Effect of Soil Microflora on the Interaction of Three Plant-Parasitic Nematodes with Celery

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The authors acknowledge the assistance of R. M. Lumsden in the identification of *Pythium polymorphon*, that of A. M. Golden in the identification of *Paratylenchus projectus*, and that of H. H. Lyon in the preparation of the photographic plate.

Accepted for publication 2 March 1976.

ABSTRACT

STARR, J. L., and W. F. MAI. 1976. Effect of soil microflora on the interaction of three plant-parasitic nematodes with celery. Phytopathology 66: 1224-1228.

In growth chamber tests, celery roots grown in organic soil collected from celery fields (field soil) and inoculated with 10,000 *Meloidogyne hapla* eggs developed extensive root galling and root necrosis. The amount of root necrosis observed after a 4-week incubation period depended upon the initial concentration of *M. hapla;* 500 eggs per seedling caused no increase in root necrosis over that observed on noninoculated roots, but 5,000 or more eggs per seedling increased it significantly. Root necrosis was not observed on nematode-infected roots in autoclaved soil. *Pythium polymorphon*, which was isolated from necrotic galls and roots, was the primary cause of necrosis of *M. hapla*-infected roots from nonautoclaved field soil. In autoclaved field soil, *M. hapla*-infected roots inoculated with *P. polymorphon* developed more necrosis than did roots not infected by the nematode at similar fungal inoculum levels. Celery growth was reduced more in the same nonautoclaved field soil than in autoclaved field soil infested with *Pratylenchus penetrans*. The low level of root necrosis in nonautoclaved field soil was not increased by inoculation with the nematode. More nematodes were recovered from roots in nonautoclaved field soil infested with *P. penetrans* than from roots in autoclaved field soil. Celery growth was not reduced in either soil when seedlings were inoculated with *Paratylenchus projectus*.

Additional key words: plant pathogenic nematodes, secondary invaders.

Plant pathogenic nematode interactions with other plant pathogens in disease complexes have been demonstrated many times (1, 12, 13). Some root disease complexes also are caused by interactions between pathogenic nematodes and organisms not individually pathogenic to the plant. Powell et al. (14) demonstrated that inoculation of *Meloidogyne incognita*-infected tobacco roots with fungi which did not cause necrosis in the absence of the nematode resulted in significant root necrosis and greater reduction in growth than did the nematode alone. Mayol and Bergeson (10) found that *M. incognita* caused a greater reduction in the growth of tomatoes in field soil than in autoclaved soil under aseptic conditions. They suggested that this was related to the activity of secondary invaders of the root-knot galls, especially bacteria. These reports suggest that a large part of the necrosis on plants infected by root-knot nematodes may be caused by the activities of organisms not pathogenic in the absence of the nematode.

Roots of field-grown celery heavily galled by *M. hapla* often become necrotic. The objectives of this study were to determine the role of the root-knot nematode in the production of root necrosis and to determine whether other organisms are necessary or involved. The effect of soil microflora on the interaction of celery and *Pratylenchus penetrans* and *Paratylenchus projectus* also was examined. A preliminary report has been published (15).

MATERIALS AND METHODS

Meloidogyne hapla Chitwood, 1949, obtained from celery plants in the field, was maintained on celery in growth chambers at 25 C. *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans-Stekhoven, 1941, a laboratory population, was maintained on alfalfa callus tissue cultures, and *Paratylenchus projectus* Jenkins, 1956, also a laboratory population, was maintained on birdsfoot trefoil (*Lotus corniculatus* L.). Organic soil (Carlisle muck), used in all experiments to provide a source of natural soil microflora, was collected from a field with a previous history of celery cropping. This soil was essentially free of pathogenic nematodes; *M. hapla, P. penetrans,* and *P. projectus* were not detected. The autoclaved soil used in some tests was organic soil collected from the same celery field; autoclaved for 4 hours at 128 C at a pressure of 1.4 kg/cm^2, and cured for 2 months before being used. These soils will be referred to as autoclaved and nonautoclaved throughout this paper. Celery (*Apium graveolens* var. *dulce* 'Golden Detroit' and 'Utah 52-70') was the host plant. All tests were conducted at 21 C with a 12-hour photoperiod at 21,520 lux. Standard techniques (2, 4) were used to extract nematodes from soil and host roots. *Meloidogyne hapla* eggs were collected by the method of Hussey and Barker (8).

