Systemic Acquired Resistance and Susceptibility to Root-Knot Nematodes in Tomato

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ABSTRACT

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Changes in host suitability of tomato (*Lycopersicon esculentum* 'Celebrity') to host-incompatible *Meloidogyne incognita* and host-compatible *M. hapla* were determined after concomitant and sequential inoculations of split-root assays. Initially, infective second-stage juveniles (J2) of *M. hapla* or *M. incognita* were applied to one-half of split-root systems, and 0, 5, 10, 15, or 20 days later, the other half was challenge-inoculated with the same or other species. Each challenge-inoculation had a corresponding control in which the same nematode species was applied to only one-half of a split-root system. Host suitability, based on nematode eggs (*Pf*) per unit of initial inoculum density (*Pi*) of 2,000 J2, was determined 60 days after challenge-infection. Prior inoculation with *M. incognita* significantly suppressed reproduction of challenge *M. hapla* applied 5 days after or later. Reproduction ratios (*Pf*/*Pi*) of challenge *M. hapla* were 20, 13, 6, 5, and 4, whereas corresponding controls were 21, 18, 17, 15, and 12. Concomitant inoculations with both species did not alter host suitability to either species nor did sequential inoculations with *M. incognita* as both prior and challenge species. Prior inoculation with *M. hapla* significantly enhanced reproduction of challenge *M. incognita* about four times relative to controls. These results indicate that prior infection of plants with incompatible or compatible nematode species induced systemic resistance or susceptibility, respectively, to later nematode infections.

Additional keywords: induced resistance, induced susceptibility, predisposition.

Infective juveniles (J2) of root-knot nematodes, *Meloidogyne*, generally locate and penetrate roots of susceptible and resistant plants in equal numbers (8,11,14). Whereas the majority of those that enter susceptible plants establish and multiply, the majority of those that enter resistant plants fail to establish and often egress from the roots 3 to 5 days later. Herman et al. (7) reported that 87% of *M. incognita* (Kofoid & White) Chitwood egressed from resistant soybean (*Glycine max*) within 5 days, compared to 4% from susceptible soybean. The emigration of juveniles was attributed to the accumulation of defensive substances that purportedly inhibited establishment of the nematodes. Veech and McClure (21) observed an association between the expression of incompatibility in cotton (*Gossypium hirsutum*) to *M. incognita* and post-infection increase in phytoalexins, such as methoxy-substituted terpenoid aldehydes. Much lower levels of the compounds were detected in compatible interactions. If host defensive substances that inhibit nematode development accumulate in incompatible plant-nematode interactions, it is probable that prior inoculation of plants with an incompatible nematode species could suppress later infection by a compatible species. Such induced resistance to fungal, bacterial, and viral pathogens after prior inoculation of plants with weakly aggressive strains, avirulent, or incompatible forms of the disease-causing organisms has been reported (2,5,20).

Although there is little information on direct induction of plant resistance to nematodes, several studies on interspecific interactions indicate mutual suppressive or synergistic effects among species (4,8,11,12,15). Ibrahim and Lewis (10) reported that prior inoculation of *M. incognita* on *M. arenaria*-susceptible soybean decreased root galls and egg-mass production by *M. arenaria*. Eisenback (3) also reported that tobacco cv. NC95 resistant to *M. incognita* race 1 lost resistance when *M. arenaria* or *M. hapla* Chitwood was applied 3 weeks earlier. Prior inoculation with *M. javanica* or *M. incognita* race 4 had no effect. These observations suggest that host susceptibility to two or more nematode species could be altered by manipulating the sequence of infection. Our objective in this study was to monitor the direction and magnitude in change of host susceptibility of tomato (*Lycopersicon esculentum* Mill.) to root-knot nematodes after concomitant and sequential inoculation with a compatible and an incompatible species.

