Root-Knot Nematode Development and Root Gall Formation in Sugarbeet[†]

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ABSTRACT

Development of *Meloidogyne incognita* and formation of root galls on greenhouse-grown sugarbeet *(Beta vulgaris* L.) seedlings grown in sand was examined at 4-day intervals over a period of 40 days. The penetration and development of root-knot nematode on sugarbeet was asynchronous and multiform. The majority of second-stage juveniles (J2) entered the roots through the root tip region, including the root cap. Before growth began, body length and width of the invading J2 decreased about 10%. Infected root segments initiated galls within 4 days and galls became stainable within 6 days after infection. The males usually developed in groups. Body length of the vermiform adult males was approximately 5 times that of the J2. Mean size (length X width) of adult males was 1.9 X 0.05 mm and of females was 0.8 X 0.5 mm. Diameter of the females increased 16-30 fold between 8 and 40 days after infection of roots. In the same period, diameter of root galls increased 3 fold when plants were grown in sand; the size of root galls responded to the level of nutrients.

Additional Key Words: *Beta vulgaris* L., *Meloidogyne incognita,* giant-cells, penetration.

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Sugarbeet, *Beta vulgaris* L., is one of the top two sucrose producing plants. Root-knot nematodes, *Meloidogyne* spp., are economically important pathogens of sugarbeet. Even though root-knot nematodes are not so widely spread as cyst nematode *(Heterodera schachtii* Schm.), they can be a serious problem in regions where they do occur. Root-knot nematodes are parasitic to a wide variety of plants, including monocotyledons, dicotyledons, and herbaceous and woody plants. Consequently, control of *Meloidogyne* spp. via crop rotation and cultivation practices becomes ineffective, and management of the nematodes in sugarbeet fields becomes more challenging than for *H. schachtii.* Fumigation was the most reliable means of root-knot nematode control, but environmental concerns have restricted nematicides. In California, the application of soil fumigant Telone (1,3-dichloropropene) (Dow Chemical Co., MI) has been prohibited since 1990. Planting nematode-resistant sugarbeet varieties would be desirable; however, *Beta* germplasm with resistance to root-knot nematode was not identified until recently (Yu, 1995).

Reports of *Meloidogyne* spp. and their parasitic relationships with non-*Beta* host plants are numerous (e.g., Triantaphyllou and Hirschmann, 1960; Roberts, 1992). Many studies have emphasized penetration and subsequent development (Herman et al., 1991; Schneider, 1991), variability in reproduction (Swanson and Van Gundy, 1984; Roberts and Thomason, 1986), characterization of nematodes (Eisenback and Triantaphyllou, 1991; Rammah and Hirschmann, 1993), and identification of resistance (Gilbert and McGuire, 1956; Omwega et al., 1989) on economic plants. However, information on root-knot nematode infection of sugarbeet, subsequent root gall formation, and nematode reproduction is lacking. In searching for genetic control and alternative management strategies, an understanding of parasitism of sugarbeet by this pathogen is important, even though its life cycle has been studied in several other plants, e.g., tomato and lettuce (Triantaphyllou and Hirschmann, 1960; Wong and Mai, 1973). This study was undertaken to obtain general information on the development of *M. incognita* in sugarbeet and the resultant root gall formation.

MATERIALS AND METHODS

Meloidogyne incognita (Kofoid and White) Chitwood race 1, originally isolated from common bean *(Phaseolus vulgaris* L.) in San Joaquin County, CA, was cultured on tomato *(Lycopersicon esculentum* Mill. 'Tropic') in the greenhouse. Eggs and egg masses removed from the root gall surfaces were incubated at room temperature on

milk filter disks (Kleen Test Products Inc., WI) in a shallow metal pan containing tap water. Emerging second-stage juveniles (J2) were collected from decanted pan water daily and used as inoculum.

Host plants were progeny of a cross of a genetically stable sugarbeet line C17 (McFarlane et al., 1971) and a commercial garden beet, 'Detroit Dark Red.' Seedlings were developed in 3 x 17 cm "cone-tainers" (Ray Leach Cone-tainer Nursery, OR) containing 110 cm^3 sterilized sand. One sugarbeet seed was planted per cone-tainer, and was inoculated at the 4- to 6-leaf stage by pipeting 1000 newly hatched J2 in 1 ml suspension. Plants were rearranged weekly and maintained in the greenhouse at 24-27° C, and fertilized at weekly intervals with approximately 40 ml/plant of an aqueous solution of 20-20-20 (N-P-K) diluted 250X.

