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Optimal Levels of *Meloidogyne incognita* Inoculum for Infection of Tomato and Peach in Vitro[1]

GHAZALA HASHMI,[2,3,4] ROBIN N. HUETTEL,[2] FREDDI A. HAMMERSCHLAG,[3] AND LORIN R. KRUSBERG[4]

Abstract: Penetration of second-stage juveniles (J2) of *Meloidogyne incognita* into tomato root explants and in vitro propagated peach plantlet roots were compared. Five inoculum levels were used: 25, 50, 75, 100, and 200 J2 for tomato; and 50, 100, 200, 500, and 1,000 J2 for peach. The greatest root penetration into tomato was 30% at the 75 J2 level, but the maximum penetration into peach roots was only 8% at the 200 J2 level. The difference ($P = 0.05$) in penetration of *M. incognita* at all inoculum levels into these two hosts indicates that penetration versus inoculum density for in vitro studies need to be determined for different plant species.

Key words: inoculum level, in vitro culture, *Lycopersicon esculentum*, nematode, *Prunus persica*, root-knot nematode.

Tissue culture technologies have been used to propagate peach (*Prunus persica* (L.) Batsch) scion cultivars (4,5), and to regenerate resistant plants by selecting at the cellular and screening at the whole plant levels for resistance to *Xanthomonas campestris* pv. *pruni* (causal agent of bacterial spot) (7–9) and to *Pseudomonas syringae* pv. *syringae* (causal agent of bacterial canker) (9). Although peach cultivars have been evaluated in vitro for resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood (13), optimum inoculum levels have not been determined. In addition, peach regenerants have yet to be evaluated for resistance to this nematode.

The initial inoculum level of a pathogen affects the rate and (or) degree of infection in a host plant. This is important to consider when developing genetically modified plants with tolerance or resistance to *M. incognita* under in vitro conditions. Determining an optimum nematode inoculum level would facilitate efficient and accurate assessment of the levels of infection during disease evaluation. Quantitative data on optimum inoculum levels of *M. incognita* second-stage juveniles (J2) for different crop plants under in vitro culture conditions are sparse. Much of the research on the penetration and development of *Meloidogyne* species has concerned the comparison of resistant and susceptible cultivars of the same crop under greenhouse conditions (10,11,19,21). However, an in vitro screening procedure was recently developed to test tissue culture-propagated peach cultivars for their resistance to plant-parasitic nematodes (13). In vitro studies of *M. incognita* penetration and development in cucumber root explants (15), and tolerance in soybean to *Heterodera glycines* Ichinohe (14) and in grapes to *Pratylenchus vulnus* Allen & Jensen (17) have also been conducted.

The main objectives of this study were i) to compare penetration of *M. incognita* J2 into tomato root explants and roots of peach regenerants and ii) to investigate optimum J2 inoculum levels for in vitro-propagated peach regenerants.

Materials and Methods

Tomato culture: Seeds of tomato, *Lycopersicon esculentum* Mill cv. Rutgers, were immersed in 95% ethyl alcohol for 3 minutes, transferred into 0.5% sodium hypochlorite for 10 minutes, and germinated on 1.0% water agar at 28 C (12). After germination, three 2–3-cm-long root tips were excised and transferred to each 15 × 100-mm-d petri dish containing Gamborg's B5 medium (2) supplemented with 1.5% Noble

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[2] Nematology Laboratory, USDA ARS, Beltsville, MD 20705-2350.

[3] Plant Molecular Biology Laboratory, USDA ARS, Beltsville, MD 20705-2350; author to whom correspondence should be addressed.

[4] Department of Botany, University of Maryland, College Park, MD 20742-5815.

agar and incubated for 72 hours in a growth chamber at 28 C.

Peach culture: Peach regenerant 156-12, derived from callus of cv. 'Sunhigh' embryo #156 (8), was propagated and rooted in vitro as described previously (6). After rooting, single plantlets, each with 3–4 roots, were transferred to quarter strength Murashige and Skoog salts medium (16) without growth regulators and supplemented with 2% sucrose and 0.6% Phytagar (Gibco, Grand Island, NY).

Nematode inoculation: Meloidogyne incognita race 1 was obtained from stock cultures maintained on sterile root explants of tomato cv. Rutgers. Egg masses were hand picked and the eggs hatched at 28 C in 12-well tissue culture plates containing 2 ml of sterile distilled water per well. Second-stage juveniles (J2) were collected aseptically and counted after 48 hours. Five petri dishes of tomato root explants were inoculated around the root tips with 0.1 ml of sterile aqueous suspension containing 25, 50, 75, 100, or 200 J2. Petri dishes were sealed with Parafilm and incubated in a controlled environmental growth chamber in the dark at 28 C. Five peach plantlets with roots about 2 cm long were inoculated around plant bases, on the agar surface in the same manner with 50, 100, 200, 500, or 1,000 J2. Plants were incubated at 25 C under a 16-hour photoperiod of about 100 $\mu E \cdot s^{-1} \cdot m^{-2} \cdot$ irradiance provided by cool white fluorescent lamps. The experiments were set up according to a randomized block design. Each experiment was conducted three times.

