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Effect of Soybean Root Tip Removal on Penetration and Development of *Heterodera glycines*[1]

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Abstract: On a few occasions, soybeans with broken root tips were included in tests to evaluate resistance to *Heterodera glycines*. Although females developed on these plants, the numbers tended to be lower than on similarly treated intact roots. To test the possibility that removal of the root meristem affected nematode development, a culture system using pruned soybeans was devised that permitted access to the roots without disturbing the plants. Treatments included removal of 2 mm of root tip at various times ranging from 24 hours before to 10 days after inoculation, or roots left intact. In each experiment, all roots were inoculated at the same time with equal numbers of freshly hatched second-stage juveniles of *Heterodera glycines*. No differences in nematode development were detected in plants with root tips removed after inoculation compared to the control. When tips were removed at or before inoculation, fewer juveniles entered roots and relatively fewer nematodes developed. Penetration levels and development correlated with root tip removal such that progressively fewer nematodes entered roots and relatively greater numbers of nematodes remained undeveloped as the time interval between root tip removal and inoculation was increased.

Key words: cyst nematode, *Glycine max*, *Heterodera glycines*, nematode, penetration, soybean.

In greenhouse experiments for evaluation of soybean (*Glycine max* (L.) Merr) resistance to the soybean-cyst nematode (SCN) (*Heterodera glycines* Ichinohe), soybean seedlings frequently are transplanted to pots for testing. During transplanting roots may be broken or damaged. Preliminary investigations indicated that nematode counts obtained from broken roots were lower than from similar unbroken roots. This paper reports the results of experiments designed to determine whether or not removal of the root meristem affects the penetration and (or) development of SCN in soybean.

Materials and Methods

A wood-burning pencil was used to melt four notches in one side of plastic culture dishes (150 × 20 mm) and corresponding notches in the lids. The dishes were filled with moistened fine sand. Soybean seedlings (cv Williams) with roots 5–9 cm long were excised below the cotyledonary node and positioned in the dishes with the hypocotyls protruding through the notches. Tap roots were gently pressed into the sand but left exposed at the surface. Plastic dividers (70 × 20 × 2 mm) were pressed into the sand between the root tips (Fig. 1). With the lid removed, root tips were accessible without disturbing the plants. At various intervals, the apical 2 mm of root tip was cut from plants with a scalpel and removed. Control plants were not cut.

The same *H. glycines* population and inoculation procedures were used in all experiments. Three drops of an aqueous suspension containing ca. 200 freshly hatched second-stage SCN juveniles (J2) were deposited on the apical portion of each root. In each experiment, all plants were inoculated at the same time (designated as time = zero (0)). The time when root tips were removed was calculated relative to the inoculation time; thus root tips excised at the same time as inoculation were designated as 0 time, root tips excised before inoculation were designated with a negative (−) time unit, and root tips excised after inoculation were designated with a positive (+) time unit. Except for inoculation and root tip removal, culture dishes were held in a vertical position in a growth chamber at 27 C under fluorescent light (cool white) 16 hours per day. Supplemental moisture was added as needed by directing a fine stream of water to the base of the hypocotyls.

In the first experiment, 45 plants were randomly assigned to one of five treat-

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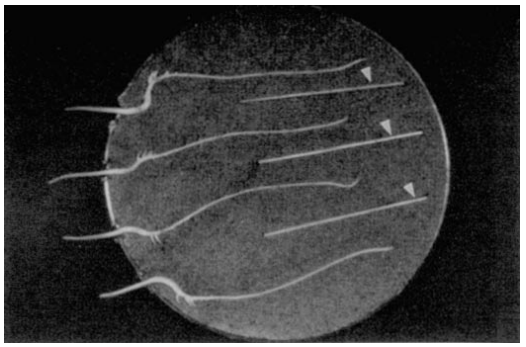

FIG. 1. Modified culture dish showing arrangement of soybean seedlings and plastic dividers (arrows).

ments (9 plants per treatment). The treatments included the control and removal of root tips at inoculation or 2, 5, or 10 days after inoculation (control, 0, +2, +5, and +10 days). Fourteen days after inoculation, the sand was rinsed from the roots and mature females were counted.

In the second experiment, plants were randomly assigned to one of six treatments (17 plants per treatment). The treatments included the control and root tips removed at inoculation, 24 hours before, or 5, 24, or 48 hours after inoculation (control, −24, 0, +5, +24, and +48 hr). Eight days after inoculation, the plants were transferred to a hydroponic culture system and held at 27 C (4). All plants having the same treatment were placed in the same tube. Fifteen days after inoculation, visible females and egressed males were counted.