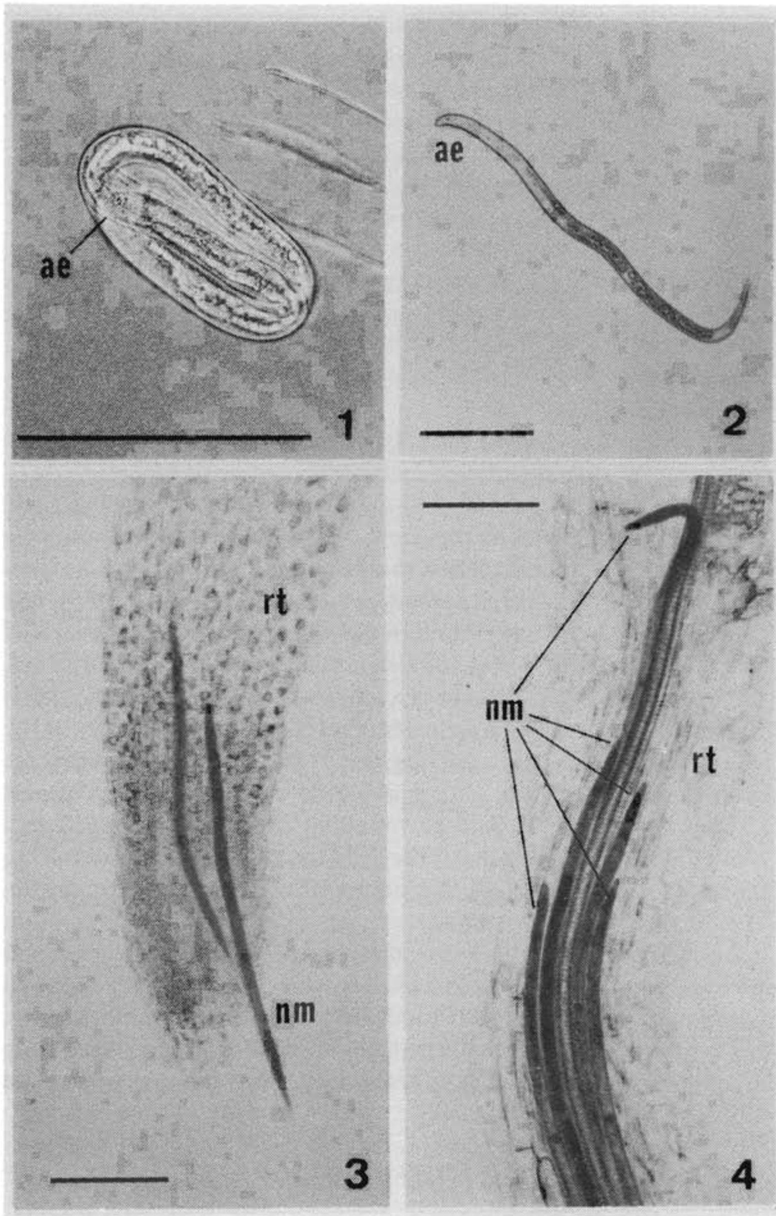
A minimum of eight arbitrarily selected plants was harvested beginning 4 d after inoculation and subsequently at 4 d intervals up to 40 d. Additional inoculated plants grown in a 9:1 (v/v) sand and soil mixture were maintained in the greenhouse as described above and were harvested 40 d after inoculation to compare gall sizes with those from 100% sand culture. At each harvest, prior to clearing and staining, gall diameter of 40 galls from fibrous roots was measured with a fine scaled ruler. Chi-square tests were used to determine gall diameter differences.

The NaOCl-acid fuchsin-glycerin technique (Byrd et al., 1983; Hussey, 1990) was used to stain nematodes within root tissues for life stage analysis. Stained root segments were placed on slides for microscopic evaluation of nematode development and giant-cell formation. Giant-cells and surrounding tissues were stained red. Nematode development inside the galls was examined after removing the multi-layered surrounding tissues with forceps.

