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Observation of Root-knot Nematodes in the Root Gall Formed on Oriental Melon

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Oriental melon, *Cucumis melo* L. *cv.* Geumssaragi-euncheon, grafted on Shintozoa (*Cucurbit maxima* × *Cu. moschata*) was planted in a greenhouse infested with *Meloidogyne arenaria* and root galls were examined five months after planting. A gram of root gall was volumed at ca. 10 cm^3 and contained in an average of 363 females (170 developing and 193 matured females), 2,120 secondstage juveniles (J2), and 13,074 eggs. In addition, there was 56 J2 per cm^3 soil around the infested plant. An oriental melon had an average of 134.6 g of root gall (70% of total root weight) per 0.72 m^2 area. In a conservative estimation, an oriental melon plant could accommodate ca. 1.2×10^7 eggs and J2 per 0.72 m^2. The eggs contained in root tissues could be an important inoculum source to the next crop and the fate of these eggs are well worth further investigation.

Keywords **:** *Cucumis melo*, giant root gall, greenhouse, host suitability, developmental stages, maximum population density

Oriental melon (*Cucumis melo* L.) is a highvalued cash crop in Korea. It is transplanted in January during the winter season under a plastic greenhouse, and is first harvested in May. Then, stems are trimmed and vines are regenerated, so the harvest is extended to September. By this method, oriental melon is grown for as much as 10 months in a greenhouse per season. Because of limited land space in Korea, oriental melon has been continuously cultivated for the last 20 years in Seongju area. Its continued and extended cultivation practices have caused severe soil diseases, including rootknot nematode (*Meloidogyne* spp.) infestation. Oriental melon roots infested with rootknot nematodes develop heavy galls and plants die early in July. During the harvest of oriental melon, it is not uncommon to find as much as 500 g sized giant galled root (Fig. 1A).

Because oriental melon roots are heavily galled, many egg masses are embedded deep within root tissue, and egg mass presence cannot properly be checked by the Phloxin B staining method (Hartman and Sasser, 1985). There are studies on life cycle, development, and ecology of root-knot nematodes (Ferris et al., 1978; Pinkerton et al., 1991; Starr and Jeger, 1985). However, there is an absence of information on how many nematodes, in what stages, and in what proportions, are contained in root galls of oriental melon. The objective of the present study was to determine stages, numbers and proportions of nematodes contained in root gall of oriental melon.

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Materials and Methods

The investigation was conducted at Seongju Fruit Vegetable Experiment Station, Korea. Oriental melon, *Cucumis melo* L. *cv.* 'Geumssaragi-euncheon', grafted on Shintozoa (*Cucurbit maxima* × *Cu. moschata*) was used in the experiment. To examine nematodes contained in root galls, microplots were prepared in a 400-m^2 greenhouse where the soil was infested with *Meloidogyne arenaria* race 2. Within a microplot, 42-day-old 'Geumssaragi-euncheon' oriental melon seedlings were transplanted in 40-cm intervals (Kim, 2001). Grafting is a common practice to growing oriental melon during the winter season in Korea to increase cold tolerance and to prevent fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *melonis*.

After transplanting, 187 Kg/ha nitrogen, 63 Kg/ha phosphate, and 109 Kg/ha potassium, and 30 ton/ha manure were applied in October. Soil in the greenhouse was ridged to 20 × 200 cm row (height × width). The ridge was mulched with black plastic film (0.02 mm thickness); herbicide had not been applied. The row was framed with iron wire and covered with clear plastic film (0.02 mm thickness) (Kim, 2001). During the night, additional blanket (thickness=400 gram/m^2) was used over a plastic film to preserve heat. The row was drip irrigated (dripper flow =1.49 l/hr; Netafim Co.). Insects and diseases were controlled with nonsystemic pesticides, such as pyrazophos, dichlofluanid, and carbosulfan. The soil had 20 J2/ 100 cm^3 before planting (Southey, 1986).

Observation of nematodes in galled roots. Five months after planting, roots were carefully dug out and washed

gently under running tap water to remove all soil particles. Galled and healthy roots were separated and weighed. Galled roots were sectioned to ca. 1 mm thickness and nematodes embedded within root tissues were stained by using Bleach stain method (Byrd et al., 1983) and observed under a microscope.

Number of eggs and J2 in galled roots. Galled roots of 5 to 7 grams were placed in a commercial blender with 200 ml of 1% NaOcl solution and blended at high speed for 1 minute (Barker, 1985). The resulting suspension of eggs, J2 and root debris was filtered through a 75-μm-aperture sieve over a 28-μm-aperture sieve. The eggs and J2 retained on the 28-μm-aperture sieve were rinsed well with running tap water and counted under a stereomicroscope. Three root batches were processed for each plant and a total of three plants were examined.

