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Mulberry Damages Caused by a Root-Knot Nematode, *Meloidogyne* mali Indigenous to Japan

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Abstract
A root-knot nematode, Meloidogyne mali was described by Itoh et al. (1969) as a new species infecting apple roots in Japan. Any incidence caused by this species has not been recorded in other countries since then. It was first considered that M. mali distributed only in the northern part of Japan and it was parasitic to woody plants. M. mali has been regarded as one of the most important nematodes injuring apple trees in Japan. It is now recognized that this nematode distributes in most of the mulberry fields throughout Japan, parasitizing mulberry as well as several kinds of herbaceous plants including vegetables. An analysis of mulberry damages caused by M. mali indicated that the growth of seedlings and graftings of mulberry inoculated with more than 2,500 of the second stage larvae of M. mali was significantly inferior to that of the control trees without inoculation. One year after the inoculation, 30-60% of those trees infected by nematodes were dead. In 2-3 year-aged mulberry trees inoculated with nematodes, they reduced their leaf weight by 10-20%. The symptom caused by the nematode infection came out more slowly and the damages were smaller as compared with those in younger trees.

Discipline: Animal pest
Additional key words: apple, nematology, plant protection, woody plant

Introduction

An incidence caused by root-knot nematode, *Meloidogyne mali* was first detected in apple roots in Nagano Prefecture, Japan. This root-knot nematode was described by Itoh et al. in 1969 as a new species[4]. No information on this nematode has been recorded in other countries since then. It was first considered that *M. mali* distributed only in the northern part of Japan and it was not parasitic to herbaceous plants except for white clover[4]. There had been limited reports available on this nematode because of its specific ecology as mentioned above. In 1978, Inagaki reviewed results of the studies on ecology and control of *M. mali* on apple trees as well as on apple damages caused by this species in the northern region of Japan[3]. Toida also identified this nematode by its morphology at the second stage larvae and identified its host plants including some vegetable crops[7]. Toida et al. disclosed its geographical distribution in the warmer region of Japan[9,10]. It was also demonstrated that *M. mali* was the most important nematode among over 20 species parasitizing mulberry trees[9].

Damages in growth of mulberry plants caused possibly by root-knot nematodes were reported by a few researchers in Japan since early this century[1,2,5,6]. However, nematodes were not definitely identified and the damages in mulberry plants were not analyzed in detail. It is very likely that most of the mulberry damages caused by nematodes are due to *M. mali*. However, there have been few papers which confirm the mulberry damages caused by that nematode[8]. The present study deals with the damages in growth and yield of mulberry under the inoculation with larvae of *M. mali* at various growing stages of the trees.

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Materials and method

1) Growth damages of young mulberry trees inoculated with different numbers of M. mali

Seeds of a mulberry variety, Ichinose × Benikawaroso were sown in 1/5,000 a Wagner pots with soils sterilized by 120°C heat for 1 hr. When the plant height reached 15–20 cm, 10, 100, 1,000 and 10,000 of the second stage larvae of *M. mali* were inoculated into the mulberry trees. Ten plants were used in each experiment with inoculation of different numbers of the larvae. Seven months after the inoculation, the following data were taken: plant height, fresh weight of leaves, number of galls made by the nematodes on the mulberry roots and nematode density in soils.

2) Growth damages in seedlings and graftings of mulberry inoculated with M. mali

Seedlings (Inchinose × Benikawaroso) and graftings (Ichinose) of mulberry were planted in 1/5,000 a Wagner pots. One month after the planting, 10,000 larvae of *M. mali* were inoculated into each 10 pot and plant height and nematode density in soils were compared with those of non-inoculated plants intermittently for 2 years.

The second stage larvae of *M. mali* were separated from soils by Baermann funnel technique under 20–25°C. The separation of the larvae from soils was repeated three times in each pot, and nematode density was expressed by mean number of the nematodes of those three replications.