Infected sugarbeet roots were placed on water pans, and adult males were recovered after the nematodes completed the last molt and emerged from host roots. Egg masses were collected from root gall surfaces of sugarbeet plants 36 and 40 d after inoculation. Some roots, egg masses, and nematodes were examined without fixing and clearing to facilitate observation of eggs, nematodes developing within eggs, and motility of J2 and adult males.

RESULTS

Prior to hatching, the developing first-stage juveniles sometimes could be observed moving within the egg case (Fig. 1). The newly hatched second-stage juveniles (Fig. 2) were motile, migratory, and infective. Most J2 penetration of sugarbeet roots occurred in the root tip region (Fig. 3), including the root cap. Some juveniles quickly



Figures 1-4. Root-knot nematode development and juvenile penetration of sugarbeet roots. 1. The first-stage juvenile developed inside the egg; the anterior end (ae) is visible. 2. An infective second-stage juvenile. 3. Two nematode juveniles (nm) entering a sugarbeet root (rt). 4. Juveniles migrating longitudinally inside the root; juvenile at the top is changing direction. All scale bars = 0.1 mm.

established a position for feeding while others continued migrating in the cortex (Fig. 4); most settled at a permanent feeding site by the fourth day after inoculation. Multiple J2 penetrations often created tunnels of broken cells.

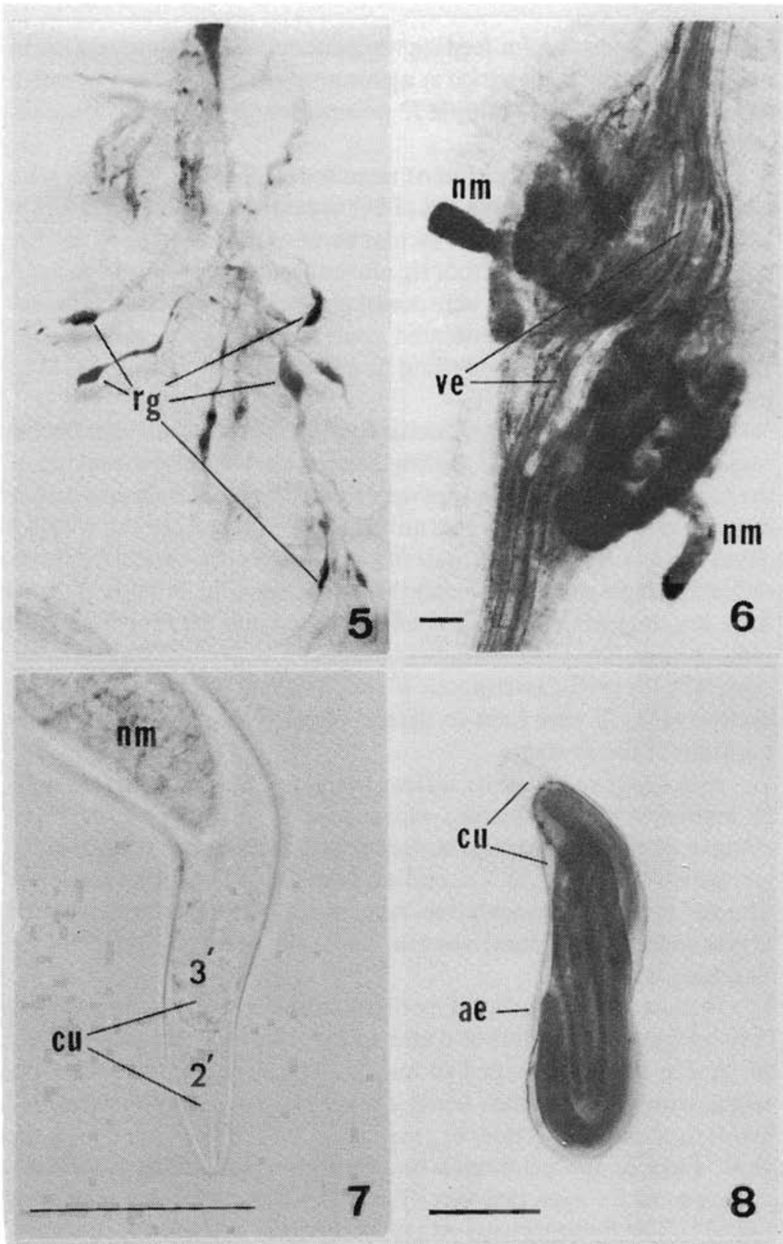
Distribution and position of nematodes inside root tissues were multiform. About 80% (304 out of 381 examined) settled in a position with the head embedded near vascular tissue of the endodermis and the body extended toward the root tip. Juveniles were not always parallel to the longitudinal axis but were occasionally curled within root tissues. Also, J2 frequently accumulated near the root apex and zone of elongation, and induced swelling or gall formation within 4 d after inoculation.

