

Induced Resistance to *Meloidogyne hapla* by other *Meloidogyne* species on Tomato and Pyrethrum Plants¹

J. L. OGALLO AND M. A. McCLURE²

Abstract: Advance inoculation of the tomato cv. Celebrity or the pyrethrum clone 223 with host-incompatible *Meloidogyne incognita* or *M. javanica* elicited induced resistance to host-compatible *M. hapla* in pot and field experiments. Induced resistance increased with the length of the time between inoculations and with the population density of the induction inoculum. Optimum interval before challenge inoculation, or population density of inoculum for inducing resistance, was 10 days, or 5,000 infective nematodes per 500-cm³ pot. The induced resistance suppressed population increase of *M. hapla* by 84% on potted tomato, 72% on potted pyrethrum, and 55% on field-grown pyrethrum seedlings, relative to unprotected treatments. Pyrethrum seedlings inoculated with *M. javanica* 10 days before infection with *M. hapla* were not stunted, whereas those that did not receive the advance inoculum were stunted 33% in pots and 36% in field plots. The results indicated that advance infection of plants with incompatible or mildly virulent nematode species induced resistance to normally compatible nematodes and that the induced resistance response may have potential as a biological control method for plant nematodes.

Key words: *Chrysanthemum cinerariifolium*, induced resistance, *Lycopersicon esculentum*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Mi* gene, nematode, pyrethrum, root-knot nematode, tomato.

When plants are infected with pathogens to which they are resistant, they normally respond by expressing diverse physiological and anatomical defense mechanisms that inhibit establishment of the pathogens (14,18,26,27). Such post-infection expression of resistance, also referred to as induced resistance, is characterized by accumulation of a wide range of pathogenesis-related (PR) substances and the hypersensitive reaction that consists of rapid and localized necrosis of cells around infection sites (15,22,25). Whereas there have been several studies on induced resistance response in plants to viruses, fungi, and bacteria (2,3,16,24,30), there is little information with regard to nematodes.

Generally, infective individuals of nematodes such as the second-stage juveniles (J2) of root-knot nematodes (*Meloidogyne*

Goeldi) locate and penetrate roots of both susceptible and resistant plants equally. Whereas they continue to establish feeding sites and multiply in the susceptible plants, the majority of those that enter resistant plants have been observed to egress 3–5 days later or die in the roots (6,8,10). Egression of the infective nematodes from roots of resistant plants shortly after penetration has been associated with post-infection production and accumulation of diverse antimicrobial compounds including phytoalexins (11,12,14,28,31).

On the basis that incompatible plant-nematode interactions result in accumulation of defensive substances that inhibit further development of the nematodes, we hypothesized that an advanced inoculation of plants with incompatible nematode species could induce resistance to challenge inoculum of normally compatible species. Thus, the purpose of this investigation was to test that hypothesis in genotypes of tomato (*Lycopersicon esculentum* Mill) and pyrethrum (*Chrysanthemum* [Syn. *Tanacetum*] *cinerariaefolium* Vis.) that are susceptible to the Northern root-knot nematode *Meloidogyne hapla* (Treub) Chitwood but resistant to some *Meloidogyne* species such as *M. incognita* (Kofoid and White) Chitwood, *M. javanica* Chitwood, or *M. arenaria*

Received for publication 12 April 1995.

¹ Portion of Ph.D. dissertation by the first author submitted to the Graduate College, University of Arizona, in partial fulfillment to the degree requirements.

² Former Graduate Student and Professor, respectively, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721. Present address of first author: Department of Nematology, University of California, Riverside, CA 92521.

The authors gratefully acknowledge financial support from The Rockefeller Foundation Sub-Saharan African Dissertation Internship Awards and The S. C. Johnson's Wax, Inc. They also thank S. D. Van Gundy and P. A. Roberts for critically reviewing the manuscript.

(Neal) Chitwood (23). Pyrethrum is a herbaceous perennial plant from which pyrethrin insecticides are obtained (4). It is produced mostly in cool and high elevation areas in Kenya and is highly susceptible to *M. hapla*, the predominant *Meloidogyne* species in such climates (20).

