibble at the proper dilution. After ca. 60 days (25 April to 24–28 June 1991; 12 February to 21–27 April 1992) plants were harvested, roots and shoots were weighed, and nematodes were extracted from roots and soil.

To determine the final nematode population (Pf), a 100-cm^3 aliquot of well-mixed soil from each pot was suspended in water and poured through nested 841- and 38-μm-pore sieves to remove plant debris and extract the nematodes. The nematode suspension from the 38-μm-pore-sieve was clarified by sucrose centrifugal flotation (7), counted, and multiplied by five to give the number per pot. The eggs and vermiform nematodes in the egg masses on roots were extracted with a 0.525% sodium hypochlorite solution (5) and counted. The total number of *R. reniformis* per pot was calculated by adding the number from the soil to the number from the roots. Because of the variation in nematode counts, data were transformed ($\log_{10}[x + 1]$) for analysis, although actual counts are presented (Table 1). Statistical analyses were conducted for each year indiviudally and for both years combined. ANOVA and Waller-Duncan k-ratio (k ratios = 100 and 500) *t* tests were calculated with SAS (SAS Institute, Cary, NC) procedures.

Results

Previously reported resistance to the soybean cyst and southern root-knot nematodes in the 30 cultivars most commonly grown in Arkansas in 1990 are presented in Table 1. The cultivar rankings for reproductive indices (Pf/Pi) in 1991 and 1992 and the combined rankings of both years generally were similar (Table 2). The combined data showed no significant cultivar × year interaction.

In our experiments, all cultivars supported at least some reniform nematode reproduction. Resistant and susceptible cultivars were identified, but many cultivars were intermediate between the two reactions. Those cultivars different from Forrest, the most resistant cultivar, and Braxton, the most susceptible cultivar, but not different ($P = 0.01$) from the remainder of either the resistant or susceptible groups were rated as intermediate. The resistant cultivar ratings were more consistent than the susceptible cultivar ratings.

In the 1991, 1992, and combined analyses, Forrest had the lowest Pf/Pi, whereas Braxton had the highest. The five cultivars Forrest, Coker 485, Centennial, Stonewall, and Sharkey had low Pf/Pi in the combined data as well as in both years of the experiment and were rated resistant.

The reproductive indices of the remaining cultivars were difficult to separate into groups. Although Braxton had the highest reproductive index in all analyses, it was not different ($P = 0.01$) from Lee 74, Coker 6955, Walters, Davis, Pioneer 9442, or Narow. Lloyd had the second highest Pf/Pi for 1992 and for the combined analyses, but differed from Braxton in 1991. These eight cultivars were rated susceptible based on their reproductive indices.

The reproductive index of *R. reniformis* varied greatly in the cultivars tested. The ranges of Pf/Pi were extremely variable for certain cultivars (Table 3). In 1991 and 1992, the ranges for the reniform nematode resistant cultivars Forrest and Sharkey; the susceptible cultivars Lee 74 and Walters; and the intermediately ranked cultivars Bedford and Tracy-M were relatively narrow. In contrast, a wide range of Pf/Pi existed within the intermediately ranked cultivars Lloyd and Leflore.

In 1992, *R. reniformis* survival in inoculated fallow pots was 71.2% after ca. 8 weeks. No consistent differences were found between the weights of the inoculated and uninoculated plants (data not shown). The average total plant weight of the inoculated plants was 20.1 g in 1991 and 32.6 g in 1992.

TABLE 1. Reported resistance to the soybean cyst (*Heterodera glycines*), reniform (*Rotylenchulus reniformis*), and southern root-knot (*Meloidogyne incognita*) nematodes of the 30 cultivars most commonly grown in Arkansas in 1990.

	Reported resistance†		Tested resistance‡				
			Riggs et al. (1988)		Riggs et al. (1991)	Hussey et al. (1991)	
Cultivar	*Heterodera glycines*	*Meloidogyne incognita*	*Heterodera glycines*	*Meloidogyne incognita*	*Heterodera glycines*	*Heterodera glycines*	*Meloidogyne incognita*
Private							
Asgro 5403	R	S	—	—	R	—	—
Asgro 5979	R	S	—	—	R	—	—
Asgro 6297	R	S	—	—	R	—	—
Asgro 6785	S	MR	S	R	S	S	MR
AT 550	R	R	—	—	R	—	—
Coker 485	R	R	R	R	R	R	R
Coker 6955	R	S	—	—	R	—	—
Deltapine 105	S	S	S	MR	S	S	S
Deltapine 415	R	S	R	S	R	R	S
Hartz 5164	R	MR	R	—	R	R	R
Hartz 6686	S	S	—	—	S	S	S
Pioneer 9442	—	—	—	—	S	—	—
Pioneer 9581	R	Tol	R	R	R	R	R
Pioneer 9592	S	MR	—	—	S	—	—
Pioneer 9641	S	MS	—	—	S	S	S
Public							
Bedford	R	MR	R	R	R	R	MR
Braxton	—	—	S	R	S	S	R
Centennial†	R	R	R	R	R	R	R
Davis	S	S	S	S	—	S	S
Forrest	R	R	R	R	R	R	MR
Hutcheson	S	S	—	—	S	S	S
Lee 74	S	R	S	R	S	—	—
Leflore	R	R	R	R	R	R	R
Lloyd	R	—	R	S	R	—	—
Narow	R	—	R	S	R	—	—
Sharkey	R	R	—	—	S	—	—
Stonewall	R	MR	—	—	R	—	—
Tracy-M	—	—	S	S	S	S	S
Walters	R	R	—	—	R	—	—
Williams 82	—	—	—	—	—	—	—

