

## Nematode Population Attrition and Histopathology of *Heterodera glycines*-Soybean Associations<sup>1</sup>

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**Abstract:** Selected populations of soybean cyst nematodes were inoculated to roots of compatible and incompatible soybeans. Rates of penetration of infective juveniles of nematode populations selected on PI 209332, PI 89772, and Pickett 71 were equivalent on compatible and incompatible soybean roots. The first two populations averaged about 10% and the last about 5% penetration in 24-hour inoculations of young seedlings. About 14% of those juveniles that entered roots in compatible combinations developed into maturing females, compared with only about 1% in incompatible combinations. Several aberrations from the pattern of syncytial development associated with mature females in compatible hosts were apparent. A rapid necrotic response occurred in both kinds of hosts but was more frequent in incompatible associations. Delayed necrosis and small syncytia were present in some combinations. Those few females that developed in incompatible soybeans were associated with a characteristic syncytium different from the kind seen in roots of compatible hosts.

**Key words:** *Glycine max*, soybean cyst nematode, syncytia, rapid necrotic response.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is a sedentary endoparasite of the soybean [*Glycine max* (L.) Merrill] and of other plants. Attempts to develop commercially useful soybean cultivars resistant to this pest have led to limited investigations of the histopathology of infections in soybean cultivars. Ross (16) found necrotic cells close to undeveloped second-stage juvenile nematodes in Peking soybeans. In these plants, females did not develop beyond third-stage juveniles, but males reached fourth or adult stages. Endo (4,5) compared the histopathology of the SCN-susceptible 'Lee,' resistant 'Peking,' and partly resistant F-4 progeny of a backcross Lee × Peking. Early development of syncytia at feeding sites was comparable in Peking and Lee. In Peking, the syncytial cells around the nematode's head became necrotic and the nematodes degenerated within 4-5 days. Both nematodes and syncytia persisted longer in the F-4 backcross progeny seedlings, although results were variable. Corresponding to the degree of syncytial growth and persistence, nematodes developed partly or completely to adult males and females. Collapsed syncytia showed necrosis and replacement by

parenchyma tissues. Necrosis of syncytia in sugarbeet resistant to *H. schachtii* (17) and in cereals resistant to *H. avenae* (1) have been observed. Partial development of syncytia followed by degeneration was observed in four clones of potato resistant to the potato cyst nematode. Details of syncytial breakdown differed among the clones (7).

Phillips et al. (14) compared invasion and development of *Globodera pallida* in a susceptible, a moderately resistant, and a highly resistant clone of potato. Sprouts of the three clones were planted in infested soil and lifted at intervals for counts of the nematodes in roots. Invasion by juveniles was lowest in the most resistant roots and progress toward the adult stage was substantially retarded. Both resistant clones had a higher ratio of males to females than the susceptible clone. The difference in entry of juveniles may result either from difference of attraction and ease of penetration or from difference of the various clones in stimulation of juveniles to hatch from eggs in soil.

Rates of nematode population attrition within plants are virtually unknown. Kerry (10) reported that of 100 hatched juveniles of *H. avenae* inoculated on barley 25 entered roots, 11 reached the J-3 stage, 8 reached J-4, 5 became adult males, and 3 developed to adult females. Our purpose was to compare population attrition and histopathology in compatible and incompatible nematode-soybean associations.

