Correlation of Resistance in Soybeans to Heterodera glycines and Rotylenchulus reniformis

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ABSTRACT

All *Heterodera glycines* (soybean cyst nematode)-resistant soybean cultivars tested were also resistant to *Rotylenchulus reniformis* (reniform nematode). Resistance of Dyer, Custer, and Pickett is derived from Peking. D66-12394, D66-12392 and two F_4 cultivars of PI90763 × Hill crosses also carry genes for resistance to both nematodes. Resistant cultivars are attacked by the reniform nematode larvae, but female development is subsequently inhibited. Histological effects of parasitism of female reniform nematodes on Lee soybean closely resembles those reported with the soybean cyst nematode. The histology of susceptible and resistant soybeans to *R. reniformis* was studied. No relationship was established between *R. reniformis* and *Meloidogyne incognita* (root knot nematode) resistance in soybeans. This is the first report in which a crop has been shown to have a common genetic source of resistance to nematodes from different genera. It is also the first report showing the histology of *R. reniformis* parasitism on soybeans. Phytopathology 60:695-700.

The first soybean (*Glycine max* L. Merr.) cultivars reported to be resistant to the reniform nematode were Pickett and Dyer (14). Lee was reported as susceptible at that time. This evidence led us to hypothesize the following: (i) Peking, the common source of resistance to the soybean cyst (*Heterodera glycines* Ichinohe, 1952) nematode for Pickett and Dyer, is resistant to the reniform (*Rotylenchulus reniformis* Linford & Oliveira, 1940) nematode; and (ii) a relationship probably exists between parasitism of reniform and soybean cyst nematodes and resistance in soybeans.

Hartwig (9) summarized the problems encountered in isolating and transferring resistance to *H. glycines* into desirable soybean cultivars. Consequently, by demonstrating resistance and physiological relationships between the two nematode species, we can take advantage of previous work, and also facilitate future studies in parasitism and resistance. This work was undertaken primarily to determine if such relationships exist and if soybean resistance screening results are interchangeable with regard to the species under consideration. This does not necessarily extend to other crops such as cotton, which is not a host for the soybean cyst nematode but is an economically important host for the reniform nematode (1, 10, 12). Auburn 56 cotton has been used at Auburn University to rear *R. reniformis,* while Epps & Chambers (7) have used this cultivar in rotation studies to reduce field populations of *H. glycines.*

Materials and Methods.—Tests for soybean resistance to *H. glycines* were conducted in Tennessee using methods previously reported (6, 15). Root-knot nematode resistance evaluations were also conducted in Tennessee unless otherwise indicated.

A screening test with *R. reniformis* was conducted in the spring of 1968 at Auburn by the following procedure. A raised transite greenhouse bench was filled to a depth of 12 cm with a heat-treated fine sandy loam soil. The surface of the soil bed was leveled and punched with rows of holes 1 cm deep and 5 cm apart. Each row of holes was spaced 10 cm apart. To each hole was added a 1-ml aqueous aliquot containing approximately 1,500 reniform nematode larvae that had been surface-sterilized for 30 min in 0.001% quinolinol-sulfate (3) and subsequently rinsed with tap water over a nylon filter (14 μ pore size). Soybean seeds were treated for 10 min with commercial 5.25% sodium hypochlorite solution and water (1:4), rinsed in running tap water for 30 min, and sprinkled with a commercial nitrogen-fixing bacterium (*Rhizobium* spp.). One seed was then added to each hole in the soil surface. Each cultivar (Table 1) was divided into four replications of five seeds. Since this was the first time we used this screening method with *R. reniformis,* a nematode-free control was included to determine nematode movement and infectivity between rows under these conditions. The plants were grown for 7 weeks at soil temperatures ranging from 18 C to 33 C. Roots were recovered by removing each replication of five plants as an intact soil block and gently washing the soil away. The washed roots were photographed, weighed, fixed in hot lactophenol acid fuchsin for 30 sec, and cleared in lactophenol. Larvae and mature females with egg masses on the stained roots were counted separately. A female matrix which contained one or more eggs was considered an egg mass for resistance evaluation purposes (Table 1, 2). For histological studies, roots were fixed at 4 C (2 hr each fixative) in either 1% osmium tetroxide, 4% neutral formalin, or in neutral formalin postfixed with osmium tetroxide, and paraffin-embedded. Sections were cut at 10, 15, and 20 μ and double-stained with either fast green and safranin-O or Delafield's hematoxylin and eosin-Y.