The soybean cyst nematode resistance noted is for race 3 only. The southern root-knot resistance is for *Meloidogyne incognita* gall indices of 1.5 or less; moderate resistance is for gall indices of 1.6 to 1.9; and susceptible is for indices of 2.0 or greater. S = susceptible, MS = moderately susceptible, R = resistant, MR = moderately resistant, Tol = tolerant, and — = not reported.

† Reported resistance is from USDA Soybean Variety Release Notices for private cultivars and cultivar registrations for public cultivars. Centennial is the only cultivar with reported resistance to the reniform nematode (*Rotylenchulus reniformis*).

‡ Tested resistance is from Riggs, Hamblen, and Rakes, 1988 (12); Riggs, Rakes, and Elkins, 1991 (13); and Hussey, Boerma, Raymer, and Luzzi, 1991 (6).

DISCUSSION

Resistance in soybean to *R. reniformis* is closely linked to soybean cyst nematode (*H. glycines*) resistance and does not appear to be related to southern root-knot nematode (*Meloidogyne incognita*) resistance. Cultivars Lloyd and Walters have resistance to *H. glycines* race 3; however, like the cultivar Mack they are susceptible to *R. reniformis*. The parentage of Lloyd and Walters include the *R. reniformis*-resistant cultivars Centennial and Forrest, respectively. Several soybean cultivars with *H. glycines* race 3 resistance are moderately resistant or moderately susceptible to *R. reniformis*. None of the cultivars susceptible to *H. glycines* race 3 were resistant to *R. reniformis*. The cultivar Braxton has a high degree of resistance to southern root-knot nema-

TABLE 2. Reproductive indices of *Rotylenchulus reniformis* on the 30 soybean cultivars most commonly grown in Arkansas in 1990 from greenhouse studies in 1991 and 1992.