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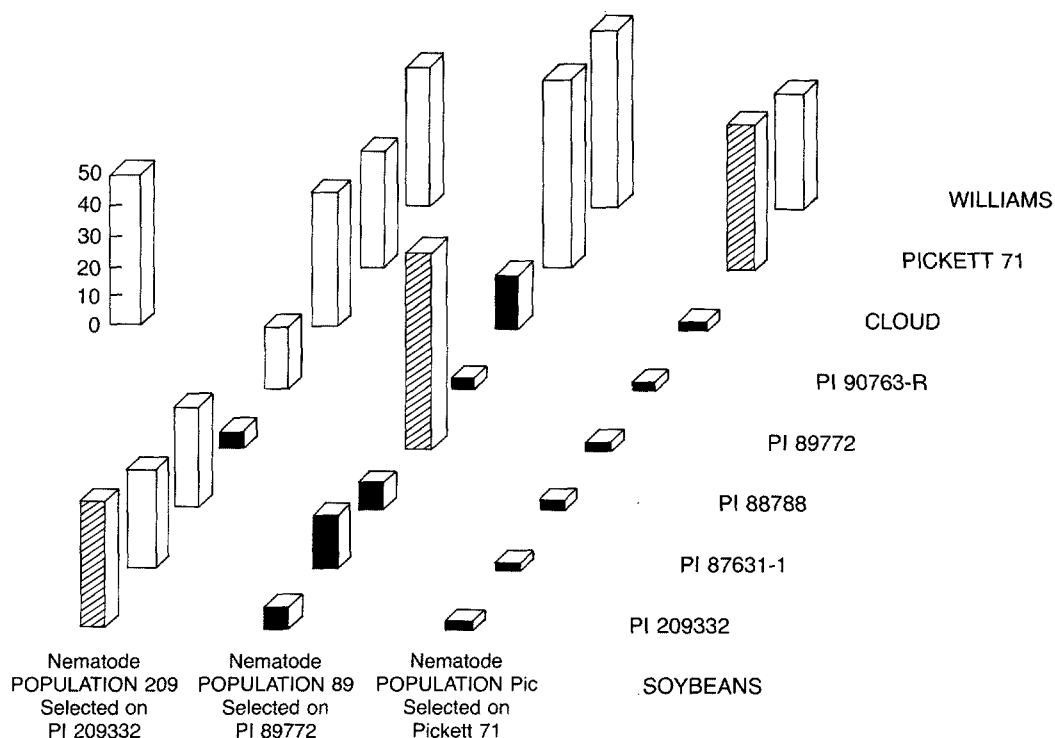


FIG. 1. Reproduction of selected populations of *Heterodera glycines* on resistant soybean lines. Height of bars represents mean number of cysts per plant 25 days after inoculation with 500 larvae (data from 13).

#### MATERIALS AND METHODS

Three populations of SCN, selected and maintained in greenhouse cultures on PI 209332, PI 89772, and Pickett 71, were used in the present study. Figure 1 compares reproduction of the three populations on a set of soybean lines and cultivars. Population 209 reproduces on PI 209332 soybeans but not on PI 89772; population 89 reproduces on PI 89772 but not on PI 209332; and population Pic reproduces well on Pickett 71 but poorly on Peking.

Seeds were tumbled in water for 4 hours and germinated on paper. Seedlings were selected for uniform length of radicles (3 cm) and spaced 3.5 cm apart on a double layer of moistened germination paper. Root tips were covered with 0.4 cc of washed Ottawa silica sand and a drop of water added. Inoculum, delivered in a second drop of water, consisted of 200 infective juveniles hatched within the preceding 48 hours in 0.01 M  $\text{ZnSO}_4$ . Roots were covered with a layer of moist germination pa-

per and overlaid with a strip of glass. The entire assembly was enclosed in two plastic trays taped together. These were incubated for 24 hours at 25 C in a horizontal position. Seedlings were then washed free of sand and some were stained in 1:1 glacial acetic acid and ethanol containing 0.0175% acid fuchsin, then cleared in lactophenol for determination of larval penetration. The remainder were transferred singly to 3 × 15-cm plastic pipes containing moistened, fumigated Hodge fine sand (Typic Udipsament). Pipes were randomized in 17 × 19-cm crocks and maintained at 27 C in a water bath in the greenhouse. Soluble fertilizer (20-10-20) was added at 10-day intervals. Because it was difficult to synchronize soybean germination and juvenile hatch, seedlings were inoculated in groups of five or more each day to accumulate the series shown in Table 1.

Plants were lifted at intervals and processed for histopathology. Washed roots were examined at 25× magnification and

TABLE 1. Penetration and development of three populations of *Heterodera glycines* in compatible (C) and incompatible (I) hosts.

Combination		Development					
		Days after inoculation					
		1 J-2 <sup>a</sup>	10		♂:♀ ratio	15	20
Nematode population	Host		♀	♂		♀	♀
P-209	PI 209332 (C)	20.4 (37)	3.1 (22)	5.3	1.7	3.1 (29)	2.3 (30)
	Essex (C)	19.1 (36)	4.6 (22)	7.8	1.7	2.7 (28)	3.8 (18)
	PI 89772 (I)	19.8 (37)	1.1 (22)	4.3	3.9	0.07 (30)	0.3 (24)
P-89	PI 89772 (C)	19.9 (33)	3.4 (22)	7.1	2.1	4.3 (19)	3.0 (22)
	Essex (C)	18.6 (33)	4.0 (22)	9.2	2.3	3.8 (19)	2.6 (19)
	PI 209332 (I)	17.7 (30)	3.3 (22)	5.0	1.5	3.3 (18)	0.2 (23)
P-Pic	Pickett 71 (C)	10.3 (15)	1.0 (15)	3.6	3.6	1.6 (14)	...
	Essex (C)	10.0 (15)	1.1 (15)	2.4	2.2	1.4 (17)	...
	Peking (I)	9.6 (15)	0.5 (15)	1.3	2.6	0.6 (15)	...