To determine the role of *M. hapla* in root necrosis, 4-week-old celery seedlings were transplanted singly to 10-cm diameter pots of autoclaved or nonautoclaved soil and inoculated with a suspension of nematode eggs. The root

necrosis that developed was estimated by a necrosis index in which 0 = none, 1 = less than 10%, 2 = 11 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, 5 = 76 to 100% of the root system necrotic. Fresh roots and dried shoots were weighed to determine growth responses.

Fungi were isolated from necrotic gall tissue (that had been surface-sterilized for 2 minutes in 1% NaClO and rinsed three times in sterile distilled water) by culturing the tissue on acidified potato-dextrose agar (PDA) or corn meal agar (CMA) containing pimaricin (10 mg/liter) and penicillin (50 mg/liter). Bacteria were isolated from necrotic gall tissue without surface sterilization, and on nutrient agar (NA) using a dilution technique (11).

Pathogenicity of the organisms was determined by inoculating celery seedlings growing in autoclaved soil with 2 ml of a suspension of the test organism prepared by comminuting a 2-week-old PDA slant culture, or a CMA culture for *Pythium* sp. in 50 ml of sterile distilled water. The bacterial inoculum was prepared by suspending the cells from a 24-hour NA slant culture in 15 ml of sterile distilled water. The actual propagule concentration was not determined for either the fungal or bacterial inocula. In all cases, the inoculum was distributed to three depressions in the soil spaced 3 cm equidistant around the base of the seedling. All organisms were tested on seedlings that had been inoculated 1 week earlier with 10,000 *M. hapla* eggs and on noninoculated control seedlings. After a 4-week incubation period, the plants were harvested and the roots were examined for necrosis.

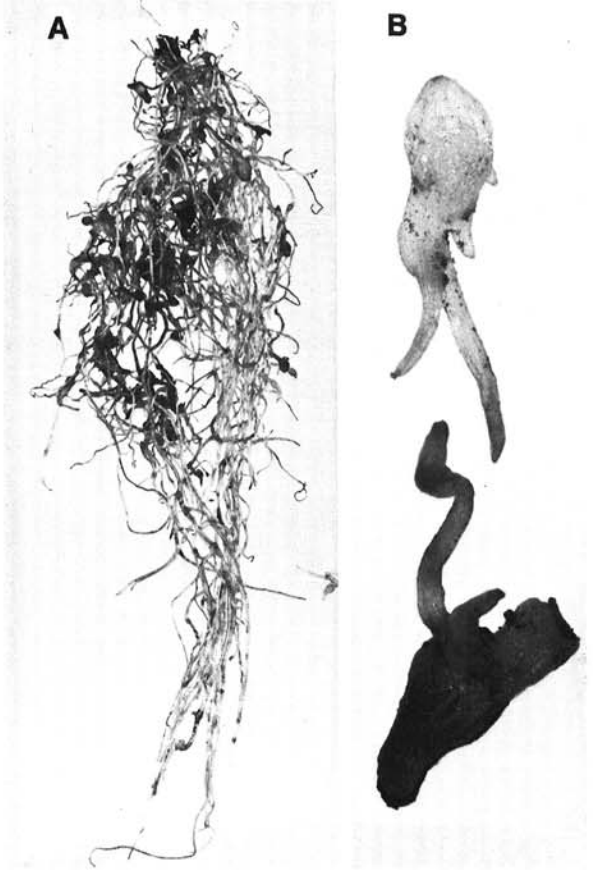

Fig. 1-(A, B). Typical root necrosis of *Meloidogyne hapla*-infected celery from nonautoclaved soil in growth chamber tests. **A)** A necrotic root system 4 weeks after inoculation with 10,000 *M. hapla* eggs. **B)** Two *M. hapla* galls; the lower one has typical necrosis.