3) Damages in growth of mulberry trees inoculated with M. mali

(1) Growth of mulberry trees planted in big pots

A mulberry tree of 2-year age was planted to the soils sterilized by chloropicrin in a concrete pot of 80 cm in diameter and 70 cm in depth. One year after the planting, 30,000 larvae of *M. mali* were inoculated into the plants. Nine plants were used in each experiment with or without inoculation of nematodes. Measurements on growth of the plants and density of the nematodes in the soils were taken periodically for 5 years.

(2) Growth of mulberry trees planted in concrete frames

A 3-year-aged mulberry tree was planted in a concrete, bottomless frame (200 cm long, 200 cm wide and 150 cm deep), which was buried in the ground. Soils in the frame were sterilized by chloropicrin before planting. Two years after the planting, 50,000 larvae of nematode were inoculated into the trees. Ten plants were used in each experiment with or without inoculation of the nematodes. Observations on the growth of plants were carried out intermittently for 3 years.

Ichinose (mulberry variety) was used in both (1) and (2) experiments. Nematode density in the soils in each pot was surveyed by number of the larvae per 30 g of soils collected from each pot, with the procedure replicated 3 times in both experiments. In these experiments including 1), 2) and 3), plant height or branch length was measured once a month from May to October. After mulberry trees stopped growing in October, the branches were cut every year.

Table 1. Comparison in growth of mulberry seedlings inoculated with different numbers of larvae of *Meloidogyne mali*

No. of larvae inoculated	Fresh weight of leaves (g)	Rate of plant height[a)]	Fresh weight of rootlets (g)	No. of galls per 1 g rootlets	No. of larvae per 30 g soil	Rate of dead plants (%)
0	20.5	11.1	10.3	0	0	0
100	21.3	10.8	10.5	1.0	2.5	0
500	18.8*	9.2	8.4	15.7	109	10
2,500	13.6*	8.5*	7.2*	70.3	409	30
12,500	10.0*	4.6*	5.5*	213.0	1,048	60

a): Maximum plant height of mulberry in the year/plant height before the inoculation of nematodes.
* Indicating statistical significance of trend.
Each value is a mean of 10 replications.

Results and discussion

1) Growth damages of young mulberry trees inoculated with different numbers of M. mali

Table 1 shows that fresh weight of leaves of the mulberry plants inoculated with 1,000 and more larvae of *M. mali* is significantly smaller than that of the trees with 10 and less larvae. It also shows that plant height and fresh weight of rootlets of mulberry inoculated with 10,000 larvae are less than those of the trees with 1,000 and less larvae. Number of galls on rootlets and the population density of the nematode increase logarithmically with

Table 2. Growth of mulberry seedlings with and without inoculation of *Meloidogyne mali* larvae for 2 years

		Inoculation	Year of inoculation	1 year after inoculation	2 years after inoculation
Fresh weight of leaves (g)	Mean	With	8.2	27.8	40.3
		Without	13.6	44.9	64.0
	Difference[a)]		−5.4±2.7*	−17.1±6.4*	−23.7±11.9*
Fresh weight of stems (g)	Mean	With	7.3	23.8	25.2
		Without	12.8	34.1	45.7
	Difference[a)]		−5.5±2.0*	−10.3±8.3*	−20.5±8.2*
Fresh weight of rootlets (g)	Mean	With	13.2	41.3	21.3
		Without	15.8	43.1	44.0
	Difference[a)]		−2.6±5.6	−1.8±9.6	−23.3±10.0*
No. of galls[b)] per 1 g rootlet		With	87±20.7	66±16.1	94±31.0
		Without	0	0	0
No. of larvae[b)] per 30 g soil		With	92.3±36.7	285±63.1	244±64.7
		Without	0	0	0
Rate of dead plants (%)		With	10.0	20.0	40.0
		Without	0	0	0

a): ± in difference; Dunn-Sidák's *t* interval test.
b): ± in no. of galls and larvae; Standard deviation.
* Significant at 0.05 level.
Each value is a mean of 10 replications.