After 2 weeks of incubation, tomato and peach roots were removed and stained with 0.05% cotton blue in a solution of distilled water, lactic acid, and glycerol (1:1:1) (v:v:v). Roots were gently crushed and the numbers of developing nematodes were recorded. The number of J2 that penetrated the roots out of the total J2 inoculated was used to calculate percentage penetration.

Data analysis: Data on nematode counts from all three experiments were combined and analyzed by factorial ANOVA. Treatment means were separated by least significant difference (LSD). Only significant ($P = 0.05$) data will be discussed unless stated otherwise.

RESULTS

Nematode penetration into tomato root explants: Total number of *M. incognita* infecting tomato roots were generally lowest ($P = 0.05$) at the initial inoculum level of 25 J2, and reached a maximum number at 75 J2, although tomato roots were invaded at all inoculum levels (Fig. 1). The numbers

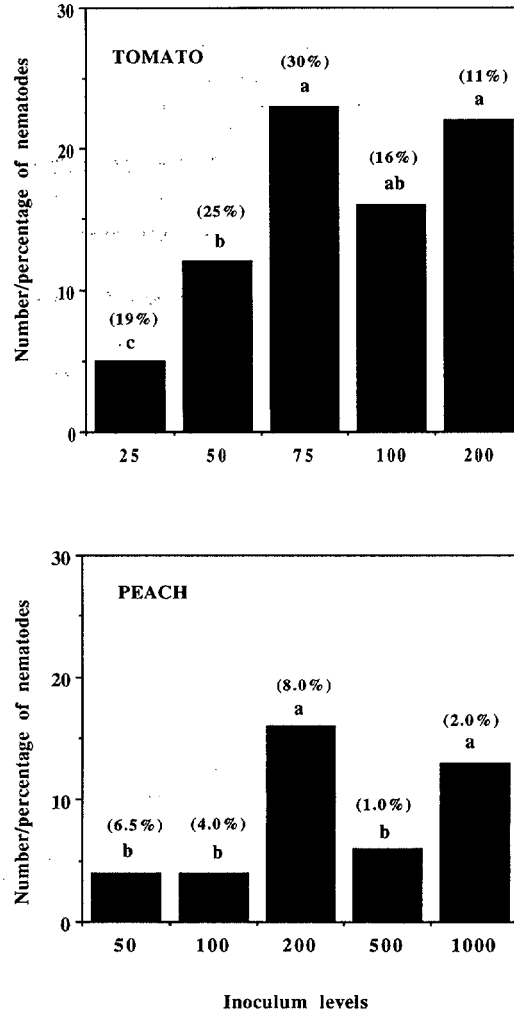

FIG. 1. Numbers and percentages of *Meloidogyne incognita* J2 penetrating tomato explant roots and peach regenerant roots at five nematode inoculum levels after 14 days. Bars labeled with the same letter do not differ significantly ($P = 0.05$).

of nematodes penetrating tomato roots using inoculum levels of 75, 100, and 200 J2 were not significantly different. There was a linear relationship between inoculum level and percentage J2 penetration in tomato roots up to the 75 J2 level (Fig. 1).

Nematode penetration into peach regenerate roots: The maximum number and percentage of *M. incognita* J2 penetrated at an inoculum level of 200 J2, although invasion occurred at all inoculum levels (Fig. 1).

Discussion

Maximum numbers of *M. incognita* juveniles penetrated tomato explant roots at an inoculum level of 75 J2, whereas 200 J2 provided the greatest invasion of peach regenerant roots. When studying penetration of *M. incognita* J2 into excised cucumber roots using four different inoculum levels, McClure and Viglierchio (15) also observed that the numbers of nematodes present in a gall increased with increasing levels of inoculum; however, they did not establish maximum inoculum levels. We found that nematode inoculum levels greater than 75 J2 with tomato root explants or greater than 200 J2 with peach regenerants resulted in decreased penetration rates, which suggests that under in vitro conditions plant roots provide only a limited number of nematode feeding sites, as proposed by Sayre (20). In cucumber, the number of available root tips failed to alter significantly the number of J2 infecting roots (15). Additional observations by Blake (1) suggested that up to a certain point there was a linear relationship between the inoculum level and the number of *Ditylenchus dipsaci* penetrating oat seedlings.

The numbers of *M. incognita* J2 penetrating peach roots was different from the numbers penetrating tomato roots. The maximum penetration of 8% of inoculated nematodes was achieved for peach, whereas maximum tomato penetration was 30%. An important factor to consider in this regard is the possible role of root attractants in J2 penetration. However, in this work, no attempt was made to identify this factor. It has been shown that *Meloidogyne* J2 are attracted to plants in response to stimuli released from roots (3,18). Weiser (22) studied the attractiveness of *M. hapla* to several host plants and found excised roots of bean somewhat repellent, and those of eggplant and soybean varied in their attractiveness to J2. In summary, the significant differences in penetration rates among host plants and inoculum levels leads us to suggest that plants propagated under in vitro culture conditions for quantitative nematode studies should first be screened to determine the optimum numbers of nematodes needed as inoculum.

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