RESULTS

Influence of Meloidogyne hapla on root necrosis.—Celery seedlings grown in nonautoclaved field soil and inoculated with 10,000 *M. hapla* eggs developed severe root galling and root necrosis (Fig. 1) which resembled that observed on heavily galled roots in the field. The necrosis, which initially was associated with the root-knot galls, could be detected 2 weeks after the seedlings were inoculated with *M. hapla;* root necrosis was not detected on noninoculated plants until 4 weeks after transplanting in the nonautoclaved soil (Table 1). At all time periods there was more necrosis on cultivars infected with *M. hapla* than on those not inoculated with the nematode.

The amount of root necrosis that developed on both cultivars during the 4-week incubation period in nonautoclaved soil depended on the initial concentration of *M. hapla*. There was no significant increase in root necrosis and only a slight decrease in shoot dry weight over that of the noninoculated plants with 500 or 1,000 nematode eggs per seedling, but with 5,000 or 10,000 eggs per seedling, root necrosis increased and shoot dry weight decreased significantly (Table 2).

The effect of *M. hapla* on celery in nonautoclaved soil then was compared to the effect in autoclaved soil. Plants in autoclaved soil that had been inoculated with 10,000 *M. hapla* eggs had little or no root necrosis 4 weeks after inoculation and no reduction in shoot dry weight, although the roots were heavily galled (Table 3). In nonautoclaved soil inoculated with *M. hapla,* roots were heavily galled and had significant necrosis and a decreased shoot dry weight. Noninoculated plants in nonautoclaved soil were free of root-knot galls, but had more root necrosis and a lower shoot dry weight than noninoculated plants in autoclaved soil (Table 3).

TABLE 1. Development of root necrosis on two celery cultivars grown in nonautoclaved field soil and inoculated with *Meloidogyne hapla*

Time after inoculation (weeks)	Root necrosis index[a] of celery cultivars:			
	Utah 52-70		Golden Detroit	
	Check	Inoc.[b]	Check	Inoc.[b]
2	0.0	1.3*	0.3	1.0*
3	0.0	1.8*	0.0	1.3*
4	0.6	2.0*	1.3	2.6*
5	1.3	2.3*	1.6	2.8*

[a]Root necrosis index: 0 = no necrosis, 1 = 0-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100%.

[b]Values are means of six seedlings inoculated with 10,000 *M. hapla* eggs; means followed by an asterisk (*) are significantly different according to Duncan's multiple range test ($P = 0.05$) from noninoculated checks observed at the same time.

Identity and pathogenicity of organisms isolated from necrotic root galls.—Six different genera of fungi were isolated from necrotic tissue of 52 plants inoculated with *M. hapla* and grown in nonautoclaved soil in the growth chamber; *Fusarium* spp. and *Pythium* spp. were isolated most frequently. Numerous different bacterial colony types also were recovered. All fungal isolates and a random selection of 54 bacterial isolates, representing several different colony types, were tested for pathogenicity by inoculating each isolate individually onto *M. hapla*-infected and noninfected plants. Of these, only a single *Pythium* sp., *P. polymorphon* Sideris, 1932, was pathogenic to celery infected or noninfected with *M. hapla*. On plants inoculated 1 week earlier with *M. hapla*, *P. polymorphon* caused greater root necrosis, and decreased shoot dry weight more than on plants not inoculated with the nematode (Table 4). The severity of root necrosis 4 weeks after inoculation with *P. polymorphon* depended on the initial *Pythium* inoculum concentration. Furthermore, in growth chamber tests, *P. polymorphon* was recovered with greater frequency from *M. hapla*-infected roots in nonautoclaved soil than from roots not inoculated with the nematode. *Pythium polymorphon* was recovered from 24 of 49 root-knot galls, but from only 8 of 48 samples (1- to 3-cm long root sections) from roots not inoculated with the nematode.