A second test (autumn 1968) was set up essentially as the first with the following exceptions. The test was conducted in 8-inch clay pots containing a fine sandy soil. Ten seed of a cultivar were planted into each pot and inoculated with *R. reniformis.* Three pots were used for each variety. At the end of the growth period,

150 cc of soil were taken from each pot, and the nematodes extracted and counted (16).

RESULTS AND DISCUSSION.—In resistant roots, the maximum female reniform nematode development commonly encountered on Peking is depicted in Fig. 2-A and B. Female development often terminates with the production of a single egg which may ultimately hatch, trapping the larvae in her body cavity. Less often a female was surrounded by a clear matrix which contained fewer than 10 eggs and averaged about 4. Egg masses on Hood and Lee roots, under the same conditions, had up to 114 eggs and averaged 57 and 61 eggs/egg mass, respectively. The egg counts were averaged from 10 egg masses taken from three separate root systems. Very rarely are egg masses of more than 10 eggs found on resistant roots. On one occasion, a reniform nematode was observed on Peking with a well-developed egg mass containing about 50 eggs. This poses questions not answered here. Was this female capable of breaking resistance? Or was she feeding in a root area less hostile to her development? Or was this particular root slightly less resistant for some undetermined reason?

Data from the spring test on numbers of *R. reniformis* females with egg masses, infective females without egg masses, and a soybean-resistance evaluation for the soybean cyst and reniform nematodes is presented in Table 1. Similar data gathered from the fall test plus the combined count of *R. reniformis* larvae, males, and infective pre-adult females (third stage) obtained from the soil at the end of the test is presented in Table 2. Cultivars may vary significantly ($P = .005$) in the numbers of *R. reniformis* egg masses developing on a root over a 6-week period. However, only Peking, Pickett, Dyer, Custer, D66-12392, D66-12394, and both F_4's of Hill × PI90763 crosses are considered highly resistant to *R. reniformis* (Fig. 1), which compares a relatively nematode-free Peking root with a susceptible Hill root bearing numerous egg masses. Both cultivars were grown under the same conditions in nematode-infested soil. In the presence of resistant cultivars, *R. reniformis* soil populations declined significantly ($P = .005$) during the test period from an initial inoculum level of 10,000 to less than 2,040 nematodes/gal of soil (Table 2). The slight differences in results between the spring and fall tests are probably due to one or more of the following variables: soil type; inoculum virulence; seed vigor; or seasonable variables such as light and temperature. The *R. reniformis* resistance evaluations are slightly lower than those reported for *H. glycines*. One reason for this, other than generic differences, is that stained roots were used to evaluate *R. reniformis* as opposed to unstained roots in *H. glycines* evaluations. Undoubtedly, if we had used unstained roots as in previous work (14) we would not have seen some egg masses, consequently giving the reniform nematode resistant cultivars a higher rating. Although highly resistant cultivars received a high resistance rating of from 2 to 3, they did not differ significantly in the numbers of females with egg masses per root.

From our data and a previous report (14), Peking is the probable source for reniform nematode resistance in Dyer, Custer, and Pickett. Peking already has been established as the source for resistance to the soybean cyst nematode (15). Therefore, it seems reasonable to

TABLE 1. The number of reniform (*Rotylenchulus reniformis*) nematode egg masses, larvae, and infective females without eggs per root, and the correlation of resistance to the soybean cyst nematode (*Heterodera glycines*) with resistance to the reniform nematode, spring 1968

