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Heterodera glycines–Soybean Association: A Rapid Assay Using Pruned Seedlings[1]

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Abstract: A 16-day bioassay to determine compatible and incompatible associations of soybeans and cyst nematodes is described. This permits large-scale experiments in the laboratory. Seedlings are placed between two sheets of moist paper towelling so that the root tips are even with the bottom edge. The towelling is then rolled and set on the surface of cyst-infested soil. Radicles are permitted to grow through the soil for 24 hours after which cotyledons and apical portions are trimmed to retard root growth. The plants are assembled in bundles and maintained in aerated water in test tubes for 15 days before counts of emerged nematodes are taken. This system is economical in both space and labor.

Key words: cyst nematodes, soybeans, hydroponics, rapid bioassay, *Heterodera glycines*, *Glycine max*.

Breeding soybeans for resistance to the soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe requires bioassay of plant–nematode association. Low numbers of adult females at the end of one life cycle indicate resistance. Seeds are sown in small pots usually containing field soil with a high population density of nematodes. After about 30 days, roots lining the pots are scored for presence of fresh adult females. This is an expensive, labor-intensive operation requiring greenhouse or field evaluation of large numbers of young plants. At this time, genetic analysis of the association has not advanced appreciably, partly because of the amount of labor required.

The resistant response of plants to phytoparasitic nematodes appears to be a characteristic of the roots. Nirula and Pushkarnath (5) used rooted leaflets of *Solanum* spp. to find resistance to root-knot nematodes. Hesling and Ellis (2) grafted scions from a susceptible tomato cultivar on rootstocks of susceptible or resistant tomatoes. Plants were grown in soils infested with graded levels of the potato cyst nematode. Plant growth and yield, as well as cyst nematode population densities, were compared in self-rooted and grafted plants. Yields of plants on susceptible rootstocks declined with increasing population densities of nematodes, but yields of those on resistant rootstocks did not. Nematode populations increased on susceptible but declined on resistant rootstocks. Lauritis et al. (3) also illustrated the independence of root host–parasite phenotypes. They showed that excised roots of soybeans in culture and intact plants in soil displayed the same resistance to SCN.

Our objective was to investigate the use of pruned soybeans in hydroponics as a bioassay for SCN development.

MATERIALS AND METHODS

All experiments were performed with nematodes selected and maintained on soybean introductions PI 209332 or PI 89772, both known to be resistant to certain isolates of SCN. These PI were planted in a microplot containing cysts assembled from all the major soybean growing areas of the United States. This gene pool was continuously maintained on the susceptible soybeans Essex, Corsoy, and Williams. SCN lines isolated on PI 209332 and on PI 89772 were passed for nine generations on the selecting host by transfer of single cysts at each generation. Populations were then permitted to expand on the selecting host and were maintained in mass cultures under continuous selection in the greenhouse. At approximately 30-day intervals, each culture was replanted with new seedlings of the appropriate host. Cysts were collected from 30-day infections.

Inoculum consisted of eggs or hatched second-stage juveniles (J2) (1). J2 were hatched from eggs incubated in a modified Baermann apparatus; after 3 days in water at 30 C, eggs were transferred to aqueous 0.01 M $ZnSO_4$ at 25 C for 12 days (6).

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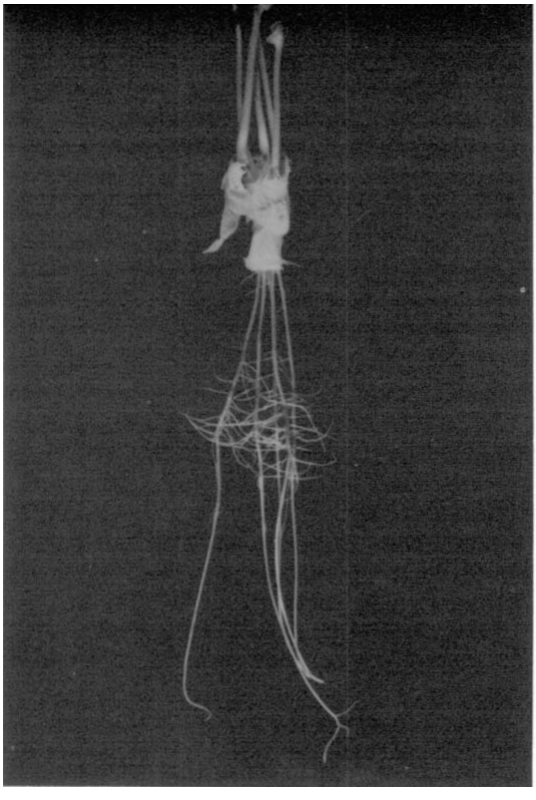