Effect of Pratylenchus penetrans and Paratylenchus projectus on celery in nonautoclaved and autoclaved soil.—Seedlings were transplanted to 10-cm diameter pots of nonautoclaved or autoclaved soil and inoculated with a suspension of *P. penetrans* or *P. projectus*. After 4 weeks, celery growth in nonautoclaved soil inoculated with 11,000 *P. penetrans* was reduced more than in autoclaved soil (Table 5) but there was no significant increase in root necrosis on plants inoculated with the nematode. *Pythium polymorphon* was not recovered from root samples (1- to 3-cm long) from autoclaved soil; neither was it recovered from roots inoculated with *P. penetrans* in nonautoclaved soil. *Pythium polymorphon* was recovered from seven of 48 root samples from plants not inoculated with the nematode in nonautoclaved soil. There were no consistent differences between soil population levels of *P. penetrans* in nonautoclaved or autoclaved soil, but numbers were 10-fold greater in roots of plants in nonautoclaved soil than in roots from autoclaved soil (Table 5).

Inoculation with 10,000 or 18,000 *P. projectus* per pot caused no reduction of growth in either soil at 4 weeks, even though preliminary tests had shown that *P. projectus* populations increased from 100 nematodes/100 ml soil to 8,900 nematodes/100 ml soil during an 84-day incubation period.

DISCUSSION

Results from this study and those reported by Mayol and Bergeson (10) clearly show that root-knot nematodes caused more damage in the presence of a natural soil microflora than in autoclaved soil. Since both *M. hapla* and *M. incognita* caused increased root necrosis in

TABLE 2. Effect of *Meloidogyne hapla* inoculum concentration on severity of root necrosis and shoot dry weight of two celery cultivars in nonautoclaved field soil after a 4-week incubation period

Inoculum concentration (eggs/seedling)	Utah 52-70[a,b]		Golden Detroit[a,b]	
	Root necrosis index	Shoot dry wt (g)	Root necrosis index	Shoot dry wt (g)
0	0.8 xy	0.317 x	0.6 w	0.304 w
500	0.6 x	0.307 x	0.7 w	0.312 w
1,000	0.9 xy	0.272 xy	0.9 x	0.251 x
5,000	1.3 y	0.247 yz	2.6 y	0.159 y
10,000	2.0 z	0.212 z	3.3 z	0.110 z

[a]Each value is the mean of ten plants. Root necrosis index: 0 = no necrosis, 1 = 0-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100%.
[b]Means followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

TABLE 3. Effect of *Meloidogyne hapla* on root necrosis and growth of two celery cultivars in autoclaved and nonautoclaved field soil

Treatment	Utah 52-70[a,b]		Golden Detroit[a,b]	
	Root necrosis index	Shoot dry wt (g)	Root necrosis index	Shoot dry wt (g)
Autoclaved soil:				
Check	0.3 x	0.265 x	0.0 w	0.593 x
Inoculated[c]	0.6 x	0.302 x	0.3 x	0.508 x
Nonautoclaved soil:				
Check	1.0 y	0.160 y	0.9 y	0.355 y
Inoculated[c]	3.0 z	0.081 z	2.9 z	0.164 z

[a]Each value is the mean of ten plants. Root necrosis index: 0 = no necrosis, 1 = 0-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100%.
[b]Means followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.
[c]Each seedling inoculated with a suspension of 10,000 *M. hapla* eggs.

combination with natural soil microflora, it is probable that other *Meloidogyne* spp. do likewise. Powell et al. (14) suggested that this increased damage resulted from increased susceptibility of *Meloidogyne*-infected roots to organisms not normally pathogenic in the absence of the nematode. We observed that the root-knot galls became necrotic first, indicating a greater susceptibility of galled tissues than of other portions of the roots. Golden and Van Gundy (7) found that the root-knot galls were the primary infection court for *Rhizoctonia solani* on okra and tomato infected with *M. incognita*.

