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Inocula and Media Affect Root-knot Nematode Infection of Peach Seedling Roots

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Abstract

Six initial population densities (Pi) (0, 2000, 4000, 6000, 8000 and 10,000 eggs per 1200 cm^3 soil) of two root-knot nematode species [*Meloidogyne incognita* (Kofoid & White) (Mi) and *M. javanica* (Treub) (Mj)] and four potting media (sand, sand/vermiculite, vermiculite, and Fafard) were used to evaluate nematode parasitism of ramets of seedlings of 'Lovell' peach [*Prunus persica* (L.) Batsch] rootstock under greenhouse conditions. There were no significant differences in plant height, shoot dry weight, and root dry weight among different Pi treatments and different medium treatments for either nematode species, except that the ramets grown in Fafard medium had greater ($P \leq 0.05$) plant height, shoot dry weight, and root dry weight. Our results indicated that a Pi of 4000 Mi or Mj eggs per 1200 cm^3 soil was needed to produce reliable nematode infections on peach roots for the evaluation of host susceptibility, and sand/vermiculite was a suitable medium for root-knot nematode infection and reproduction on susceptible peach roots under greenhouse conditions.

Root-knot nematodes (*Meloidogyne* spp.) cause significant economic damage to peaches in many countries [21, 27]. The continued evaluation of peach germplasm for resistance to root-knot nematodes is necessary for development and utilization of resistant rootstocks throughout the world. However, the long generation time and large plant size of peach trees limits the efficiency of resistance evaluation under conventional field trial conditions [14]. *In vitro* testing methods have been developed to increase the evaluation efficiency, but they require tissue culture expertise and facilities. In addition, somaclonal variations of nematode resistance exist among plantlets under *in vitro* conditions [7, 11]. Therefore, evaluation with juvenile seedlings of peach (or other *Prunus* species) under controlled greenhouse conditions would be a more practical and reliable approach for determining the resistance to root-knot nematodes [3, 19, 20, 24].

The expression of plant resistance to root-knot nematodes can be modified by environmental conditions and developmental stages in many crops [26, 29]. The initial population density (Pi) of *Meloidogyne* spp. [6, 30]; soil texture, pH value and nutritional status [9, 32]; soil temperature [8, 31]; and type, age and endogenous hormone levels of plant tissue [2, 9, 13, 16] are major factors that can affect nematode infection and plant growth. Thus, experimental conditions must first be standardized in order to consistently assess plant resistance to root-knot nematodes. From a practical standpoint, more rigorous evaluation would allow the selection of peach rootstocks that maintain nematode resistance under environmental stress [2].

Pi and soil medium are two major factors that influence the evaluation of nematode resistance in peach rootstocks. The Pi of root-knot nematodes greatly affects the rate and degree of infection in a host plant, whereas the optimum inocula for nematode infection vary between plant species [1, 10]. Soil type, texture, and pore size also influence nematode migration, penetration, and reproduction, in addition to plant growth and development [5, 30, 32]. The major objectives of this study were to compare the effects of different Pi levels of *Meloidogyne* spp. and different potting media on nematode infections and peach vegetative growth, and to determine

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the most efficient and reliable Pi level and optimum medium for evaluating resistance in peach rootstocks under greenhouse conditions.

Materials and Methods

Selfed seeds from 'Lovell,' a commercial peach rootstock that originated as a chance seedling from California in 1882 and is homozygous susceptible to both *M. incognita* (Mi) and *M. javanica* (Mj) [17], were collected from the USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory (Byron, GA) in Summer 1995 and stratified at 4°C for two months. The germinated seeds were planted in 12-cm-diameter plastic pots filled with approximately 1200 cm^3 sand/vermiculite media (50:50 v/v), and seedlings were grown in the greenhouse. Twenty-four uniform seedlings were selected for vegetative propagation. Vegetative propagation by herbaceous stem cuttings in vermiculite [23] was used to produce either 4 or 6 ramets of each seedling for treatment replications. Since susceptibility to these two root-knot nematodes are due to homozygous recessive genes and Lovell is a susceptible genotype, all selfed 'Lovell' seedlings should be root-knot susceptible and were considered the "same" susceptible genotype in this root-knot infection study.