Table 3. Growth of mulberry gratfings with and without inoculation of *Meloidogyne mali* larvae for 2 years

		Inoculation	Year of inoculation	1 year after inoculation	2 years after inoculation
Fresh weight of leaves (g)	Mean	With	28.3	48.4	40.3
		Without	38.4	60.9	63.7
	Difference		−10.1±7.6*	−12.5±9.9*	−23.4±10.4*
Fresh weight of stems (g)	Mean	With	11.0	13.8	23.0
		Without	16.2	27.5	39.4
	Difference		−6.2±3.6*	−13.7±4.6*	−19.4±2.4*
Fresh weight of rootlets (g)	Mean	With	27.6	41.2	36.2
		Without	32.2	45.4	55.3
	Difference		−4.6±10.4	−4.2±3.4*	−19.1±3.6*
Rate of dead plants (%)		With	0	10.0	20.0
		Without	0	0	0

* Significant at 0.05% level in Dunn-Sidák's *t* intereval test.
Each value is a mean of 10 replications.

augmentation of the nematodes inoculated. When the mulberry trees were inoculated with 1,000 and 10,000 larvae, 20% and 50% of the trees died, respectively. From these results, it is concluded that more than 20% of leaf weight, 15% of rootlet weight and over 10% of plant height of a young mulberry tree planted in a 1/5,000 a pot become reduced, in case where nematode density in soils is about 400 larvae per 30 g sample, or the level of infestation is about 70 galls per 1 g rootlet. When the nematode density is around 1,000 larvae per 30 g soil, or the gall density is about 200 per 1 g rootlets, much greater growth damages of mulberry take place, resulting in 50% of the trees dead.

2) Growth damages in seedlings and graftings of mulberry inoculated with M. mali

Growth of mulberry seedlings during the period of 2 years after inoculation of *M. mali* and nematode densities in soils are shown in Table 2. One year after the inoculation, fresh weight each of leaves and stems of the trees inoculated with nematode was significantly smaller than that of the trees without inoculation. Two years after the inoculation, weight of rootlets of mulberry with nematodes was smaller than that of the tree free from nematode. Twenty percent of the trees inoculated with *M. mali* were dead within 1 year, with a total mortality reaching 50% in 2 years. Although number of galls on mulberry rootlets did not change for 2 years, number of the second stage larvae of *M. mali* rapidly increased, reaching its peak in 1 year after the inoculation. In 5 months after the inoculation, plant height of mulberry was slightly lower than that of the control trees. A significant difference was seen however in the plant height between with and without nematodes in 2 years. Regarding the length of newly grown branches of mulberry trees, there was no significant difference between with and without inoculation in 1 year after the treatment.

Growth of mulberry graftings with and without inoculation treatment is shown in Table 3. Weight of leaves and stems of the graftings with nematodes was significantly lower than the control even within 1 year after the treatment. The plant height was also lowered by inoculation in 1 year. However, the difference in growth of the graftings between with and without nematodes was smaller than that of the seedlings. Less trees were dead in the graftings than in the seedlings under inoculation. Density of the nematodes in a pot increased, reaching the peak in 2 years as the case in the seedlings. Most of the withering of seedlings and graftings caused by *M. mali* took place after pruning of their branches in spring or in winter under non-irrigation. This result suggests that young mulberry trees inoculated with

Table 4. Growth of mulberry trees with and without inoculation of *Meloidogyne mali* larvae in large cocrete pots for 5 years