	Reniform nematode				
				Resistance evaluation[a]	
Cv.	Mean no. egg masses/root[b]	Larvae and infective females/root without eggs	Avg wet root wt, g	*R. reniformis*	*H. glycines*
Peking	3.1 a	50	1.7	2	1 < 2
Pickett	3.4 a	23	1.0	2	1 < 2
D66-12394[c]	6.6 a	54	1.1	2	1 < 2
Custer[d]	15.9 a	24	0.7	3	1 < 2
Dyer	21.6 a	20	1.2	3	1 < 2
D64-4636[e]	180.3 b	53	0.9	4	4
Hill	324.2 c	54	1.3	4	4
Dare	333.6 c	23	1.1	4	4
Lee	455.7 d	47	1.3	4	4
Lee (non-nematode-inoculated	25.7	8.9	1.0		

[a] Resistance evaluation according to Ross & Brim (15): The rating refers to the number of stained *R. reniformis* egg masses per root or the number of white unstained cysts per root. 1 = none; 2 = 1 to 10; 3 = 10 to 25; 4 = 26 or more.

[b] Figures followed by different letters are significantly different at the 1% level ($P = .005$) Duncan's multiple range test.

[c] D66-12394 is an experimental strain having Dyer as a parent.

[d] Custer has Peking as its source of resistance to the cyst nematode and is highly susceptible to *Meloidogyne incognita*.

[e] D64-4636 is an experimental strain highly resistant to *M. incognita*.

TABLE 2. The number of reniform (*Rotylenchulus reniformis*) nematode egg masses, larvae, and infective females without egg masses found on the roots. The number of nematodes found free in the soil and the correlation of resistance to the soybean cyst nematode (*Heterodera glycines*) with resistance to the reniform nematode, fall 1968

	Reniform nematode				Resistance evaluation[a]	
Cv.	Mean no. egg masses/ root[b]	Infection female/ root without eggs	Avg wet root wt, g	*R. reniformis*/ gal soil[c]	*R. reniformis*	*H. glycines*
Peking	1.1 a	19	3.7	2,040 a	2	1 < 2
Hill × P190763-1)[d]	1.5 a	35	3.7	1,710 a	2	1 < 2
Hill × P190763-2)[d]	1.7 a	44	2.4	1,670 a	2	1 < 2
D66-12392[e]	2.6 a	35	2.2	1,880 a	2	1 < 2
Lee	209.2 b	10	2.6	19,830 b	4	4

[a] See Table 1.
[b] See Table 1.
[c] The numbers of nematodes extracted from 150 ml of soil corrected to 3785 ml (1 gal), the soil content of one 8-inch pot. The count includes free living larvae and adults.
[d] F_4 of the crosses having resistance to both the Tennessee and Holland, Virginia, strains of soybean cyst nematodes.
[e] D66-12392 is an experimental strain highly resistant to *Meloidogyne incognita*.

assume that it is also the source of resistance in D66-12394 and D66-12392, as these cultivars derive their resistance from Dyer.

PI90763 is equal to Peking in its resistance to most populations of the soybean cyst nematode (15); but it has better resistance to the Holland, Virginia strain than Peking (2). Unfortunately, a pure source of PI90763 was not found for these tests, so we cannot state with certitude that PI90763 was also a source of resistance to the reniform nematode. However, the two PI90763 × Hill crosses tested here appear about equal to or slightly superior to Peking in reniform nematode resistance (Table 2).