Mi was originally isolated from a peach orchard in Georgia, and Mj from tobacco plants in North Carolina. These two isolates were maintained as pure greenhouse cultures on roots of 'Rutgers' tomato at Clemson University. Egg enoculum collection procedures followed the NaOCl method described by Hussey and Barker [15]. Eight Rutgers tomato were inoculated with 6000 Mi or Mj eggs to check their viability during experiments.

To observe Pi effects, 72 ramets from 12 'Lovell' seedlings were transplanted in 12-cm-diameter plastic pots filled with approximately 1200 cm^3 sand/vermiculite media (50:50 v/v). Six Mi or Mj inoculum treatments (0, 2000, 4000, 6000, 8000 and 10,000 eggs per 12cm^3 soil) were applied to the potted ramets one month after transplanting by adding eggs to 3 holes in the soil of each pot. Each Mi inoculum treatment had one ramet from each of six seedlings for a total of 6 different seedling ramets per Pi treatment and 36 ramets total in the experiment. Each Mj inoculum treatment had one ramet from each of 6 other seedlings for a total of 6 different seedling ramets per Pi treatment and 36 ramets total in the experiment.

For medium effects on peach and *Meloidogyne* spp., 48 ramets (4 per seedling) from 12 other 'Lovell' seedlings not used for the Pi experiments were individually transplanted into 12-cm-diameter plastic pots filled with one of four types of media: sand, sand/vermiculite (50:50 v/v), vermiculite, or Fafard potting mix No. 2P (Conrad Fafard. Agawam, MA). Sand was sterilized before use, and vermiculite and Fafard media were purchased from a commercial garden center. Each potted ramet received 6000 Mi or Mj eggs one month after transplanting as described above. Each of the four media treatments with Mi eggs consisted of six pots that included one ramet or pot each from six seedlings. The same media treatments with Mj eggs consisted of six pots that included one ramet or pot each from six seedlings different than those used with the Mi treatments.

The experimental design was completely randomized, and individual treatments were replicated 6 times by using one ramet each from six seedlings. Potted ramets were kept in the greenhouse (20-30°C) for approximately one month (May, 1996) after inoculation, then transferred to a shade-house (30% sunlight, 16-38°C) for three months (June to August, 1996). Osmocote fertilizer (N-P-K = 14-14-14) was supplied for mineral nutrition, and pots were watered when needed. No systemic insecticides were applied to the peach ramets during the experiments. The number of galls and egg masses per root system [12], and plant height, dry root and shoot weight (dried at 50-60°C in paper bags a few weeks until no more loss in weight) per ramet were recorded 120 days after inoculation. Data were subjected to analysis of variance with the GLM procedure of SAS (SAS Institute, Cary, NC).

Results

1. Pi effects on peach and *Meloidogyne* spp.

No significant differences were detected among the different *M. incognita* Pi treatments for plant eight (ranging from 28.4 to 35.6 cm), shoot dry weight (ranging from 2.9 to 4.2 g), and root dry weight (ranging from 3.9 to 5.0 g) (Table 1). However, a greater ($P \leq 0.05$) number of root galls and egg masses were produced at a Pi $\geq$ 4000 eggs per 1200 cm^3 soil than at 2000 eggs per 1200 cm^3 soil and the non-inoculated control. No significant differences were detected between the 2000 eggs per 1200 cm^3 soil treatment and the non-inoculated control.

Differences in plant height (ranging from 28.3 to 35.7 cm) and shoot dry weight (ranging 3.3 to 4.9 g) were not detected among any of the *M. javanica* Pi treatments (Table 1). However, root dry weight was less ($P \leq 0.05$) for seedling ramets initially inoculated with 2000 eggs per 1200 cm^3 soil (3.4 g) than for the 6000 eggs per 1200 cm^3 soil (5.7 g) treatment. There were no significant differences on root gall and egg mass numbers between 0 (control) and 2000 eggs per 1200 cm^3 soil treatments, or among the 4000, 6000, 8000 and 10,000 eggs per 1200 cm^3 soil treatments. However, a greater ($P \leq 0.05$) number of galls were recorded at Pi $\geq$ 4000 eggs per 1200 cm^3 soil as compared to Pi $\leq$ 2000 eggs per 1200 cm^3 soil. Furthermore, a greater ($P \leq 0.05$) number of egg masses were produced on roots of seedling ramets inoculated with 10,000 eggs per 1200 cm^3 soil than on the ramets inoculated with 2000 eggs per 1200 cm^3 soil.