<sup>a</sup> Numbers in the columns indicate the mean numbers of nematodes per plant. Numbers in parentheses indicate numbers of seedlings inoculated with 200 juveniles each.

portions with lesions were cut into 0.5-cm segments. These were fixed in 3% glutaraldehyde-acrolein fixative, postfixed in 0.5% OsO<sub>4</sub>, and embedded in Spurr's resin (2). Serial sections 3–5 μm thick were cut on a rotary microtome and stained with 0.1% toluidine blue. Whole root samples were fixed and stained with the same technique described for studies of penetration. Nematodes were recovered by dissection of the roots and scored for stage of development at 100× magnification.

## RESULTS

**Penetration:** About 10% of the second-stage infective juveniles (J-2) of P-209 and P-89 penetrated into soybean roots within 24 hours; juveniles of P-Pic were less infective (Table 1). No differences were noted between penetration in compatible vs. incompatible nematode–soybean associations. The data are variable, both within subsets of a single day and between days. Two- to three-fold differences in the number of juveniles in a root were commonly observed within the set of roots of a host-parasite combination inoculated with nematodes that emerged on a single day.

**Nematode development:** The first molt from J-1 to J-2 occurs within the egg. In compatible hosts at 27 C, molts occurred between days 7 and 13 after inoculation. At day 8, the second molt from J-2 to J-3 was completed. On the next day, the cu-

ticle of J-3 was observed to have ridges, whereas the second cuticle was smooth. Cuticular ridges became prominent at day 10 and the cuticle of J-4 began to appear at the anterior portion of the body. The molt to adult female nematodes was completed on day 12 or 13. By day 13 to 15, the adult female contained many eggs. The first egg in the egg sac was observed on day 16. On days 18 to 20 the egg sac contained an average of 16 eggs (4–42 per egg sac) and at day 22 an average of 123 eggs was recorded. At this time, the adult female began to change color from pearly white to light yellow. At day 25 a few small cysts were brown and at day 35 most cysts were brown. An occasional cyst was yellowish-brown with the egg sac still attached.

Sexes could not be distinguished at 5 days after infection, but by 7 days the nematodes were swollen into sausage shapes of the late J-2 and early J-3 stages and sexes could be distinguished. Beginning with day 8, females were observed to be breaking through a yellowish necrotic concavity at the root surface. Males were smaller and deeper within the tissues. Males began to uncoil within the fourth cuticle on day 9. By day 10 most males were partly out of this cuticle. The first vermiform males were observed on day 11.

Sex ratios shown in Table 1 were collected only on day 10. By day 15 some males had left the roots so that reliable numbers

were not obtainable. In every combination, both compatible and incompatible, males outnumbered females. No general pattern of sex ratios is apparent in these data.

**Attrition:** Nematode attrition began with penetration and continued through development. In roots of soybeans compatible with P-209 and P-89, the mean number of females on day 10 was 3.8. The average male/female ratio was 1.95. Therefore, the average number of males was 7.4 and the total developing nematodes averaged 11.2. Nematodes present on day 10 represent 57% of the juveniles (weighted mean) present in roots on day 1, of which females comprised 19% and males 38%. Between days 10 and 15 there was little further loss. On day 15 the mean number of females was 3.4 and on day 20 it was 2.8. Thus, about 14% of those juveniles that entered roots in compatible soybean–nematode associations reached the maturing female stage at 20 days after inoculation.