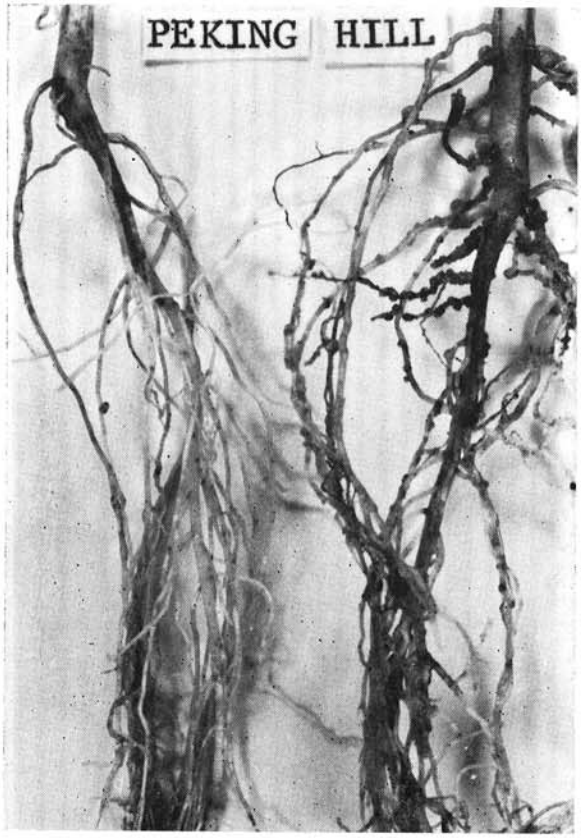

Fig. 1. Peking and Hill soybean roots grown for 7 weeks in the presence of reniform nematodes. Note numerous large egg masses on highly susceptible Hill.

R. reniformis larvae penetrate soybean roots intercellularly, and they are almost always oriented perpendicular to the central axes of the root (Fig. 2-A,B,C,D). Unlike may *H. glycines* larvae (4), reniform nematode larvae rarely become completely buried in host tissue unless the root cortex thickness exceeds the larval length. In soybeans, the female reniform nematode penetrates the roots as a larva or preadult; then the posterior portion of her body, which is outside the root, begins to swell. As the swelling increases, the external root cells rupture first (Fig. 2-C). The cell walls of the penetrated cortical cells thicken slightly and stain darker in the presence of osmium tetroxide. Mechanical damage to cortical cell walls occurs almost exclusively as the result of direct penetration and growth of the nematode. In giant cell formation, the cell walls appear to grow and thicken with the cells. Little mechanical breakdown or dissolution of giant cell walls was noted, except for a few cells immediately next to the feeding cell.

Optimum *R. reniformis* female development, egg mass production, and giant cell formation were associated with feeding in the pericycle tissue adjacent to the outermost xylem vessel of the protoxylem pole. The inability of larvae and preadult females to develop, produce egg masses, and cause giant cell formation was associated with feeding at the protophloem poles (Fig. 2-D). The pericycle cell in which the female head finally comes to rest and feed as she enters the sedentary and reproductive phase of her life develops into the largest of the giant cells. It was estimated that as many as 100 to 200 adjoining giant cells may be associated with the feeding of one female. These adjoining giant cells are located in the pericycle region, and ex-