Number of females in galled roots. *Meloidogyne* females embedded in galled roots were separated by using Jeffrey's solution (10% nitric acid plus 10% chromic acid; Southey, 1986). Galled roots of 2 to 3 grams were placed in a 20-ml volume vial and prefixed with FG41 (Southey, 1986) for 1 to 2 days. Subsequently, roots were placed in Jeffrey's solution for 15 to 20 hours. Root prefixed in FG41 hardened female cuticle and reduced female shrinkage (data not shown). After 15 to 20 hours., the vials were shaken vigorously for about 20 seconds to separate females from disintegrated roots tissue. The resulting suspension was filtered through a 200 μm-aperture sieve over a 75 μm-aperture sieve and females retained on each sieve were counted separately under a stereomicroscope; females retained on 200 μm-aperture sieve were counted as mature females, while those passed through 200 μm-aperture sieve and retained on 75 μm-aperture sieve were counted as young developing females. Females appeared as dark spherical objects among translucent irregularly-shaped root debris, and were easily countable. Three root batches were processed for each plant and a total of three plants were examined.

Number of J2 in soil. At the time of root digging, the soil around the roots were also sampled. Soil was throughly mixed in plastic bag and 300 cm^3 soil were processed by sieving and centrifugation-flotationfor J2 extraction (Southey, 1986). Two soil samples were processed from each plant and total of three plants was examined.

Results and Discussion

A five months old oriental melon plant infested with *M. arenaria* had an average of 134.6 g of galled root which composed 70% of total root weight. A gram of galled root was volumed ca. 10 cm^3 and contained 363 females (170 developing and 193 matured females), 2,120 J2, and 13,074 eggs (Table 1). Thus, in a conservative estimation, a plant root may contain ca. 49,000 females and 2 million eggs and J2 per plant. Root gall of 134.6 g can be found commonly for five months old plant. In later season, it is not infrequent to find 300-500 g sized galled roots in farmer's fields (Fig. 1A).

Table 1. Number of nematodes contained in root gall of oriental melon infected by *Meloidogyne arenaria* race 2

Nematode stages	Number of nematodes		
	per gram	per plant[a]	Range
In root gall			
Female (total)	363	4.9×10^4	4.1×10^4~5.8×10^4
developing[b]	170	2.3×10^4	1.4×10^4~3.6×10^4
matured	193	2.6×10^4	9.7×10^3~4.1×10^4
Eggs & Juveniles	15,193	2.0×10^6	1.5×10^6~2.6×10^6
eggs	13,074	1.8×10^6	1.2×10^6~2.3×10^6
juveniles	2,120	2.9×10^5	1.6×10^5~4.0×10^5
In soil[c]			
juveniles	56	1.0×10^7	4.4×10^6~2.7×10^7
Total eggs & J2/plant/0.72 m^2		1.2×10^7	

[a] 5-month-old oriental melon plant infected with *M. arenaria* had 134.6 g of galled root which composed 70% of total root weight.
[b] Females retained on a 200-μm aperture sieve were counted as mature female, while those passed through 200-μm aperture sieve and retained on 75-μm aperture sieve were counted as developing females.
[c] Soil volume: length × width × depth = 40 × 180 × 25 cm.

In soil around the infested roots, 56 J2/cm^3 soil were found. An oriental melon plant occupies ca. 0.72 m^2 area (40 × 180 cm) and J2 distribute in a uniform and horizontal spacing to the soil depth of 25 cm (Kim, 2001); in a rough estimation, considering 20% isolation efficacy of centrifugation-flotation method (Viglierchio and Schmitt, 1983), there was approximately 60 million J2 in soil around a plant. Males were rare in this particular study and were not examined further.

In general, high nematode population level causes plant damage, results in food shortage, and limits nematode multiplication. Egg masses embedded in root tissues of five month old oriental melon appeared white which are typical of a developing population in younger roots, in contrast to brown colored egg masses which are overwintering populations from older roots (Ishibashi, 1969); evidently, galled root contained 47% of developing young females (Table 1). The result suggests that a 5-month-old oriental melon root infested with 49,000 females still provides enough nutrients for those nematodes. Therefore, we could assume that nematode population density in oriental melon in later month could be higher than reported here.