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MATERIALS AND METHODS

Assay plants and nematode inocula. Test plants consisted of commercial tomato cv. Celebrity that has the *Mi* gene for resistance to the root-knot nematodes *M. incognita*, *M. javanica*, and *M. arenaria* but not to *M. hapla* (15,18). To exclude competition for infection sites between different nematode species, each root system was split into sides designated A and B. Seedlings were initially grown in steam-sterilized sandy soil in 15-cm-deep 400-cm^3 pots. Thirty days after seeding, plants were removed gently from pots, and each root system was separated into two equal portions that were planted in two adjacent plastic pots. The unsplit upper portion of the root system and adhering soil were enclosed in an inverted pot with its bottom removed (Fig. 1).

Root-knot nematodes *M. hapla* race A and *M. incognita* race 3 were used as the host-compatible and -incompatible test species, respectively. Both species were obtained from greenhouse cultures

at the University of Arizona, Tucson, and were maintained on eggplant (*Solanum melongena*) cv. Black Beauty. Species identity was confirmed by female perineal patterns (6) and genetic analysis of mitochondrial DNA by polymerase chain reaction (16). Nematode-infected roots were macerated in 0.2% sodium hypochlorite for 30 s to extract eggs (1). The eggs were laid on wet filter paper over water in pans for 4 days to hatch into the second-stage juveniles (J2) that were used for inoculation.

Inoculation procedures. Inoculation of plants began 10 days after replanting of the split-root systems. Initially, 2,000 J2 of *M. hapla* or *M. incognita* were applied to one-half of the split-root system as the prior or inducer inoculum. After prior inoculation (0, 5, 10, 15, or 20 days), 2,000 J2 of *M. hapla* or *M. incognita* were applied to the other half of the roots as challenge inoculum. Each challenge-inoculation had a corresponding control in which the same nematode species was applied to only one-half of a split-root system. There were six main treatments: (i) *M. incognita* as prior inoculum and *M. hapla* as challenge inoculum, (ii) *M. incognita* as both prior and challenge inocula, (iii) *M. hapla* as prior inoculum and *M. incognita* as challenge inoculum, (iv) *M. hapla* as both prior and challenge inocula, (v) *M. incognita* control, and (vi) *M. hapla* control. Within each main treatment were five subtreatments consisting of challenge-inoculations 0, 5, 10, 15, or 20 days after the prior inoculation. Each treatment was replicated five times.

The plants were placed on greenhouse tables in a completely randomized design. Host suitability, based on nematode-induced root galls and egg production, was determined 60 days after challenge-infection. Root gall counts were abandoned later because several galls did not have nematodes in them. Nematode eggs were extracted from root tissues by sodium hypochlorite. Egg counts (*Pf*) were divided by initial inoculum density (*Pi*) to obtain a reproduction ratio (*Pf*/*Pi*).

Data analysis. Data was analyzed by SuperAnova computer software (Abacus Concepts, Berkeley, CA). Treatment effects within sets of subtreatments were compared by linear factorial analysis of variance. The relationship between reproduction ratios of challenge nematodes and time elapsed since inoculation of prior nematode species was determined by regression analysis (13). Each main treatment was repeated at least two times.

RESULTS

Induced resistance. Inoculation of half a split-root system of tomato plants with host-incompatible *M. incognita* five or more days before inoculating the other half with compatible *M. hapla* significantly ($P \leq 0.01$) suppressed reproduction of the latter species. Reproduction ratios (*Pf*/*Pi*) of challenge *M. hapla* inoculated 0, 5, 10, 15, or 20 days after *M. incognita* were 20, 13, 6, 5 and 4, respectively, compared to 21, 18, 17, 15, and 12 for *M. hapla* alone (Fig. 2A). The *Pf*/*Pi* of challenge *M. hapla* decreased as the time elapsed between prior and challenge-inoculation was increased from 0 to 10 days but thereafter remained about the same through 15 to 20 days. Regression analysis indicated that the relationship fit a second-degree polynomial decay curve ($R^2 = 0.997$, $P \leq 0.01$) (Fig. 2A). Prior inoculation with *M. incognita* did not significantly change host suitability to itself (challenge *M. incognita*) (Fig. 2B). *M. incognita* as prior inoculum or by itself reproduced poorly, as was expected, and had an average *Pf*/*Pi* = 5 in all the treatments. Apparently, challenge infections did not significantly affect reproduction of nematode species used in initial infections, at least within our test period.