Two different patterns are apparent in the incompatible combinations. Nematodes of P-209 disappeared more rapidly from roots of PI 89772 than did nematodes of P-89 from PI 209332. Of the P-209 juveniles present in roots of PI 89772 on day 1, 27% remained on day 10; females comprised 5.5% and males 21.5%. Females represented 0.35% of the invading P-209 juveniles by day 15 and 1.5% by day 20. Nematodes of P-89 in roots of PI 209332 on day 10 represented 47% of the invading juveniles; females made up 19% and males 28%. There was no further loss of females between days 10 and 15, but by day 20 the females were only 1% of the original group in the roots. Thus, mortality was delayed in this combination. Although the data are limited, and are variable among separate batches of roots, they are nevertheless convincing. On day 15, a total of 60 P-89 female nematodes were present in 18 roots of PI 209332, whereas a total of only two P-209 female nematodes were present in 30 roots of PI 89772. The histopathology reflects these different patterns of mortality.

**Histopathology:** Two conspicuous phenomena were observed in the sections of infected roots. 1) Early degeneration of feeding sites in association with P-209 nematodes in PI 89772 soybeans was more

common than those in association with P-89 nematodes in PI 209332 plants. 2) A characteristic type of syncytium was associated with those few female nematodes that reached full development to maturity in incompatible associations.

A healthy soybean root is shown in cross section in Fig. 2A. The syncytium associated with female nematodes in compatible hosts forms a wedge pointed toward the center of the stele. The large cells seen in cross section (Fig. 2B) are filled with moderately dense cytoplasm and the walls are heavily stained. In longitudinal section (Figs. 2D, E), the internal walls are seen to be perforated (cf. 9). Figures 2D and E compare a syncytium induced in a susceptible commercial cultivar (Williams) with one in PI 89772 induced by a nematode from P-89. Both consist of hypertrophied cells joined together in an elongated structure extending longitudinally in the stele. Figures 2B and C compare syncytia associated with male and female nematodes in compatible hosts. Male feeding sites are usually found in the cortex and pericycle, and their cytoplasm stains more deeply than that of female feeding sites. The male unit of Fig. 2C appears to be at an early stage of degeneration (day 9), whereas the syncytia that support females remain functional until the nematode completes its life cycles at about day 22 under our conditions. Thereafter, syncytial tissue is replaced by parenchyma cells.

Incompatible combinations result in various aberrations of syncytial development. Figure 3A shows the rapid necrotic response of a few cells in close proximity to the nematode's head. The second-stage juvenile that penetrated the root remained close to this feeding site but did not grow. Figure 3B illustrates the appearance of such a necrotic response in a cleared and stained root. In Fig. 3C we see that the nematode made some growth and molted by day 18 but did not reach the adult stage seen at this time in a compatible association. The feeding site is necrotic; presumably, this occurred later than illustrated in Fig. 3B. But not every aberrant syncytium is necrotic. Figures 3D and E compare a syncytium of a compatible combination fixed on day 10 and one of an incompatible combination on day 9. The latter is small and

the nematode is correspondingly retarded. The syncytium illustrated in Fig. 4A reached a large size but degenerated before day 19 and was replaced by parenchyma cells.

In all roots a number of infective juveniles remain without growth. Table 2 summarizes observations on 111 roots fixed on day 10 to assess the importance of the necrotic reaction on the nematode's arrested development. The table shows the following:

1. The necrotic reaction is not the sole block to parasite success.
2. In incompatible combinations of P-209 and P-89 nematodes in soybeans, almost all the undeveloped juveniles were associated with necrosis, but in combinations with P-Pic the proportion of necrotic reactions was lower.
3. In compatible soybeans inoculated with P-209 and P-89 nematodes, about half of the undeveloped juveniles were associated with necrosis, but in inoculations with P-Pic nematodes, the incidence of necrosis was lower.

About 20 juveniles of P-209 and P-89 and 10 of P-Pic penetrated each root (Table 1). The 74 seedlings of the compatible combinations had 105 undeveloped juveniles representing 7% of the total nematodes that penetrated. The incompatible combinations comprise 37 seedlings with

129 undeveloped juveniles, representing 17% of the total invading juveniles.

In every incompatible combination, a few females become adult and produce at least a few eggs. Such nematodes can be selected to give rise to a thriving population on a host which was resistant to the population before selection (13). The syncytia associated with the few selected nematodes are distinctly different from all the examples given of compatible and incompatible associations. Figures 4B and C show two such syncytia. They are large, located within the stele, and have the typical cytoplasm of functional feeding sites. However, they are not elongated. The incorporation of a series of adjacent cells as illustrated in Figs. 2D and E, and in Fig. 3D, did not occur in the growth of the ones shown in Figs. 4B and C. We have seen this in every case examined in our series.