2. Medium effects on peach and *Meloidogyne* spp.

Inoculated with *M. incognita,* the seedling ramets grown in Fafard medium had greater ($P \leq 0.05$) plant height (44.7 cm), and shoot dry weight (5.7 g) than those in the other media (Table 2). Root dry weight (4.8 g) in Fafard was also greater ($P \leq 0.08$) than those in sand and vermiculite, but not in the sand/vermiculite (3.4 g) media. As for numbers of root galls and egg masses, there were no significant differences ($P \leq 0.05$) among the sand, sand/vermiculite, and vermiculite media. However, a greater ($P \leq 0.05$) number of root galls were produced in vermiculite (41.0 per ramet) than in Fafard (17.3 per ramet). Furthermore, fewer ($P \leq 0.05$) number of egg masses were found on roots of ramets grown in Fafard (7.3 per ramet) than in sand (21.7 per ramet).

Inoculated with *M. javanica,* the seedling ramets grown in Fafard medium had greater ($P \leq 0.05$) plant height (61.9 cm), shoot dry weight (8.3 g) and root dry weight (7.7 g) than those grown in the other media (Table 2). Based on Duncan's multiple range test ($P \leq 0.05$), root gall and egg mass production by *M. javanica* did not differ among any of the media tested. However, based on T tests, there were significant differences ($P \leq 0.05$) in gall number between Fafard (21.5) and sand (36.5), and in egg mass number between Fafard (12.0) and vermiculite (23.3) (Table 2).

Discussion

Root-knot nematodes can reduce peach tree growth, vigor, yield, and the ability to withstand environmental stress under field conditions of not controlled [22]. However, this study showed no statistical differences in plant heights, shoot and root dry weights between control seedling ramets and infected seedling ramets after 120 days. Similar results were also observed in a F_2 population of peach rootstocks [18] and Myrobalan plum [4]. It appeared that there may be different interactions of peach roots with root-knot nematodes between greenhouse tests and field trials. Under greenhouse conditions, nematodes feeding in the roots did not reduce the vegetative growth of young peach seedlings in small pots after 4 months, so it may be possible that peach seedlings compensate for the root-knot nematode parasitism because of favorable environmental conditions. Further research is warranted to determine if optimum environment, fertilizer, and water explain this absence of growth reduction in peach seedlings parasitized by root-knot nematodes growing under greenhouse conditions.

Table 1. Effects of egg inocula density of *Meloidogyne* spp. on vegetative growth and nematode infection of ramets of 'Lovell' peach seedlings after 120 days.[z]

Nematode species	Inoculum (eggs/plant)	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Gall number	Egg mass number
M. incognita	0 (control)	29.0	2.9	4.0 a	0.0 a	0.0 a
	2000	33.3	4.2	4.0 a	11.2 a	4.2 a
	4000	28.4	3.4	4.2 a	27.5 b	15.1 b
	6000	35.6	4.1	4.2 a	27.7 b	17.5 b
	8000	33.0	3.5	3.9 a	36.8 b	17.8 b
	10,000	28.6	3.7	5.0 a	34.2 b	18.3 b
M. javanica	0 (control)	28.3	3.9	4.2 ab	0.0 a	0.0 a
	2000	29.6	3.3	3.4 b	11.5 a	5.2 ab
	4000	33.4	4.9	5.0 ab	30.0 b	13.3 bc
	6000	33.8	4.6	5.7 a	33.0 b	13.2 bc
	8000	35.5	4.3	4.0 ab	27.2 b	12.5 bc
	10,000	35.7	4.2	5.1 ab	28.8 b	17.0 c