Induced susceptibility. Prior inoculation of tomato plants with host-compatible *M. hapla* significantly ($P \leq 0.01$) enhanced reproduction of previously host-incompatible *M. incognita*. The reproduction ratios of challenge *M. incognita* applied 0, 5, 10, 15, or 20 days after *M. hapla* were 6, 16, 20, 20, and 19, respectively, compared to 5, 5, 4, 4, and 3, for *M. incognita* applied alone. The *Pf*/*Pi* of challenge *M. incognita* increased as the time elapsed between prior and challenge-inoculation was increased from 0 through 5 to 10 days but thereafter remained about the same through 15 to 20 days. Regression analysis indicated that the relationship fit a second-degree polynomial growth curve ($R^2 = 0.978$, $P \leq 0.01$) (Fig. 2B). Prior inoculations with *M. hapla* significantly enhanced reproduction of challenge *M. hapla* (Fig. 2A). Reproduction ratios of *M. hapla* as prior inoculum or by itself averaged 20, which was relatively high, as was expected.

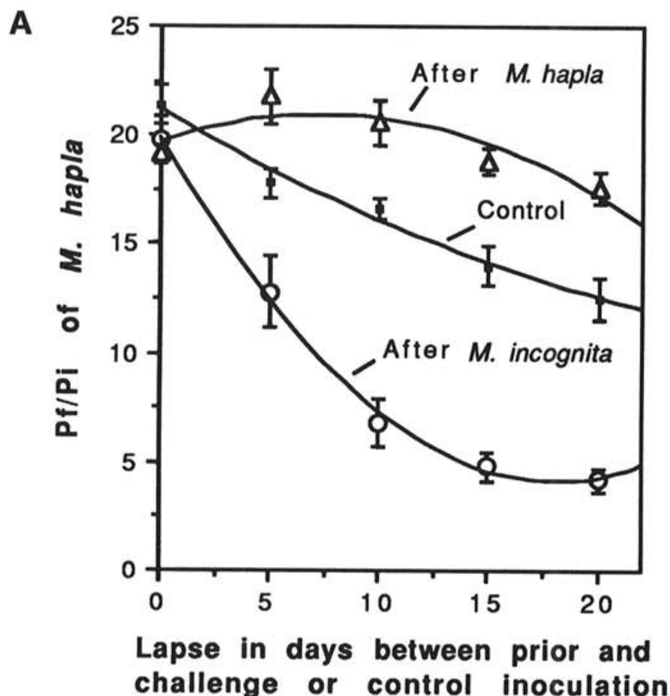
DISCUSSION

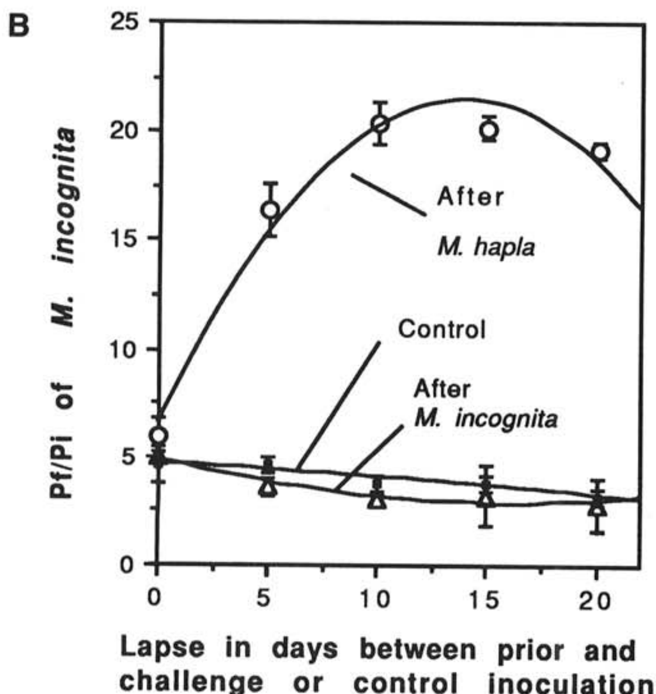
Prior inoculation of tomato cv. Celebrity with incompatible *M. incognita* induced resistance to previously compatible *M. hapla*, whereas prior inoculation with *M. hapla* induced susceptibility to *M. incognita*. Concomitant inoculations with both species did not significantly alter host suitability to either nor did sequential inoculations with *M. incognita* as both prior and challenge species. However, prior inoculation with *M. hapla* significantly enhanced reproduction of challenge *M. hapla*. Other antagonistic mechanisms, such as competition for space and infection sites, were excluded by employing a split-root assay that separated prior and challenge nematode species in different portions of single root systems. The split-root system demonstrated the systemic nature of factors associated with changes of host suitability presumably elicited in one side of a root system and expressed in the other side. Other studies on nematode interactions attribute changes in host suitability to competition for nutrients and plant stress due to multiple infections (4,17,19). These mechanisms do not seem to have played major roles in our study, because reproduction ratios of inducer nematode species were not significantly different from control infections, which did not have corresponding infections on the other half of the root systems.