Nematode reproduction should be examined when

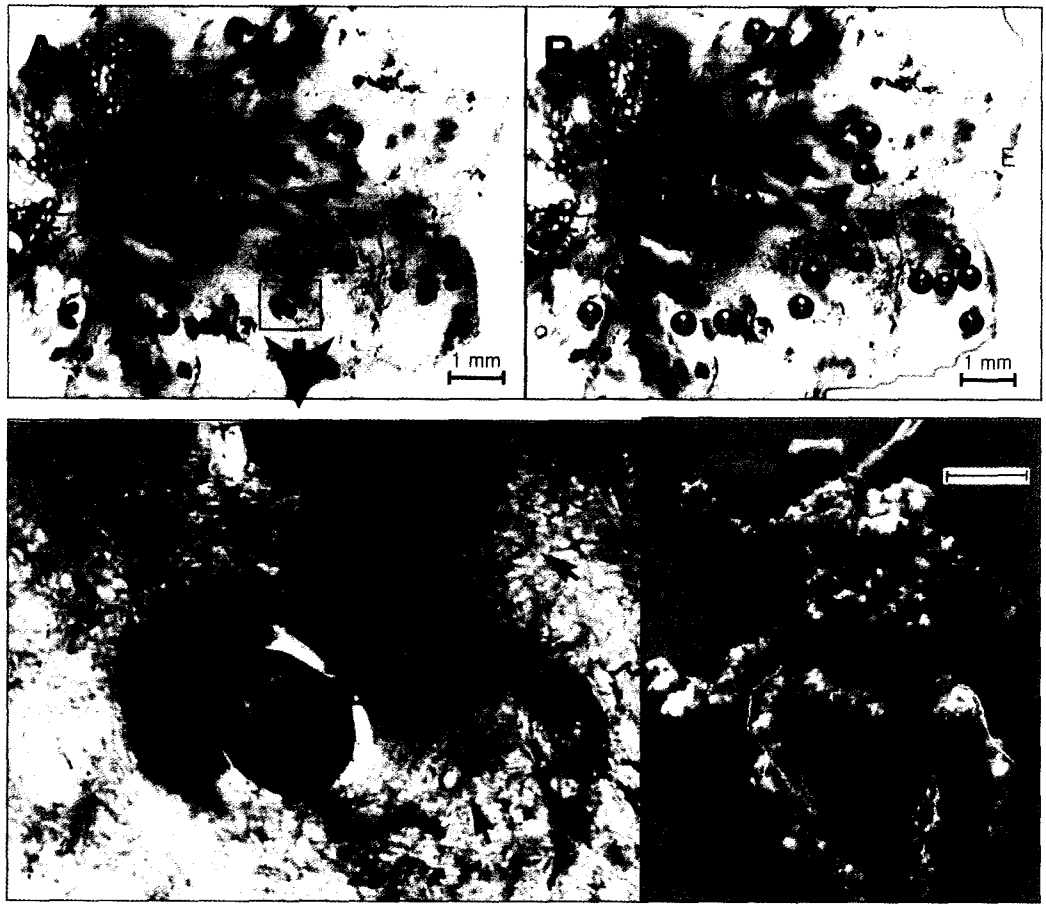

Fig. 1. A. Section of a root gall showing embedded nematodes of *Meloidogyne arenaria* (red color) in root tissue. **B.** Schematic drawing of Fig. 1. 'A' showing position of females ( ). C = center of root, E = epidermis, X = xylem, = female. **C.** Enlargement of a section in the root of Fig. 1. 'A'. EM = eggmass, F = female, GC = gianit cell, ← = juveniles. **D.** Heavy root gall of oriental melon caused by *M. arenaria.*

studying host susceptibility or development of *Meloidogyne* species. Reproduction of *Meloidogyne* species usually is checked by staining egg masses exposed to root surfaces (Hartman and Sasser, 1985). However, a large portion of egg masses on oriental melon roots infested by root-knot nematodes were embedded within root tissue. Therefore, Phloxin B staining could not properly be used to examine root-knot nematode reproduction.

Females were embedded in root section everywhere (Fig. 1B). It is uncertain how the females embed deep in the center of root tissue; we, however, assume that young root tissue infected with root-knot nematodes enlarges faster and surrounds developing females. And later, mature females produce eggs within root tissue, J2 hatch from those eggs, and those J2 re-establish in adjacent root tissue without leaving roots. Embedded eggs in root tissues probably are safer than those eggs exposed to the root surface. If J2 could complete their life cycle within root tissue without leaving the root, it should escape from adverse soil environments such as extreme temperature and moisture or from natural enemies.

Large number of eggs and J2 contained in root tissues would be an important inoculum source to the next crop and the fate of those eggs are well worth of further investigation.

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