FIG. 1. View of pruned seedlings on day 20 of culture in hydroponics. Note abundance of lateral roots in the inoculation portion.

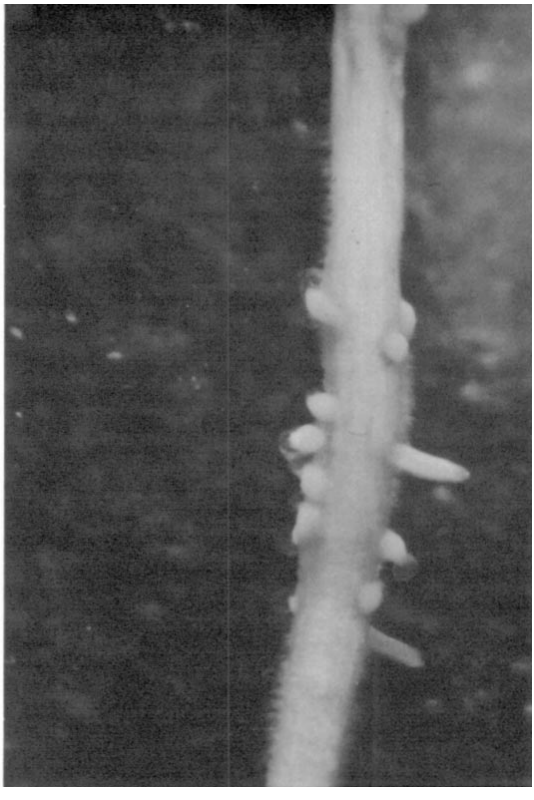

FIG. 2. Closeup of adult *Heterodera glycines* females on a root shown in Figure 1. Note that root hairs are present only in the infected region.

Inoculum consisted of very fine sandy loam containing 1,000 eggs/cm^3. The soil was held in a double-walled container made from sections of two plastic beakers fitted into each other securing a single layer of cheese cloth. Three-day-old seedlings with 2–3-cm-long roots were sandwiched in a roll of two sheets of moist germination paper so that root tips were even with the bottom edge. The roll was placed upright on the surface of the SCN-infested soil. The entire assembly was held at 100% relative humidity for 24 hours after which the seedlings were lifted from the soil, washed, and transferred in bundles of 5 or 10 to hydroponics.

Inoculated soybeans were maintained in test tubes (20 × 3 cm) with aerated deionized water. A single layer of cheesecloth fastened to the top held the groups of seedlings. Aeration was provided by an aquarium pump connected to a length of latex tubing clamped at one end. Short lengths of tubing bled air through hypodermic needles from the main supply and delivered it to individual test tubes by Pasteur pipettes. The test tubes were assembled in racks partly submerged in a water bath at 27 C. Light was provided 16 hours each day by a 40-watt fluorescent unit suspended over the water bath. To control root growth, seedling shoots were pruned upon transfer to hydroponics. Two degrees of pruning severity were employed: 1) the hypocotyl was severed just below the cotyledons or 2) 75% of each cotyledon was removed, leaving the apical meristem intact, and as the apical portion elongated, it was pruned back to the cotyledonary node. Each method retarded root growth, but the second produced a more robust plant. Once transferred to constant temperature hydroponics, plants survived well with only an occasional addition of water (Fig. 1). After 15 days at 27 C, females were observed on root surfaces (Fig. 2). Counts of females were made under 8× magnification. Males were retrieved by sieving the fluid of the test tubes. This system permitted large-scale experiments in a small

TABLE 1. Numbers of females recovered from pruned and nonpruned soybean plants inoculated with compatible and incompatible populations of *Heterodera glycines*.