The successful establishment of feeding sites at cell differentiation regions, and the start of J2 feeding, were evident from the swelling of the root (Fig. 5). The gall tissues were stained by basic fuchsin 6 d after inoculation. Swelling of the vermiform body of the J2 began within 8 days (Table 1). By 16 days, a majority of the juveniles were in the third- or fourth-stage, embedded inside the gall tissues (Fig. 6; Table 1). At 20 days, majority of the juveniles had developed into the fourth- or fifth (adult)-stage. The shed second-stage cuticles were readily observable, especially the spiked tails (about 40 μm); however, the shed third-stage cuticles (Fig. 7) were hard to detect, partially because of the short duration of the J4 stage.

Meloidogyne incognita males usually differentiated in root galls or segments where infection was caused by several juveniles. The elongated bodies of the male larvae entwined within cast cuticles at the late fourth-stage (Fig. 8). The earliest J4 males (only in few cases) were detected at 16 d post-inoculation. Adult males were approximately 0.05 mm in width and 1.88 mm in length, which was 5 times the length of the J2 (Table 1).

Females differentiated two gonads (Fig. 9) during the J2 development stage. Within 16 d after inoculation, the pear-shaped body of females (Fig. 10) started to appear. The sizes and shapes of the females varied. The adult females (Fig. 11) were entirely embedded inside root tissues, but their egg masses usually were secreted from the galls. Eggs in the gelatinous matrices were at various embryonic developmental stages, and were 87 μm in length and 40 μm in diameter (Table 1). The juveniles hatched asynchronously at room temperatures.

A single nematode feeding induced a group of giant-cells which resulted in formation of a root gall or swollen area within 4 d after inoculation (Table 2). The number of detectable galls per sugarbeet seedling root system ranged from 11 to over 200. Under multiple larval infections, many galls coalesced and became gall complexes of various sizes and shapes (Figs. 5 and 6).



Figures 5-8. Formation of root galls and metamorphosis of developing root-knot nematodes. 5. Root galls (rg) on sugarbeet 12 days after inoculation with juveniles. (ca. 1.2 X actual size). 6. Two clusters of giant cells stimulated by feeding of nematodes 12 days after inoculation; vascular elements (ve) are twisted and a root gall is formed. 7. Anterior end of the old second- and third-stage (2' and 3') cuticles (cu) of a developing fourth-stage juvenile 20 days after inoculation. 8. A fourth-stage male larva enclosed inside cast cuticles 24 days after inoculation. Scale bar = 0.1 mm (Figs. 6, 8); scale bar = 0.05 mm (Fig. 7).

Table 1. Development of *Meloidogyne incognita* race 1 on sugarbeet in the greenhouse at 24-27° C[†].

Days after inoculation	Life stage[‡]	Gender	Length (μm) Whole body	Body + cuticle	Swollen section	Width (μm)
0	J2 (in inoculum)		395 ± 21			15 ± 9
4	J2		358 ± 25			14 ± 5
8	J2		342 ± 32			16 ± 3
	J3		350 ± 24			30 ± 8
12	J2		352 ± 47			16 ± 8
	J3/J4		367 ± 32	385		37 ± 10
16	J2		363 ± 33			16 ± 4
	J3/J4		377 ± 26	399		55 ± 27
20	J3/J4		418 ± 37	433		116 ± 29
	Adult	Female	464 ± 67		432	189 ± 48
24	J3/J4	Male	370 ± 37	406		72 ± 17
	Adult	Female	521 ± 83		434	257 ± 53
28	J4	Male	288 ± 56	372		74 ± 26
	Adult	Female	653 ± 100		424	349 ± 68
32	Adult	Female	689 ± 93		444	391 ± 85
	Adult	Male	1877 ± 255			46 ± 6
36	Adult	Female	695 ± 102		452	415 ± 77
40	Adult	Female	775 ± 96		519	492 ± 63
—	Eggs		87 ± 7			40 ± 3
—	J1 (in egg cases)		89 ± 6			41 ± 1

[†] Plants were grown in sand; values are means of 10 or more samples ± one standard deviation.