[z]Data were means of 6 replications with mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Compared to using the second stage juveniles (J2) as an initial inocula, the egg mass inoculation is a simple and fast method for the evaluation of resistance to root-knot nematodes in plants [15]. In this study, seedling ramets inoculated with 2000 Mi or Mj eggs per 1200 cm^3 soil produced root galls and egg masses, but were not statistically different from the control. Peach seedling ramets inoculated with 4000 eggs per 1200 cm^3 soil can produce reliable nematode infection. In addition, it appeared that there was not much difference in nematode infection among all treatments with Pi $\geq$ 4000 eggs per 1200 cm^3 soil. Since the infective J2 stage is known to penetrate only directly behind the root [28], our results may be in part due to: (1) not all 2000 eggs hatching, thus producing insufficient number of J2s to penetrate all available root tip sites of each peach seedling ramet; (2) all available root tip sites may have been penetrated by J2s at the inoculum level of 4000 eggs per 1200 cm^3 soil; and (3) the penetration of root tips may be saturated at Pi $\geq$ 4000 eggs per 1200 cm^3 soil. We also found that the nematode penetration and infection did not increase even when the Pi were increased to 16,000, 20,000 or 24,000 Mi or Mj eggs per 1200 cm^3 soil (data not shown). Therefore, at least 4000 eggs per 1200 cm^3 soil should be applied to produce sufficient root-knot nematode infec-

Table 2. Effects of different media on vegetative growth and *Meloidogyne* infection of ramets of 'Lovell' peach seedlings after 120 days.[z]

Nematode species	Medium treatment	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Gall number[y]	Egg mass number[x]
M. incognita	Sand	27.2 a	2.5 a	1.9 a	32.7 ab	21.7 a
	S./V.	29.8 a	3.7 a	3.4 ab	31.0 ab	19.5 ab
	Vermiculite	35.0 a	3.6 a	2.2 a	41.0 a	20.2 ab
	Fafard	44.7 b	5.7 b	4.8 b	17.3 b	7.3 b
M. javanica	Sand	33.3 a	2.9 a	1.9 a	36.5	22.7
	S./V.	33.3 a	3.0 a	2.3 a	33.5	22.5
	Vermiculite	32.3 a	3.3 a	1.9 a	30.7	23.2
	Fafard	61.9 b	8.3 b	7.7 b	21.5	12.0

[z]Data were means of 6 replications with mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.
[y]Based on T tests ($P \leq 0.05$), there was a significant difference between sand and Fafard media in Mj treatment.
[x]Based on T tests ($P \leq 0.05$), there was a significant difference between vermiculite and Fafard media in Mj treatment.

tion on susceptible peach roots under greenhouse conditions.

Root-knot nematodes prefer sandy or loamy sand soil for their development since fine particles of clay and silt appear to be obstacles to their migration [25,32]. Esmenjaud et al. [5] reported that *M. arenaria* J2s directly added to perlite medium did not induce root galling in young cuttings of Myrobalan plum (*Prunus cerasifera*), whereas galls were observed in control cuttings established in sand medium. In this study, the seedling ramets grown in Fafard had more vegetative growth than those grown in the other media, thus Fafard proved to be a suitable media for vegetative growth of peach seedling ramets. In comparisons, sand, sand/vermiculite and vermiculite were better media for root-knot nematode survival, infection, and reproduction than Fafard. However, nutrients were difficult to maintain in sand medium, and the seedling ramets in vermiculite media tended to lean in pots. Therefore, of these three media sand/vermiculite (50:50 v/v) was a better medium for evaluating root-knot nematode infection on peach roots under greenhouse conditions, and the use of this medium and 4000 eggs as Pi allows separation of susceptible from resistant genotypes after 4 months in the greenhouse.

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Soil Management in Young Orchards

Grass, white clover, straw, sawdust and composted bark reduced tree growth and flower bud setting of apple the year after planting. Later trees mulched with pine bark, sawdust, polyethylene foil and *Latrasil spoonusd* showed the best growth and fruiting. Straw mulch increased rodent damage. Growing cover plants close to the tree trunk was rejected because of competition for water and poor tree growth. There was little effect of mulching on fruit quality and mineral composition of leaves and soil. From: Mika et al. 1998. J. Fruit and Orn. Plant Res. VI(1):1-13.

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