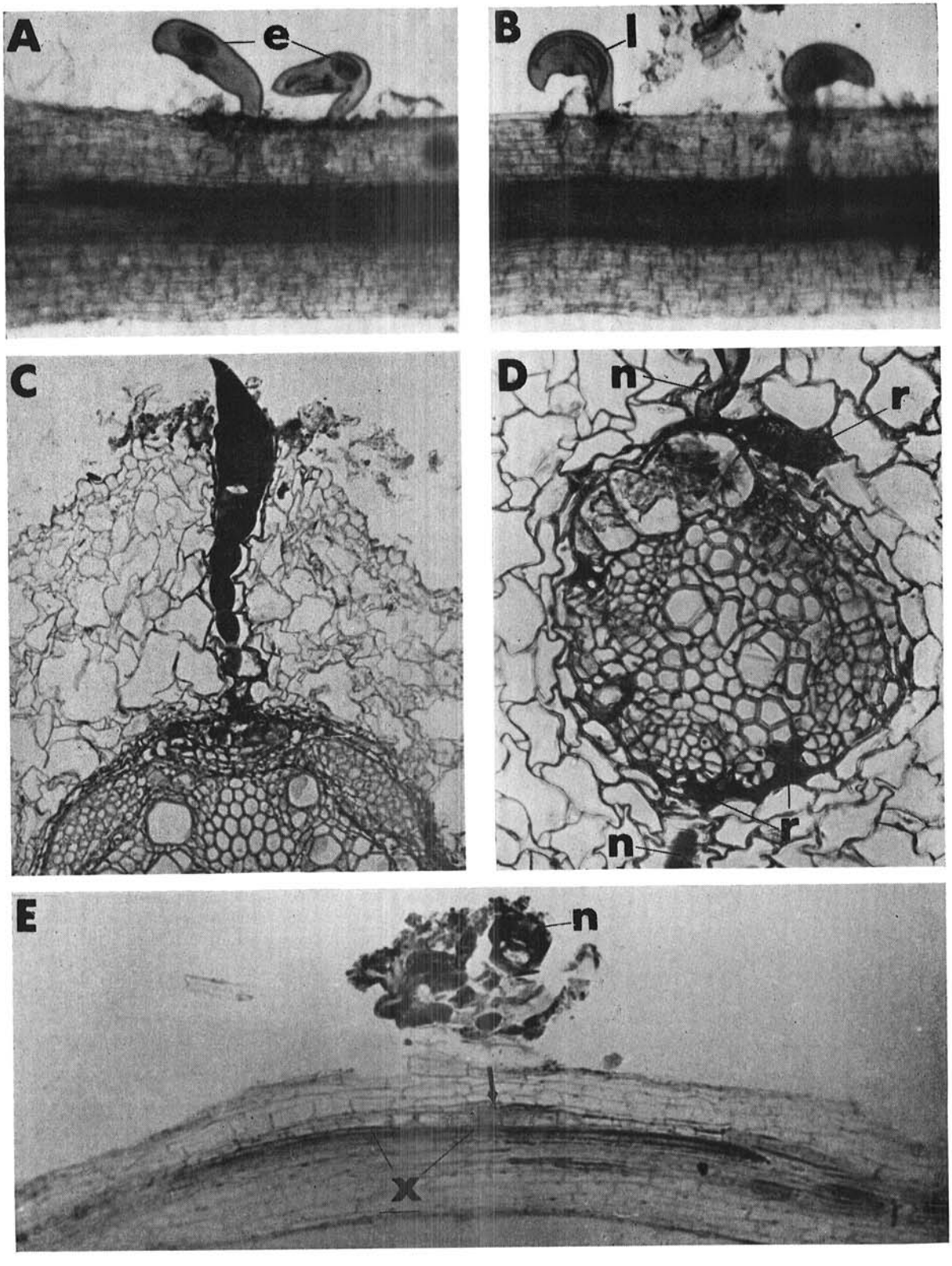
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Fig. 2. Reniform nematode in resistant and susceptible roots. **A)** Advanced females on Peking (resistant), each containing a single egg. Stele takes on dark appearance in infected area. **B)** Hatched larva trapped inside a female attached to a Peking root. **C)** Cross section showing intercellular penetration of cortical cells by a female on Lee (susceptible). A slight thickening of penetrated cell walls and early development of giant cells formed in pericycle at the protoxylem pole immediately beneath the endodermis are noted. Female swelling and development has begun. **D)** Top: cross sectional enlargement of root showing well-developed giant cells. A resistance-type reaction limited mainly to cortex is indicated by the dark-stained cortical cells. Bottom: poorly developed female penetrated at the protophloem pole. Dark stain appears in pericycle region of the stele, and giant cells are not forming. **E)** Longitudinal section showing giant cells on Lee extending in both directions (about 10 cells) from center of infection site (arrow). A dark-stained xylem ray vessel paralleling giant cells is also noted. e = Egg; l = larva; n = female nematode; r = dark stain characteristic of the resistance reaction; x = yxlem vessel.

tend about 3 to 10 cells in both directions from the protoxylem pole when viewed in cross section (Fig. 2-D), and extend about 5 to 15 cells in both directions from the invaded cell when viewed in longitudinal section (Fig. 2-E). Giant cell size diminishes as the distance from the feeding cell site increases. This bipolar direction of giant cell development from the primary feeding site may indicate that the plant's polar transport system is either interfered with, or has no effect on, the primary agents responsible for giant cell formation.

The female feeding site and the associated tissue responses found in this study on *R. reniformis* are markedly similar to that reported by Endo (4, 5) regarding *H. glycines* parasitism on Lee. Giant cell formation, enlarged and multinucleated cells, and a clumping of nuclei in the pericycle tissue near the protoxylem poles appear to be similar for both species. Some variations between the two species occur regarding male feeding at the protophloem poles and male development. *H. glycines* males have been reported to penetrate soybean roots where they feed. Development of *H. glycines* males has been associated with feeding at the protophloem poles (4). We have observed that the *R. reniformis* larvae feeding near the protophloem poles become females, but their development and egg production is inhibited much as in resistant soybean roots. *R. reniformis* males were found free in the soil or associated with an egg mass but never in root tissues. This agrees with the work of Linford & Oliveira (11) and Peacock (13), who made similar observations. In these studies, the ratio of reniform nematode males to preadult females was approximately 1:1. The above observations, the small size of *R. reniformis* males, and the vacuolations often observed in their bodies suggest that they probably do not feed and may be necessary for propagation. Indirect evidence has been reported (8) indicating *H. glycines* males are necessary for reproduction. These differences in the male life histories may be expected to account for some variations in host range and resistance-breaking capabilities.