1991			1992			Combined 1991–1992			
Cultivar	*N*	Reproductive index†	Cultivar	*N*	Reproductive index	Cultivar	*N*	Reproductive index	Rating‡
Braxton	4	26.6 a	Braxton	5	130.2 a	Braxton	9	84.2 a	S
Lee 74	5	21.6 ab	Coker 6955	5	109.4 ab	Lee 74	10	54.3 ab	S
Davis	4	17.0 abc	Lloyd	5	120.1 abc	Coker 6955	10	63.1 ab	S
A-5403	5	17.0 abc	Lee 74	5	86.9 abcd	Walters	9	53.9 ab	S
Hartz 5164	4	16.1 abcd	Walters	5	83.1 abcd	Davis	9	52.7 ab	S
Walters	4	17.5 abcd	Narow	5	82.0 abcd	Narow	9	52.7 ab	S
Coker 6955	5	16.8 abcd	Davis	5	81.3 abcd	Pioneer 9442	8	44.2 ab	S
Leflore	5	17.6 abcd	Delta Pine 415	5	78.1 abcd	Lloyd	10	67.1 ab	S
Narow	4	16.1 abcd	Williams 82	5	69.0 abcd	A-6297	9	43.3 b	I
A-6297	4	13.8 abcd	A-6297	5	66.9 abcd	Delta Pine 415	9	49.5 b	I
Pioneer 9442	3	14.6 abcd	Tracy-M	5	68.3 abcd	Hartz 5164	9	38.1 b	I
Delta Pine 415	4	13.7 abcd	Hartz 6686	5	64.1 abcd	Williams 82	9	44.3 b	I
Williams 82	4	13.3 abcd	Pioneer 9641	5	67.8 abcd	Tracy-M	9	43.7 b	I
Tracy-M	4	13.0 abcde	Delta Pine 105	5	64.9 abcd	A-5403	10	36.6 bc	I
Hutcheson	4	11.5 abcde	Pioneer 9592	5	65.3 abcd	Hartz 6686	9	40.4 bc	I
Lloyd	5	14.0 bcde	Pioneer 9442	5	62.0 abcd	Delta Pine 105	9	41.3 bcd	I
A-5979	4	10.9 bcde	A-6785	5	60.1 abcd	Pioneer 9641	8	45.1 bcd	I
Delta Pine 105	4	11.8 bcde	Hartz 5164	5	55.7 abcde	Hutcheson	9	36.4 bcd	I
Hartz 6686	4	10.7 bcde	Hutcheson	5	56.3 bcdef	Leflore	10	55.7 bcd	I
AT 550	4	11.1 bcde	A-5403	5	56.3 bcdef	A-5979	9	34.2 bcd	I
Pioneer 9581	4	11.1 bcde	A-5979	5	52.7 bcdef	A-6785	9	38.1 bcd	I
Pioneer 9592	5	9.6 cde	Leflore	5	93.8 cdef	Pioneer 9592	10	37.4 bcd	I
A-6785	4	10.6 cde	Bedford	5	40.0 def	Bedford	9	24.6 cd	I
Pioneer 9641	3	7.4 def	Pioneer 9581	5	35.0 efg	Pioneer 9581	9	24.4 cd	I
Bedford	4	5.3 efg	AF 550	5	25.5 fg	AT 550	9	19.1 d	I
Coker 485	4	3.9 fg	Sharkey	5	11.6 gh	Sharkey	10	7.6 e	R
Sharkey	5	3.5 fg	Centennial	5	12.9 gh	Stonewall	10	8.9 e	R
Stonewall	5	3.3 fg	Stonewall	5	14.6 gh	Centennial	9	8.4 e	R
Centennial	4	2.7 g	Coker 485	5	7.3 h	Coker 485	9	5.8 e	R
Forrest	4	2.1 g	Forrest	5	5.9 h	Forrest	9	4.2 e	R
CV		18.8			13.8			18.6	

Values followed by the same letter are not significantly different according to Waller-Duncan k-ratio *t* test: k-ratio = 500. CV calculated from transformed data; actual count means are given. Five hundred cm^3 soil was used in each 10-cm-d clay pot.

† Reproductive index = final nematode population density/initial nematode population density.

‡ S = susceptible, I = intermediate, and R = resistant reniform nematode rating determined by sameness in all three analyses.

TABLE 3. The reproductive index (Pf/Pi) and standard error (in parentheses) of *Rotylenchulus reniformis* for selected resistant, susceptible, and intermediate rated soybean cultivars in 1991 and 1992.

Cultivar	1991	1992
	Resistant	
Forrest	0.5–4.0 (±0.6)	3.1–9.4 (±1.3)
Sharkey	1.7–7.8 (±1.1)	9.2–14.2 (±0.8)
	Susceptible	
Lee 74	13.1–30.6 (±4.1)	65.6–105.4 (±6.4)
Walters	8.5–34.0 (±5.0)	58.6–112.4 (±8.9)
	Intermediate	
Bedford	3.2–6.3 (±0.7)	28.1–55.6 (±5.0)
Tracy-M	5.6–18.1 (±3.4)	26.0–101.0 (±12.3)
Lloyd	5.9–36.8 (±5.8)	41.0–225.8 (±31.6)
Leflore	3.5–35.5 (±5.3)	2.0–181.7 (±34.5)

Ten replications/cultivar/year.

tode, but is highly susceptible to *R. reniformis*.

The high degree of variability in intermediately ranked soybean cultivars such as Lloyd and Leflore may be due to heterogeneity resulting in segregation. Heterogenic strains could easily occur because no test for resistance to *R. reniformis* was reported in any of the release notices for any of the cultivars included in this experiment. Multiple genes responsible for *R. reniformis* resistance in soybean (2,3,17) may also help explain the difficulty in separating the resistant, susceptible, and intermediate cultivars.

The average total plant weight of inoculated plants in 1992 was greater than in 1991 and may help explain the higher numbers of nematodes that were recovered in 1992. The reason for the larger plants in 1992 is unknown.

Reniform nematode juveniles do not feed. The infective stage of this nematode is the immature female. The reniform nematode can survive and remain infective in soil for up to 1–year (15). The longevity of the juveniles and infective stage, evidenced by the high survival rate in the inoculated fallow plots in the 1992 test and the authors' unpublished observations of field samples, may be the main reason the reniform nematode generally has high populations densities during the spring planting season. The host-seeking second stage juveniles of *Heterodera glycines* and *Meloidogyne* spp. normally remain infective for a much shorter time. Although *H. glycines* survives for long periods of time, it does so as unhatched eggs within the protective cyst.

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