	Population 1*			Population 2		
	Females	Plants	Females/ plant	Females	Plants	Females/ plant
Pruned plants						
Compatible soybean	2,738	430	6.4†	4,325	415	10.4‡
Incompatible soybean	51	430	0.1	2,317	415	5.6
Intact plants§						
Compatible soybean	1,708	10	170.8	1,700	10	170.0
Incompatible soybean	7	10	0.7	1,061	21	50.5

* Population 1 selected and partly inbred on PI 209332; Population 2 selected and partly inbred on PI 89772.
† Mean of data from three separate experiments with PI 209332 as the compatible host and PI 89772 as the incompatible host.
‡ Mean of data from three separate experiments with PI 89772 as the compatible host and PI 209332 as the incompatible host.
§ Data from a greenhouse experiment with the same populations as those used with miniaturized plants.

space. Up to 750 plants were simultaneously maintained in a water table (76 × 91 cm) using 75–150 test tubes.

RESULTS AND DISCUSSION

This technique was compared with the technique of removing SCN females from plants grown in soil in the greenhouse with a jet of water (4). Table 1 shows that Population 1 females developed well on their selecting host (PI 209332) but poorly on PI 89772. Population 2 females developed better on their selecting host (PI 89772) than on PI 209332. These data were compiled from six experiments including a total of 1,690 pruned plants. In every experiment the distinction between compatibility and incompatibility of nematodes and soybeans was clear. In the laboratory the number of cysts averaged fewer than 10 per plant, whereas nonpruned plants in the greenhouse supported an average of 170 cysts. The latter were grown in soil in sections of PVC pipe (2.5 cm d × 15 cm long) and were inoculated with 1,000 eggs per pipe. As the J2 emerged, growing roots were continually available for J2 entry. However, pruned plants were exposed to J2 for only a single 24-hour period.

Use of pruned soybeans facilitates some analysis of the expression of resistance. In one experiment sizes of females from all combinations of nematodes and soybeans were compared. Pruned seedlings were placed in modified petri plates with very fine sandy loam. Each seedling was inoculated with 150 freshly hatched J2 per plant, one set of each host with nematodes of Population 1 and the other with Population 2. After 27 days at 27 C, all females were counted and sized. Table 2 shows that the association between SCN and soybeans

TABLE 2. Numbers and sizes of adult females in pruned soybeans of compatible and incompatible associations with two populations of *Heterodera glycines*.

	Soybean–nematode association	
	Compatible	Incompatible
Population 1		
No. of plants	32	32
Large females	183 (73%)	0
Small females	67 (27%)	0
Population 2		
No. of plants	31	31
Large females	142 (58%)	38 (31%)
Small females	102 (42%)	84 (69%)

TABLE 3. Numbers of males in all combinations of two *Heterodera glycines* populations and two soybeans. Corresponding data on females in these experiments appear in Table 1.

Population	Host	Plants	Males	Males/ plant
1	PI 209332 (compatible)	430	2,547	5.9
	PI 89772 (incompatible)	430	1,275	3.0
2	PI 89772 (compatible)	415	3,567	8.6
	PI 209332 (incompatible)	415	3,805	9.2

TABLE 4. Comparison of coefficients of variability (CV) of females per tube of 10 pruned seedlings vs. CV of individual plants in three compatible soybean–*Heterodera glycines* associations.

Population	Host	Females/tube (mean ± SD)	CV (tube)	Females/plant (mean ± SD)	CV (individual)
1*	PI 209332	83.7 ± 37.9	0.45	8.4 ± 6.1	0.73
	Williams	83.7 ± 24.4	0.29	8.4 ± 5.8	0.69
2†	PI 89772	99.6 ± 31.1	0.31	10.0 ± 5.9	0.59
		112.0 ± 16.9	0.15	11.2 ± 5.2	0.46
		88.2 ± 13.3	0.16	8.8 ± 5.1	0.58
		82.2 ± 6.3	0.08	8.2 ± 4.9	0.60
		106.6 ± 7.1	0.07	10.7 ± 4.5	0.42
		112.0 ± 16.9	0.15	11.2 ± 5.2	0.46
	Williams	150.2 ± 37.0	0.25	15.0 ± 7.3	0.49
		149.0 ± 23.7	0.16	14.9 ± 6.5	0.44
		76.6 ± 17.9	0.23	7.7 ± 3.7	0.48
		78.2 ± 8.3	0.11	7.8 ± 4.7	0.60
		87.6 ± 37.8	0.43	8.8 ± 6.0	0.68