In the third experiment, plants were randomly assigned to one of five treatments (20 plants per treatment). Root tips were left intact, removed at the time of inoculation, or removed at 6, 18, or 24 hours before inoculation (control, 0, −6, −18, and −24 hr). For convenience, the treatments were scheduled as two sets of inoculations with the −24 and −18 hour treatments in the first set and the −6 and 0 hour treatments in the second set. Each set of treatments had a control, but these were later combined for analyses when no differences were noted. Both sets of treatments used the same batch of J2, which were held at 10 C during the 6 hours between inoculations.

Twenty-four hours after inoculation, several roots from each treatment were stained and cleared to count the number of J2 within the roots (1). The remaining plants were transferred to hydroponics (16 plants/tube) as described above. The water from each tube was sieved after 1, 8, 14, and 16 days to collect egressed J2 and males. After 16 days, the females visible on the roots were counted and removed. Infected root segments were stained and cleared to determine the development of nematodes remaining within the root (6). The numbers of egressed males collected from culture tubes on the 14th and 16th day of hydroponic growth were added to the numbers of males found within the roots.

RESULTS

Nematode counts from the first experiment supported previous observations that root tip removal at the time of inoculation results in fewer adult females. The number of females recovered from roots with tips removed at the time of inoculation was less than from roots with tips removed after inoculation or the controls ($P = 0.05$) (Table 1).

Results of the second experiment were similar to the first; however fewer females were recovered from roots with tips removed 24 hours prior to inoculation ($P = 0.01$) (Table 1). Males were relatively abundant, except for the −24 hour treatment. Because all males from each treatment were collected as a group, these data did not lend themselves to a statistical analysis of variance. Nevertheless, the low number of males in the −24 hour treatment appeared to indicate a real difference because the numbers corresponded with the low number of females in the same treatment.

In experiment three, similar levels of J2 penetrated roots of the control and 0 time treatment, but progressively fewer nema-

TABLE 1. Number of adult *Heterodera glycines* recovered from soybean roots 14 and 15 days after inoculation with approximately 200 second-stage juveniles

	Experiment 1	Experiment 2	
Root tip removal	Females/plant	Females/plant	Males/plant
Control	8.3 a	6.7 a	13.7
−24 hour		0.2 b	1.1
0 time	2.7 b	4.9 a	11.8
+5 hour		5.9 a	16.6
+24 hour		7.4 a	7.4
+48 hour	6.9 a	6.5 a	6.5
+120 hour	7.4 a		
+240 hour	7.6 a		

Values are the means of nine replicates and 17 replicates for experiments one and two respectively. Means, within columns, followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$) experiment one and ($P = 0.01$) experiment two. Root tip removal times are relative to the time of inoculation. Controls did not have the root tip removed.

todes penetrated roots with tips removed prior to inoculation (Table 2). The numbers of J2 that left the roots were unaffected by the treatments and remained approximately proportional to the numbers that entered (Table 3). At 16 days postinoculation, differences in nematode development were observed between all except the −18- and −24-hour treatments. In general, the percentage of undeveloped J2 was correlated with the time interval between root tip removal and inoculation. This ranged from 23% of the total in the controls to 90% of the total in the −18- and −24-hour treatments. The 0- and −6-hour treatments showed a differential effect on development of the sexes: males were unaffected while female development was inhibited. The −18 and −24 hour treatments inhibited development of both sexes (Table 4).

TABLE 2. Number of nematodes counted in roots 24 hours after inoculation of soybean roots with approximately 200 second-stage juveniles (J2) of *Heterodera glycines*.

Root tip removal	No. of plants	J2/plant
Control	6	88.8 a
0 time	4	90.2 a
−6 hour	4	54.0 ab
−18 hour	4	17.5 bc
−24 hour	4	9.7 c

Means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.01$). Root tip removal times are relative to the time of inoculation. Controls did not have the root tip removed.

DISCUSSION

Previous papers have reported on the use of modified culture systems such as pruned plants, hydroponics, or excised roots to study the interaction of *H. glycines* with its host (3–5,8,9). Results from such experiments have shown that removal of part or all of the above-ground plant portion has no discernible effect on the ability of SCN to find its host or complete development. The pruned soybean culture system used in these experiments greatly reduced the amount of root growth, thus permitting access and manipulation of roots over an extended period of time. A second advantage of this technique is that the volume of root cortex tissue was reduced, which facilitated finding nematodes in stained and cleared root sections.