Fig. 1. Tomato seedlings with split-root systems within and without plastic pots. Prior and challenge-inoculum nematode species were applied to halves designated A and B, respectively. Control plants were inoculated with one nematode species only on the half root portion designated C.

The reproduction ratios of challenge nematode species under induced resistance or susceptibility decreased or increased, respectively, when the time elapsed between prior and challenge-inoculations was increased from 0 through 5 to 10 days, and thereafter was about the same through 15 to 20 days. This observation paralleled that of Zacheo et al. (22) who reported that the amount of defense-related peroxidases in tomato plants inoculated with incompatible *M. incognita* peaked about 10 days after infection. Our finding of empty galls, purportedly induced by nematodes that egressed or died soon after infection, leads us to postulate that the changes of host suitability that we observed resulted from biochemical substances produced by the plants after the initial inoculation. Several reviewers have concluded that postinfection plant incompatibility to nematodes involves induced plant defense compounds as opposed to constitutive plant compounds (8,9,11).

Two or more species of *Meloidogyne* that differ in pathogenic status commonly are found together in the same field, root system, or gall (4,17), and on the basis of our results, such interspecific and -pathogenic communities may come about when a more aggressive nematode attacks a plant first and predisposes it to secondary attack by less aggressive nematodes. From an ecological standpoint, such predisposition facilitates successful infection and, hence, survival of the less aggressive nematode species or populations in an environment. From an etiological standpoint, a plant may benefit from an earlier attack by a less aggressive nematode species that activates its physiological defenses, thereby enhancing its capacity to suppress damage from sequential infections. Therefore, postinfection induction of resistance or susceptibility in plants due to previous nematode attacks may have significant ecological and etiological roles with regard to plant tolerance and nematode population dynamics.





Figs. 2A and B, Reproduction ratios (*Pf*/*Pi*) of *Meloidogyne incognita* or *M. hapla* (i.e., egg counts [*Pf*] were divided by initial inoculum density [*Pi*] to obtain a reproduction ratio [*Pf*/*Pi*]) in one-half of split-root systems of tomato cv. Celebrity applied as challenge- or control-inoculation 0, 5, 10, 15, or 20 days after prior inoculation of the other half of the root system with either nematode species. Control plants were inoculated with one nematode species only. Inoculum density (*Pi*) was 2,000 juvenile nematodes per one-half of a split-root system. Nematode reproduction, in terms of eggs per root system (*Pf*), was determined 60 days after the challenge- or control-inoculation for each treatment. Data are means of five replications. **A,** Prior inoculum was *M. incognita*. **B,** Prior inoculum was *M. hapla*.