Of the numerous organisms isolated from necrotic root-knot galls, *P. polymorphon* appears to be the most important cause of root necrosis. Even though none of the other organisms, when tested singly, caused any necrosis in the presence or absence of *M. hapla,* this does not necessarily imply that they were not involved in the disease complex. If all of these organisms had been tested together rather than singly, they might have interacted and caused necrosis of galled roots. We do not know of previous reports of *P. polymorphon* being pathogenic to celery.

Inoculation with *P. penetrans* did not increase root necrosis in nonautoclaved soil as did *M. hapla*. We detected no increase in susceptibility of celery roots to *P. polymorphon* when measured in terms of increased frequency of its isolation from roots infected by *P. penetrans*. The greater reduction in growth in nonautoclaved soil appears to be a function of the increased number of nematodes parasitizing the roots. Edmunds and Mai (5) showed that more *P. penetrans* penetrated alfalfa roots in the presence of *Trichoderma viride* or *Fusarium oxysporum* than in the absence of these fungi. El-Sherif and Mai (6) reported that *P. penetrans* is attracted by temperature gradients as small as .033 C/cm in agar; Klinger (9) found that *P. penetrans* also is attracted by CO_2. Thus, the increased attraction of *P. penetrans* to host roots in the presence of microorganisms, possibly due to the creation of temperature and/or CO_2 gradients by the activities of rhizosphere microorganisms, may explain why more nematodes were recovered from roots in nonautoclaved soil than from roots in autoclaved soil.

Paratylenchus projectus, at the inoculum levels tested, was not pathogenic to celery, but did reproduce on celery as previously reported (3). The failure to detect any effect of the soil microflora on the interaction of celery and *P. projectus* may be due to the apparent nonpathogenic nature of the relationship.

TABLE 4. Effect of *Pythium polymorphon* inoculum concentration on root necrosis and growth of the celery cultivar Golden Detroit in the presence or absence of *Meloidogyne hapla*

Inoculum level[a]	*M. hapla*[b]	Root necrosis index[c,d]	Shoot dry wt (g)[c]
0	No	0.4 w	0.40 x
0	Yes	0.6 w	0.36 xy
6,500	No	1.0 wx	0.32 xy
6,500	Yes	2.1 xy	0.26 y
65,000	No	2.5 yz	0.27 y
65,000	Yes	3.0 yz	0.16 z
850,000	No	2.1 xy	0.26 y
850,000	Yes	3.6 z	0.11 z

[a]Number of *P. polymorphon* propagules per 10 cm pot; a mixed suspension of sporangia and oospores (2:1).

[b]Each inoculated seedling received 10,000 *M. hapla* eggs 1 week prior to inoculation with *P. polymorphon*.

[c]Each value is the mean of ten plants; means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[d]Root necrosis index: 0 = no necrosis, 1 = 0-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100%.

TABLE 5. Effect of *Pratylenchus penetrans* on the growth of celery cultivar Golden Detroit in autoclaved and nonautoclaved field soil

Treatments	Shoot dry wt (g)[a]	Root fresh wt (g)[a]	Nematodes/g root[b]
Nonautoclaved soil:			
Check	0.18 x	0.93 x	. . .
Inoculated[c]	0.10 y	0.37 y	1,626 x
Autoclaved soil:			
Check	0.25 z	1.34 z	. . .
Inoculated[c]	0.20 x	1.10 x	158 y

[a]Each value is the mean of ten plants; means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[b]Significantly different ($P = 0.01$).

[c]Each seedling was inoculated with 11,000 nematodes.

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