Figures 4D and E illustrate a secretion deposited from the stylet of *H. glycines* into the cytoplasm. In Fig. 4E the tip of the stylet is seen protruding through the cell wall. The stylet is surrounded by a seal as previously described (6). A thread of tubular secretion extends into the cytoplasm. The area surrounding the stylet appears free of organelles. In another section (Fig. 4D) the nematode's saliva has a beaded appearance, suggesting that it is viscous and is extruded in spurts. Cross sections of secretions can also be seen. We have seen

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FIG. 2. Sections of noninoculated soybean root and feeding sites of *Heterodera glycines* in compatible hosts. A) Cross section of healthy soybean root (173 $\times$ ). B) Cross section of root with syncytia associated with a female ( $\rightarrow$ ) and with a male ( $\Rightarrow$ ) (114 $\times$ ). Note that the former is within the stele and the latter is in the cortex and pericycle. C) Longitudinal section of syncytium associated with a female ( $\rightarrow$ ) and with a male ( $\Rightarrow$ ) (197 $\times$ ). The latter is more dense and located in the cortex and pericycle and the former is within the stele bordering on xylem vessels. D) Mature syncytium of P-209 in cv. Williams, a commercial, susceptible soybean, fixed on day 19 after inoculation (108 $\times$ ). E) Mature syncytium of P-89 in PI 89772, fixed on day 19 (116 $\times$ ). ( $\Rightarrow$ ) indicates adult female nematode, ( $\rightarrow$ ) indicates syncytium.

FIG. 3. Sections of SCN-feeding sites in incompatible (I) and compatible (C) soybeans. A) Rapid necrotic response, P-Pic in PI 89772 (I), day 21 (181 $\times$ ). B) Rapid necrotic response, P-209 in PI 89772 (I), day 14, whole mount showing no nematode development beyond infective stage (132 $\times$ ). C) Delayed necrotic response, P-209 in PI 89772 (I), day 18, whole mount showing partial development of female nematode (214 $\times$ ). D) Syncytium of P-209 in PI 209332 (C), day 10 (97 $\times$ ). E) Syncytium of P-Pic in PI 89772 (I), day 9 (175 $\times$ ).

FIG. 4. Sections of SCN-feeding sites in incompatible and compatible soybeans. A) Syncytium of P-89 in PI 209332 (I), day 19, showing replacement by parenchyma cells and partially developed female nematode (198 $\times$ ). B) Syncytium of P-89 in PI 209332 (I), day 19 (191 $\times$ ). The syncytium ( $\rightarrow$ ) is associated with a mature reproducing female ( $\Rightarrow$ ) in an incompatible combination. Note the absence of lateral extensions of the syncytium. C) Syncytium ( $\rightarrow$ ) of P-209 in PI 89772 (I), day 19, associated with mature reproducing female ( $\Rightarrow$ ) in another incompatible combination (194 $\times$ ). D) Nematode saliva in longitudinal section ( $\rightarrow$ ) and in cross sections ( $\Rightarrow$ ) in a syncytium of P-209 in PI 209332 (C), day 19 (1,077 $\times$ ). E) Cross section of syncytium and nematode showing stylet ( $\rightarrow$ ) and saliva ( $\Rightarrow$ ), P-Pic in Pickett 71 (C), day 14 (1,066 $\times$ ).