[‡] The eggs, J1, J2 (in inoculum), and adult males were measured without clearing and staining. The few J4 males that were barely recognizable at 16 and 20 days after inoculation were not listed.

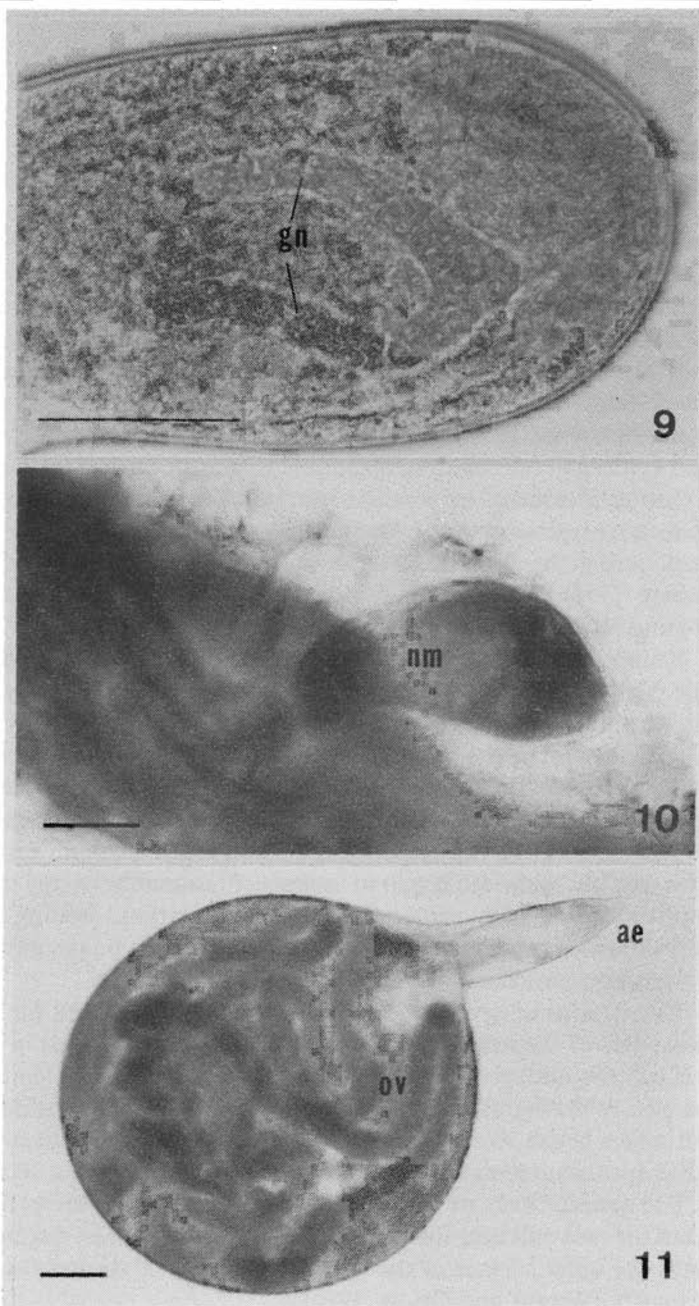
Within 8 d after inoculation, the visibility of nematodes inside the roots was hampered by presence of gall tissue. The mean diameter of nematode females increased 16-30 fold (i.e., from the 16-μm J2 and 30-μm J3 to the 492-μm adults) between 8 and 40 d after inoculation (Table 1). On the other hand, the diameter of root galls increased only 3 fold from 0.67 mm to 2.01 mm (Table 2) in sand (a low nutrient condition). In the 9:1 sand and soil mixture, the mean gall diameter was 2.7 mm at 40 d after inoculation (Table 2). The transverse gall diameters in the two types of cultures were, however, not significantly different (χ^2 : $P < 0.05$).

Table 2. Development of root galls in *Meloidogyne incognita* race 1 infected sugarbeet[†].