Regardless of where a reniform nematode penetrates the endodermis to enter the pericycle tissue, some degree of hypersensitive reaction usually occurs. When endodermal penetration takes place almost anywhere in a resistant root (Fig. 3) or in an area other than the protoxylem poles in a susceptible root (Fig. 2-D), the reaction is limited and mostly involves the endodermal and pericycle region. Several pericycle cells, about and including the nematode-invaded cell, are usually involved in a resistance reaction. The affected cells are dead and filled with a substance that stains red in the presence of safranin-O and fast green FCF. This stain reaction is characteristic of a lignin-type substance. Whether the red-staining substance causes the cells to die, or whether it is a postmortem response, was not determined. Whatever the reaction, giant cell formation is prevented and subsequent nematode development inhibited. When larvae penetrate a susceptible host at the protoxylem poles, the reaction was light or confined to a few cortical cells (Fig. 2-D) outside the stele. Under these conditions, the pericycle cells remain viable and form giant cells. In susceptible roots, the proximity of the giant cell to the outermost xylem vessel (Fig. 2-E) appears to have some important relationship. This relationship is not understood, but the xylem vessels adjacent to the giant cells stain more readily, and they may help remove excess toxins which might kill the pericycle tissue or inhibit giant cell formation after penetration by the nematode.

The following evidence shows that soybean resistance to the reniform nematode may be morphological as well as biochemical. In susceptible roots, the maxi-

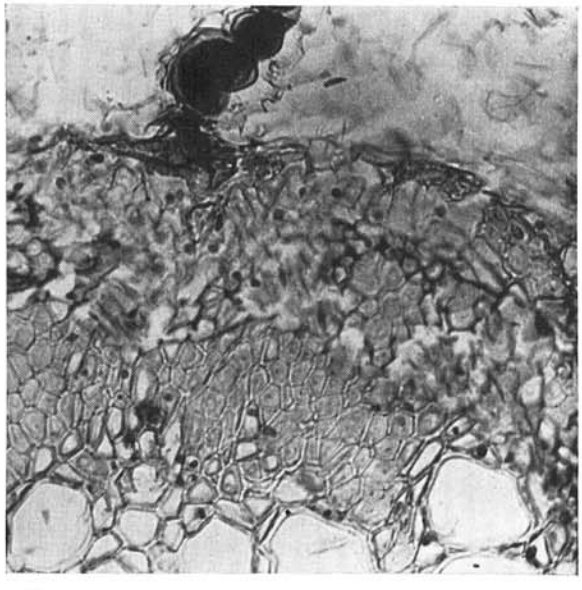

Fig. 3. Cross section of resistant PI90763 × Hill (F_4) root (resistant) showing a resistance type of reaction and lack of giant cell formation induced by a female reniform nematode. Dark-stained limited to stele area, much as in susceptible Lee (2-D, bottom).

mum female development was generally associated with feeding in pericycle tissue at the protoxylem poles. Also, the inability of *R. reniformis* to induce giant cells is not limited to resistant varieties, but appears to occur in susceptible cultivars, particularly when attached at the protophloem poles.

From the similarity in host range and feeding habits of female reniform and soybean cyst nematode, we conclude that the same gene, or linked genes, that impart resistance in cultivars developed from Peking, and possibly PI90763, are responsible for resistance to both species. Therefore, it may be possible to use either nematode to test for resistance to both nematodes. Since Custer is resistant to *R. reniformis* and *H. glycines* but highly susceptible to root knot nematodes, whereas the reverse situation exists in Hill and D64-4636, no relationship was established between reniform and root knot nematode resistance in soybeans.

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