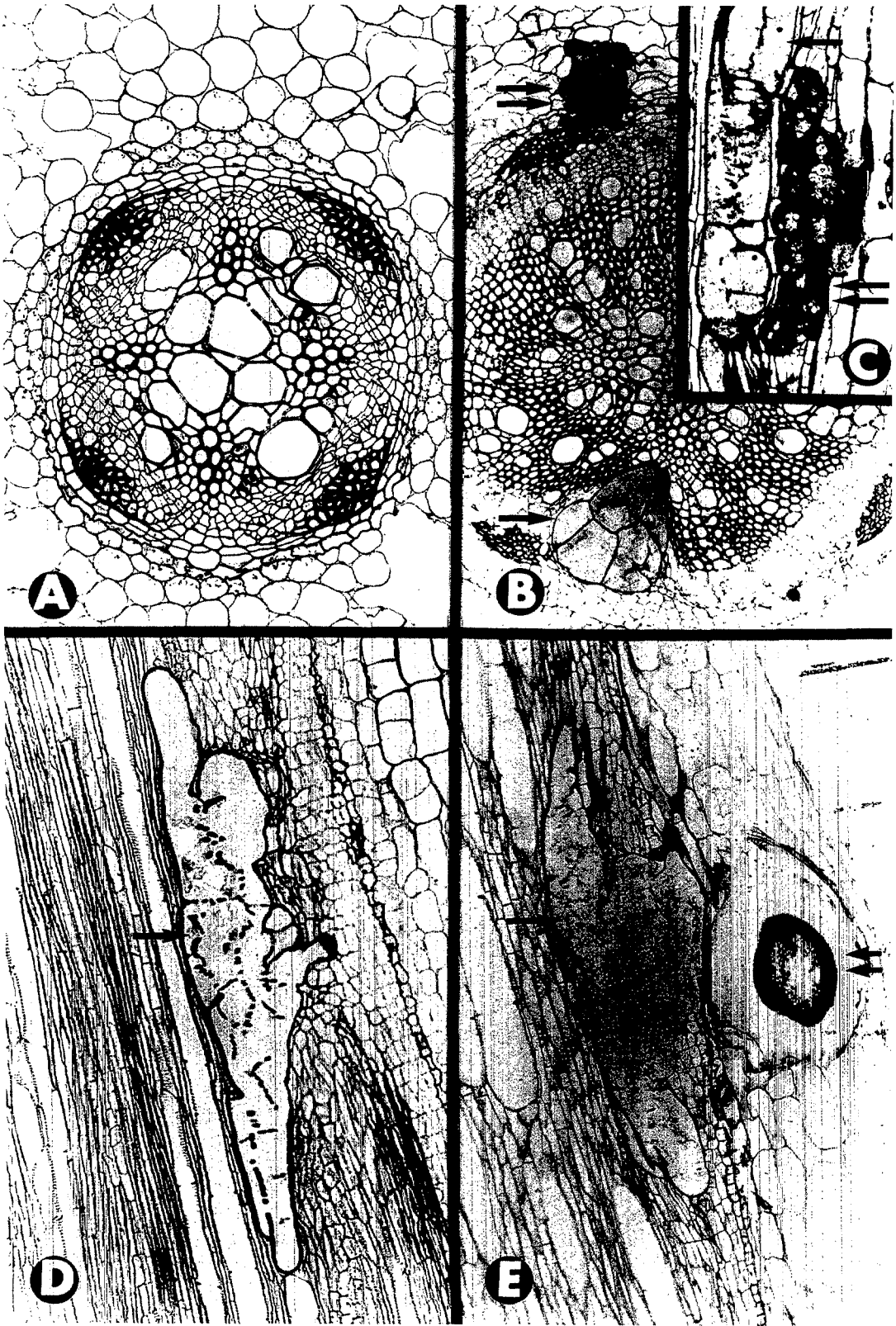


FIG. 2.