Days after inoculation	Gall diameter[‡] (mm)
	In 100% sand culture
4	0.32 ± 0.09 (Control)
4	0.51 ± 0.16 (Swollen areas)
8	0.67 ± 0.23
12	0.91 ± 0.27
16	1.05 ± 0.24
20	1.32 ± 0.27
24	1.54 ± 0.38
28	1.72 ± 0.43
32	1.80 ± 0.39
36	1.84 ± 0.43
40	2.01 ± 0.47
	In 90% sand and 10% soil mixture
40	2.72 ± 0.69

[†] Applied diluted liquid fertilizer weekly in greenhouse.

[‡] Values are means of 40 samples each ± one standard deviation.



Figures 9-11. Development of root-knot nematode females. 9. Formation of two gonads (gn) reflects female differentiation 12 days after inoculation. 10. A pear-shaped young female feeding inside the root gall 16 days after inoculation. 11. A flask-shaped adult female 36 days after inoculation; the ovary (ov) has been well developed. Scale bar = 0.05 mm (Fig. 9); scale bar = 0.1 mm (Figs. 10, 11).

DISCUSSION

Penetration and subsequent development of individual *M. incognita* juveniles in sugarbeet tissues differed considerably, which was comparable to their development on some other host plants, e.g., soybean and tobacco (Herman et al., 1991; Schneider, 1991). Nevertheless, the measurements at various developmental stages (Table 1) were generally within the range of variability for this root-knot nematode (Eisenback and Triantaphyllou, 1991). The penetration and migration habits of *M. incognita* juveniles in sugarbeet roots were similar to those exhibited by *H. schachtii* (Yu and Steele, 1981). Wergin and Orion (1980) reported *M. incognita* juveniles penetrated between adjacent epidermal cells causing no apparent damage to the surrounding tissues in tomato. In separate host-parasitic interaction study, tomato host reactions to the *Meloidogyne* spp. parasitism were initiated during the first 12 hours after infection (Williamson and Lambert, 1992). With sugarbeet I did not find this type of epidermal penetration, nor was such rapid host reaction apparent.

Nematode migration, emergence, or repenetration on host roots (Figs. 3 and 4) is largely dependent on the nematodes' ability to establish suitable feeding sites (Schneider, 1991). Four days after inoculation, *M. incognita* J2 in sugarbeet roots (stained) were about 10% smaller in both body length and width than prior to inoculation (viz., 357.7 vs. 394.7 μm and 13.6 vs. 15.1 μm, respectively; Table 1). By the eighth day, the length of the J2 was even less than on the fourth day, but the width began to increase. Presumably, energy consumption during root penetration, larval migration, feeding-site establishment, and the possible low- or non-nutrient intake during the transition period contributed to the decrease in size.

The patterns of development were dramatically different for the two genders of the nematode. Males developed single gonads in J2, but it was not until the late J4 stage that males (Fig. 8) and females were clearly distinguishable. After the fourth molt, the vermiform adult males began to emerge from roots. In contrast, the pear- or flask-shaped adult females continued to enlarge (Table 1; Figs. 10 and 11). The anterior ends of the females were embedded in the pericycle, but the root cells near their posterior regions began to be displaced toward the outer surface of the root by extrusion of the gelatinous egg matrix (Wergin and Orion, 1980).

Meloidogyne incognita egg matrices derived from sugarbeet root galls were similar to, but slightly smaller than, those from tomato cv. Tropic. Probably the number of eggs in the egg masses from sugarbeet root galls also were lower than those of tomato. Even

though the egg matrices on sugarbeet roots could be discerned by unaided eyes, especially after staining, egg masses from some pepper *(Capsicum annuum* L.) lines were even more readily detected (Yu, unpublished).

The transverse diameter of root galls (Fig. 5) enlarged steadily up to 28 d after infection. This was in comparison to the diameter and length of the females, which increased rapidly from 16 d to 32 d (Table 1). The diameter of females has been used as a criterion for the growth of *Meloidogyne* spp. (Wong and Mai, 1973).

The diameter of root galls on infected sugarbeet grown with 10% soil mixed into 90% sand was increased by 35% (from 2.01 to 2.72 mm) at 40 d after inoculation (Table 2). One possible explanation of this is that the size of root galls responded to the level of nutrients. Seedlings propagated in growth pouches with various nutrient levels available to roots could be used to test this hypothesis.

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