LITERATURE CITED

1. Barker, K. R. 1985. Nematode extraction and bioassays. Pages 19-35 in: An Advanced Treatise on *Meloidogyne*. Vol. 2, Methodology. J. N. Sasser and C. C. Carter, eds. North Carolina State University Graphics, Raleigh.
2. Dean, R. A., and Kuć, J. 1985. Induced systemic protection in plants. Trends Biotechnol. 3:125-129.
3. Eisenback, J. D. 1983. Loss of resistance in tobacco cultivar NC95 by infection of *Meloidogyne arenaria* or *M. hapla*. (Abstr.) J. Nematol. 15:478.
4. Eisenback, J. D., and Griffin, G. D. 1987. Interactions with other nematodes. Pages 313-320 in: Vistas on Nematology. J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD.
5. Fulton, R. W. 1986. Practices and precautions in the use of cross-protection for plant virus disease control. Annu. Rev. Phytopathol. 24:67-81.
6. Hartman, K. M., and Sasser, J. N. 1988. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. Pages 69-77 in: An Advanced Treatise on *Meloidogyne*. Vol. 2, Methodology. J. N. Sasser and C. C. Carter, eds. North Carolina State University Graphics, Raleigh.
7. Herman, M., Hussey, R. S., and Boerma, H. R. 1991. Penetration and development of *Meloidogyne incognita* on roots of resistant soybean genotypes. J. Nematol. 23:155-161.
8. Huang, J. 1985. Mechanisms of resistance to root-knot nematodes. Pages 165-177 in: An Advanced Treatise on *Meloidogyne*. Vol. 1, Biology and Control. J. N. Sasser and C. C. Carter, eds. North Carolina State University Graphics, Raleigh.
9. Hussey, R. S. 1985. Host-parasite relationships and associated physiological changes. Pages 143-153 in: An Advanced Treatise on *Meloidogyne*. Vol. 1, Biology and Control. J. N. Sasser and C. C. Carter, eds. North Carolina State University Graphics, Raleigh.
10. Ibrahim, I. K. A., and Lewis, S. A. 1981. Interrelations between *Meloidogyne arenaria* and *M. incognita* on tolerant soybean. J. Nematol. 18:106-111.
11. Kaplan, D. T., and Keen, N. T. 1980. Mechanisms conferring plant incompatibility to nematodes. Rev. Nematol. 3:123-134.
12. Kaplan, D. T., Keen, N. T., and Thomason, I. J. 1980. Association of glyceollin with the incompatible response of soybean roots to *Meloidogyne incognita*. Physiol. Plant Pathol. 16:309-316.
13. Little, T. M., and Hills, F. J. 1978. Agricultural Experimentation: Design and Analysis. John Wiley & Sons, New York.
14. McClure, M. A., Ellis, K. C., and Nigh, E. L. 1974. Resistance of cotton to the root-knot nematode, *Meloidogyne incognita*. J. Nematol. 6:17-20.
15. Ogallo, J. L., and McClure, M. A. 1995. Induced resistance to *Meloidogyne hapla* by other *Meloidogyne* species on tomato and pyrethrum plants. J. Nematol. 27:441-447.
16. Powers, T. O., and Harris, T. S. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. J. Nematol. 25:1-6.
17. Roberts, P. A. 1993. Integration and protection of novel nematode man-

agement strategies. Fund. Appl. Nematol. 17:203-210.
18. Roberts, P. A., and Thomason, I. J. 1989. A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including *Lycopersicon*. Agric. Zool. Rev. 3:225-252.
19. Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Annu. Rev. Phytopathol. 30:245-270.
20. Van Peer, R., Niemann, G. J., and Schippers, S. B. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WC5417r. Phytopathology 81:728-734.
21. Veech, J. A., and McClure, M. A. 1977. Terpenoid aldehydes in cotton roots susceptible and resistant to the root knot nematodes, *Meloidogyne incognita*. J. Nematol. 9:225-229.
22. Zacheo, G., Arrigoni-Lisso, R., Bleve-Zacheo, T., Lamberti, F., and Arrigoni, O. 1983. Mitochondria peroxidase and superoxide dismutase activities during infection by *Meloidogyne incognita* of susceptible and resistant tomato plants. Nematol. Mediterr. 11:107-114.