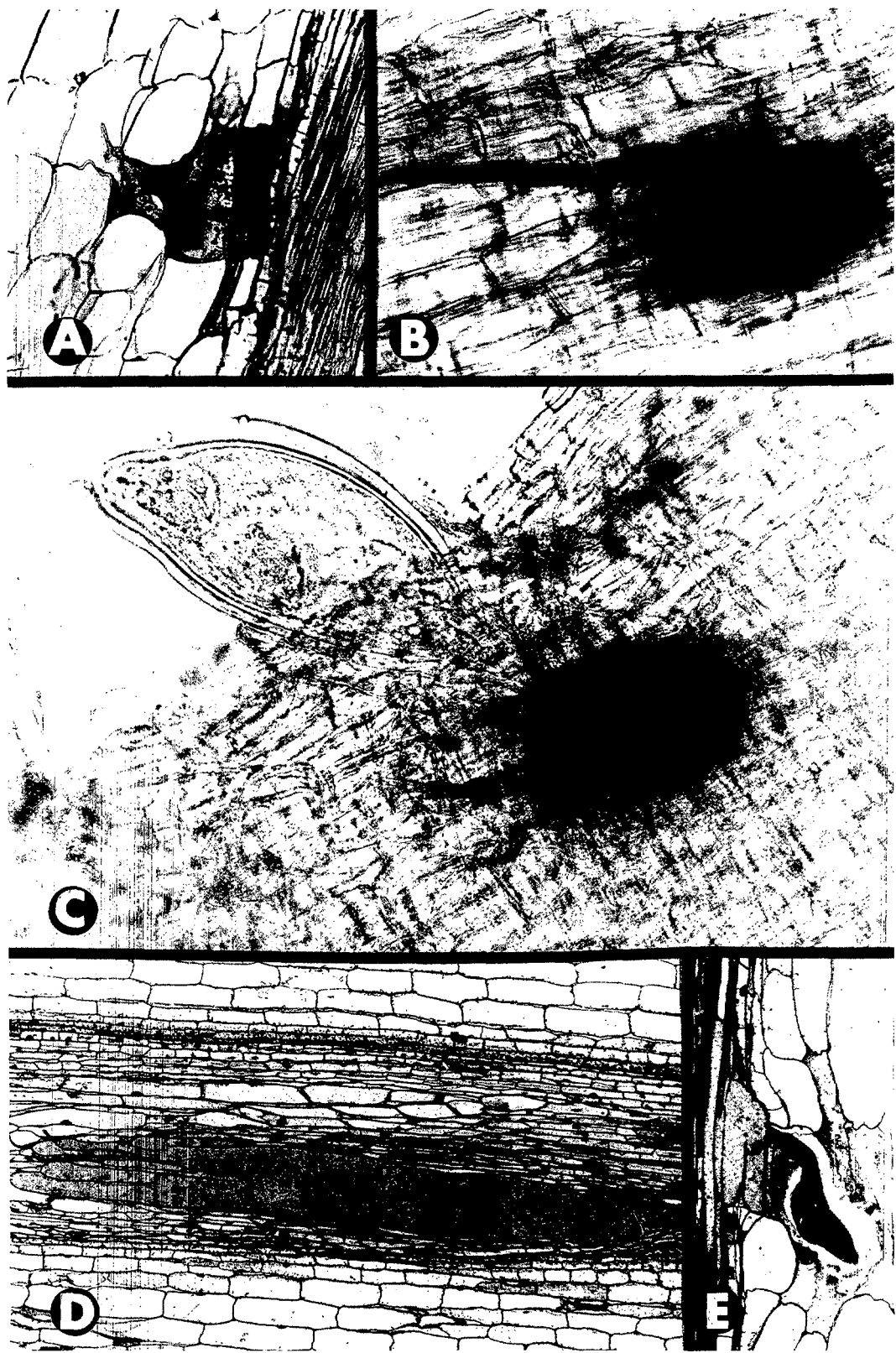


FIG. 3.