* Data from five replications of five tubes of 10 seedlings each.
† Data from one replication of five tubes of 10 seedlings each.

varied in different combinations. No adult females developed in the incompatible soybean with Population 1. In the association of nematodes from Population 2 with their incompatible host, however, there was 50% reduction of adult females, and ca. 66% of these were small females.

Our method of 24-hour infection time and maintenance of pruned seedlings in aerated water culture facilitates detailed studies of nematode development in hosts of diverse genetic origin. The method of recovering females from roots of non-pruned plants does not permit gathering information on dwarfed females as a phenotype of incompatibility. In addition, we recorded the number of males emerged from various combinations of nematodes and soybeans (Table 3). Males were counted from three experiments with each population. Females counted from these experiments are listed in Table 1. In compatible hosts both SCN populations produced relatively high numbers of males, but in the incompatible host male production by Population 1 was half that in the compatible host. Population 2 males, however, were slightly more numerous in the incompatible host than in the compatible host. Thus, resistance operates against both males and females in one combination, but only against females in the other.

Williams soybeans were included for comparison in all the experiments with pruned plants. Somewhat greater numbers of adult females and males were recovered from Williams in every instance than from the selecting hosts.

Roots of pruned plants maintained under our conditions were favorable for observation of nematodes after staining and clearing. The small quantities of stored products and the small amount of cortical tissues facilitated observations permitting collection of extensive data on nematode penetration and rates of development and attrition.

The variability in numbers of nematodes

TABLE 5. Comparison of coefficients of variability (CV) of females per tube of five pruned seedlings vs. CV of individual plants in three compatible soybean–*Heterodera glycines* associations, based on four replications of six tubes each.

Population	Host	Females/tube (mean ± SD)	CV (tube)	Females/plant (mean ± SD)	CV (individual)
1	PI 209332	12.2 ± 7.2	0.59	2.4 ± 2.7	1.13
	Williams	30.0 ± 10.6	0.35	6.0 ± 6.0	1.00
2	PI 209332	19.5 ± 10.6	0.54	3.9 ± 3.7	0.95
	PI 89772	46.4 ± 13.6	0.29	9.3 ± 5.2	0.56
	Williams	64.3 ± 22.5	0.35	12.9 ± 6.7	0.50

penetrating host plants inoculated under controlled conditions is a major difficulty in the study of nematode–plant interactions. In our technique, pruned seedlings were bundled into groups of 5 or 10 per test tube. It was therefore possible to measure variability resulting from treating a bundle of roots as one unit. A set of 13 experiments with bundles of 10 plants per tube (105 tubes and 1,050 plants) was analyzed by bundle and by individual plants, and the coefficients of variability (CV) calculated were compared (Table 4). Coefficients of variability of total females per tube ranged from 0.07 to 0.45 with the median 0.20. CV of individual plants in these experiments ranged from 0.42 to 0.73 with the median 0.58 (Table 5). Another group of five experiments had five plants per tube and a total of 120 tubes and 600 plants. In this case the CV of tubes ranged from 0.29 to 0.59 with the median 0.35. CV of individual plants ranged from 0.50 to 1.13 with the median 0.95. Clearly the variability in nematode counts is reduced by using bundles of plants as the units.

The system described here offers the following advantages over conventional methods for screening plants to determine compatibility with SCN:

1) Shorter duration of experiments (15 days post-inoculation).
2) Conservation of inoculum; the method allows repeated use of the same infested soil.
3) Localized and synchronous 24-hour SCN infections.
4) Space requirements and labor are minimized by use of hydroponics. Plants require little attention during the experiment.
5) Data collection is facilitated by the absence of soil.
6) Data on male emergence and female size is easily obtained.

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