		Inoculation	Year of inocu.	1 year after inocu.	2 years after inocu.	3 years after inocu.	4 years after inocu.	5 years after inocu.
Fresh weight of leaves (g)	Mean	With	490	646	860	452	550	680
		Without	423	737	1,451	754	1,050	1,080
	Difference[a)]		67.0±85.7	−91.0±89.3*	−591±205*	−302±110*	−500±191*	−400±204*
Length of branches (cm)	Mean	With	409	706	885	569	700	760
		Without	381	811	1,204	776	1,027	912
	Difference[a)]		28.3±67.4	−105±120	−319±115*	−207±112*	−327±167*	−152±189
Diameter of branches (mm)	Mean	With	8.8	10.0	10.3	9.5	11.0	10.7
		Without	9.4	10.7	14.0	12.1	15.4	12.9
	Difference[a)]		0.6±2.1	0.7±1.6	−3.7±3.2*	−2.6±2.1*	−4.4±2.6*	−2.2±2.1*
No. of larvae[b)] per 30 g soil			175±20.7	450±56.0	680±160	380±54.2	284±36.3	207±34.4

a): ± in difference; Dunn-Sidák's *t* interval test.
b): ± in no. of larvae; Standard deviation.
* Significant at 0.05 level
Each value is a mean of 9 replications.

Table 5. Growth of mulberry trees with and without inoculation of *Meloidogyne mali* larvae in concrete frames for 3 years

		Inoculation	Year of inocu.	1 year after inocu.	2 years after inocu.	3 years after inocu.
Fresh weight of leaves (g)	Mean	With	2,130	1,910	2,100	2,380
		Without	2,180	2,360	2,490	3,050
	Difference[a]		−50±238	−450±306*	−390±455	−220±452
Length of branches (cm)	Mean	With	231	189	206	280
		Without	228	196	217	290
	Difference[a]		3.4±22.4	−7.1±21.0	−11.5±13.4	−10.0±25.8
Leave weight per 1 m branch	Mean	With	4.6	5.5	6.9	9.7
		Without	4.8	6.0	7.5	10.5
	Difference[a]		−0.2±0.6	−0.5±0.8	−0.6±1.3	−0.8±1.6
No. of galls[b] per 1 g rootlet		With	15.0±1.3	89.3±7.6	133±13.5	156±17.9
		Without	0	0	0	0
No. of larvae[b] per 30 g soil		With	43.2±3.7	121±14.7	197±15.2	210±20.3
		Without	0	0	0	0

See the footnote of Table 2.

M. mali be tolerable under a usual condition but not when they are subjected to some stresses such as cutting of their branches or lack of irrigation water.

3) Growth damages in aged mulberry trees after inoculation with M. mali

Growing status of mulberry trees for 5 years with and without inoculation of *M. mali* is shown in Table 4. They were planted in large concrete pots. In 1 year after inoculation, no significant difference was seen in plant growth between with and without treatment. In 2 years, however, the trees with nematodes showed significantly smaller weight of fresh leaves, lower height and shorter diameter of branches than those of the control trees. During the period third to fifth year, the difference in growth with and without treatment remained nearly the same level, whereas the nematode density gradually decreased from the third year and beyond. Leaf area of mulberry with nematodes (140 cm^2) was slightly smaller than that (150 cm^2) of the trees without nematodes. Leaf weight (80 g) of mulberry per 1 m of branch was also smaller than that (100 g) of the control trees. There was no difference in number of leaves between the inoculated trees and the control. This result indicates that the decline of leaf weight in the infected trees was caused not by lessened number of leaves per tree but by reduced size of leaves as suggested earlier[8]. Growth of the mulberry trees with and without a *M. mali* inoculation in the concrete frame is exhibited in Table 5. Results obtained in this experiment somewhat differed from those in the other experiments as mentioned above. Growth difference in the trees between with and without nematodes was seen only in leaf weight in 1 year after the inoculation, disappearing in 2 years.

It is likely that the mulberry trees planted in the frame can widely spread their roots just as the case in open fields, where some roots may escape from the attack of nematodes. In addition, number of the larvae inoculated might not have been large enough to cause damages to the mulberry trees planted in frame. The data shown in Table 5 suggest that 5–20% yield decline of mulberry leaves result from the infestation of the level of 100–200 larvae per 30 g soils, or 100–150 galls per 1 g mulberry rootlets.

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