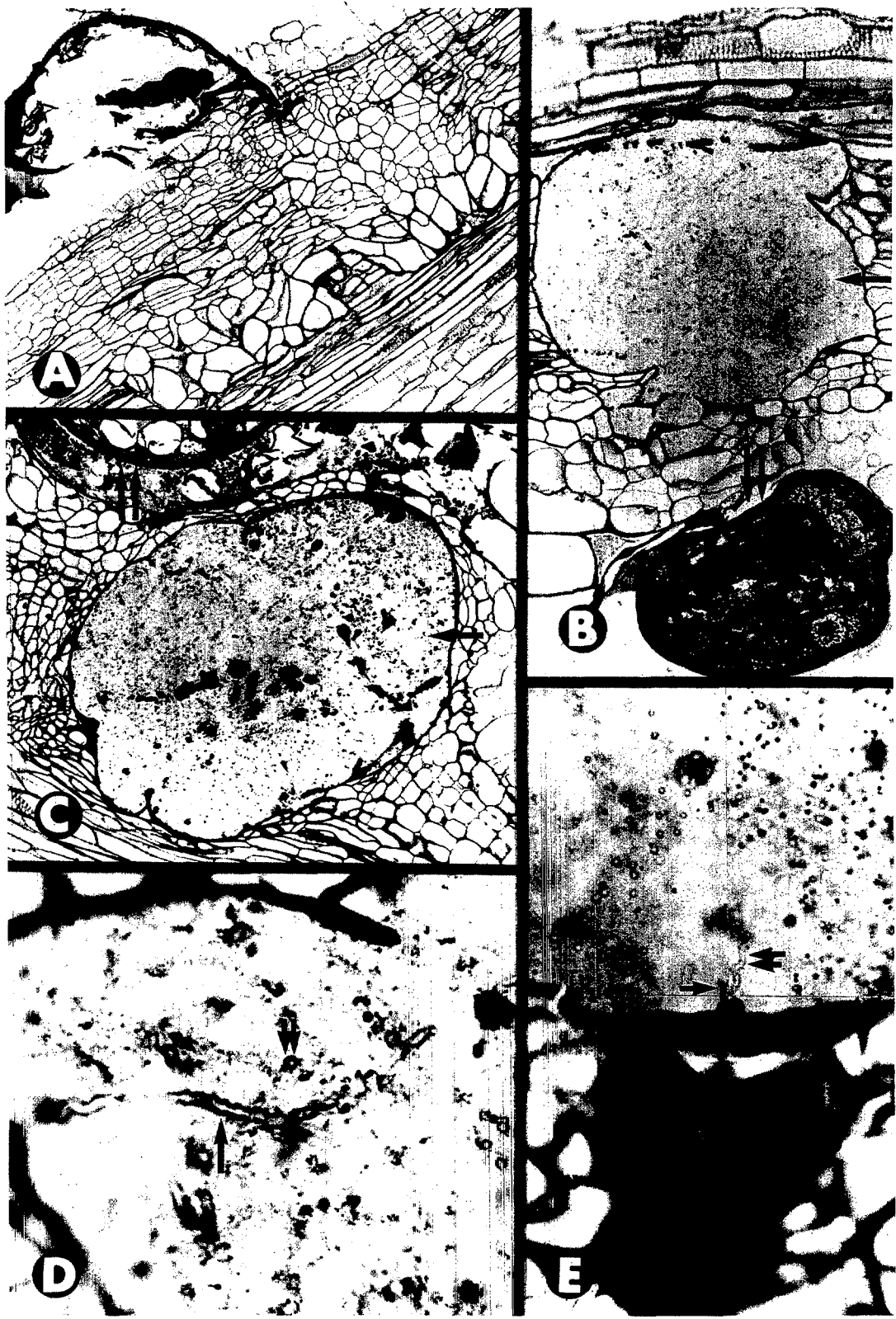


FIG. 4.



TABLE 2. Incidence of necrosis associated with undeveloped infective juveniles in various host-nematode combinations on day 10 after inoculation.<sup>a</sup>

Combination		Total nematodes in the J-2 stage	% nematodes with necrotic response
Host	Nematode		
PI 209332	P-209 (C) <sup>a</sup>	17 (11) <sup>b</sup>	47
Essex (C)		12 (11)	58
PI 89772 (I)		63 (11)	97
PI 89772	P-89 (C)	11 (11)	64
Essex (C)		14 (11)	57
PI 209332 (I)		30 (11)	100
Pickett 71	P-Pic (C)	25 (15)	36
Essex (C)		26 (15)	15
Peking (I)		36 (15)	69

<sup>a</sup> C = compatible combination; I = incompatible.  
<sup>b</sup> Inoculum = 200 juveniles per seedling. Numbers of seedlings in parentheses.

such cytoplasmic inclusions associated with the nematode's head commonly in sections of syncytia fixed on days 7–19. They are more common from day 12 onward. The numerous cross sections illustrated in Fig. 4D suggest that the nematode introduces relatively large amounts of saliva into syncytia. Similar findings have been reported by others (3,6,8,12,15).

#### DISCUSSION

The rate of attrition of nematodes in plants has received very little attention. The variability of penetration into roots is a problem inherent in any attempt to collect data of this kind. In our inoculations the number of invading juveniles per seedling within a series inoculated sequentially from the same batch of nematodes has varied within a broad range. The lowest number of nematodes per root was often one-third that of the highest number, despite our best efforts to maintain uniform conditions.

This work is part of a long-term study of genetics of the association of soybeans and *Heterodera glycines*. Our data are not surprising except that the pattern of nematode mortality differed in two incompatible associations. This difference suggests that the genetic control of the incompatible association of P-209 in PI 89772 rests

with genes that act very quickly after penetration, whereas in the association of P-89 in PI 209332, genes that act later must be involved. These differences suggest the existence of an array of genes in both nematodes and soybeans that control the success of the parasite's reproduction.

The difference between syncytia associated with reproducing females in incompatible hosts and those in selected populations in their selecting hosts also points to the existence of multiple alleles regulating the host-parasite interaction. A second selection imposed on already selected populations resulted in a change of parasitic ability (11). The fully compatible association, both in "susceptible" hosts such as Essex or Lee with unselected populations and in PI 209332 with a selected population, must result from the interactions of many genes in both host and parasite that affect the development and maintenance of the nematode and its feeding site.

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