Fewer *H. glycines* adults developed in plants if root tips were removed at or before the time of inoculation but not after. During the period 0 to −24 hours, the numbers of adults progressively decreased as the time interval between root tip removal and inoculation increased. Experiments one and two established a time frame demonstrating this relationship but did not account for the difference in numbers. Experiment three showed that the

TABLE 3. Number of *Heterodera glycines* second-stage juveniles (J2) leaving soybean roots after varying lengths of time in hydroponic culture.

Root tip removal	No. of plants	Number of J2 collected				J2/plant
		1 day	8 day	14 day	16 day	
Control	32	111	50	14	14	5.9
0 time	16	45	31	13	3	5.7
−6 hour	16	37	11	11	1	3.8
−18 hour	16	22	7	5	0	2.1
−24 hour	16	15	5	2	0	1.4

Root tip removal times are relative to the time of inoculation. Controls did not have the root tip removed.

difference resulted from two phenomena; fewer J2 entered decapitated roots, and a smaller percentage of nematodes developed to maturity.

Fewer nematodes within the root could result from reduced attractiveness of decapitated roots to J2 or from physical changes that make decapitated roots more difficult for J2 to penetrate. Although decapitated roots were in effect older at the time of inoculation because of the loss of the meristematic region, it seems unlikely that the difference of a few hours maturation should prevent J2 from penetrating. Juveniles have been observed to penetrate intact root segments that were the age equivalent of decapitated roots (based on rate of root growth in 6 or 24 hours) (pers. obs.). It seems more likely that decapitated roots became less attractive to J2 than intact roots. Perhaps the attractiveness of excised roots could be evaluated by performing a similar experiment but using agar rather than sand, thus permitting J2 movements to be monitored (12).

Reduced penetration alone did not account for the total reduction in numbers of adults. Fewer adults developed in roots with tips removed at the time of inoculation than in intact control roots, even though J2 penetration levels were the same. Also, higher percentages of undeveloped nematodes were found in all decapitated roots (−time) compared to controls, regardless of the time interval before inoculation when tips were removed. Female development was affected to a greater extent than male development when the time interval between tip removal and inoculation was short (i.e., 0–6 hours). These data suggest a reduced ability of J2 to successfully establish a feeding site in decapitated root tissue. Undoubtedly the biochemistry of decapitated roots must be different from intact roots as a result of meristem removal and the accompanying cellular changes involved in wound healing. Perhaps J2 in decapitated roots did not receive a proper biochemical signal to initiate a feeding site. An alterna-

TABLE 4. Development of *Heterodera glycines* (SCN) in soybean 16 days after inoculation with approximately 200 second-stage juveniles (J2)

Root tip removal	No. of plants	SCN/plant	Stage (%)				
			Juvenile			Adult	
			J2	J3	J4	M	F
Control	32	50.6	22.7	20.3	12.8	20.0	24.2
0 time	16	43.8	31.1**†	23.4NS	10.7NS	17.7NS	17.1**
−6 hour	16	28.0	47.4***	13.1**	12.5NS	19.6NS	7.4***
−18 hour	16	8.7	89.9***	5.1***	1.4***	3.6***	0.0***
−24 hour	16	7.3	89.6***	2.6***	0.9***	6.0**	0.9***

Root tip removal times are relative to the time of inoculation. Controls did not have the root tip removed.

† Relative number of nematodes at each stage compared to the control; significance determined by chi-square contingency table analysis of raw data, NS = not significant, * = ($P \leq .05$), ** = ($P \leq .01$), and *** = ($P \leq .001$).

tive explanation could be that cells of decapitated roots became physiologically too old to respond appropriately to the nematode's attempt at initiating a synctytium or that hormones produced in the meristem are required for proper synctytium formation (2,11). Males may have an advantage because their feeding requirements are less than that of females (7). Perhaps a poorly developed synctytium would support development of a male to maturity but not a female. A possible test of this hypothesis could be the exogenous application of cytokinins, which are noted for their ability to retard cell aging (10).

This series of experiments provided some insight into the interaction of SCN and its host and suggested several possible areas of future research. In addition, the inoculation technique developed may be useful for some types of SCN–soybean interaction studies, multiple pathogen studies, or sequential inoculation of pathogens. The information obtained from this study has practical application for experiments where root tips may occasionally be injured in the process of transplanting seedlings. It should be noted that root injury prior to inoculation may result in inaccurate data concerning SCN development.

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