MATERIALS AND METHODS

Test plants and nematode inocula: Tomato seeds of cultivars that purportedly possessed the *Mi* gene that confers resistance to root-knot nematodes *Meloidogyne incognita*, *M. javanica*, and *M. arenaria*, but not to *M. hapla* (23), were bought locally (Harlows Nurseries, Tucson, AZ). They consisted of 'Celebrity', 'Lemonboy', 'Quickpick', and 'Betterboy'. Seedlings were grown in steam-sterilized sandy soil in 500-cm³ plastic pots in a greenhouse. A slow-release granular fertilizer (N-P-K: 14-14-14) was applied at 10g/pot. Greenhouse temperature ranged between 25–30 C. The seedlings were maintained for 30 days before inoculation. Pyrethrum seedlings were obtained from The Pyrethrum Board of Kenya at a tissue-culture laboratory in Molo, Kenya, and consisted of commercial clones 223 and 487. Both were purportedly susceptible to *M. hapla*. Seedlings were maintained on steam-sterilized sandy loam soil in 500-cm³ plastic pots in a greenhouse for 4 months before inoculation.

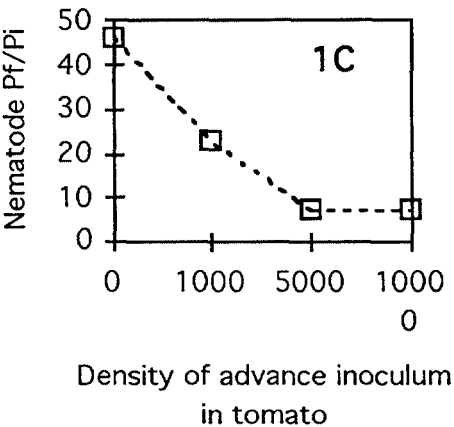
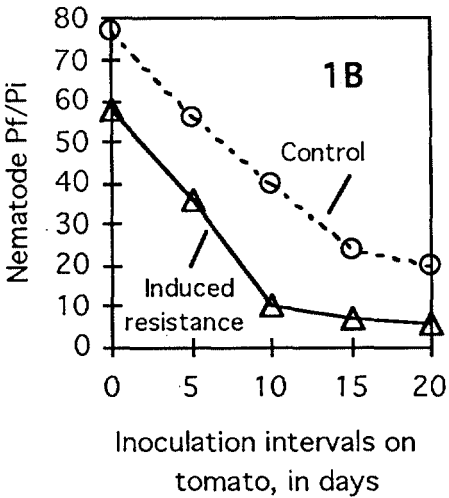
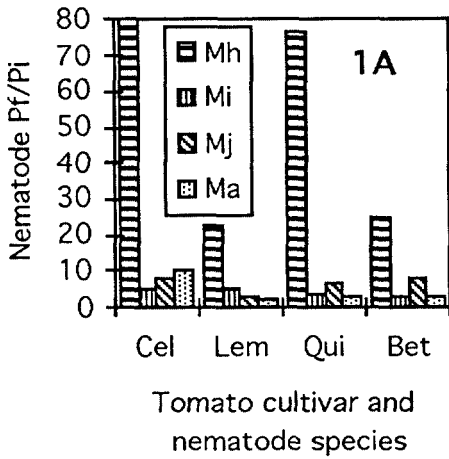
Single egg-mass populations of *M. hapla* type A, *M. incognita* race 3, *M. javanica* and *M. arenaria* race 1 were obtained from greenhouse cultures at the University of Arizona. They were maintained and multiplied on potted eggplant (*Solanum melongena*) cultivar Black Beauty. Species identities were confirmed by examination of female perineal patterns (7), and by analysis of mitochondrial deoxyribonucleic acid markers that were amplified by the polymerase chain reaction method (21). Nematode eggs were extracted from plant roots with 1% sodium hypochlorite (1). Plants were inoculated with freshly hatched J2 that were pipetted into 3-cm-deep holes in the root zone. Sixty days after the final inocu-

lation, roots were gently pulled out of the pots and washed clean. Host status of the test plant genotypes to the test *Meloidogyne* species was expressed in nematode reproduction ratios (1). A reproduction ratio was calculated by dividing the number of nematode eggs produced per root system after 60 days of infection (Pf), by the initial inoculum density (Pi).

Induction of resistance in tomato: In order to rate the relative host status of the four cultivars of tomato to the test *Meloidogyne* species, the cultivars were separately inoculated with each species at 5,000 J2 per potted plant. The treatments were replicated 5 times. Pots were arranged in a completely randomized block design on a table in a greenhouse. Plants were maintained in the greenhouse for 60 days after which they were evaluated for nematode infectivity as described earlier. The experiment was performed twice. On the basis of results from this experiment (Fig. 1A), 'Celebrity' tomato and *M. incognita* were selected for testing induction of resistance to *M. hapla*.

The reproduction ratios of *M. hapla* in 'Celebrity' tomato after inoculation with *M. incognita* was determined at increasing intervals of 0, 5, 10, 20, or 30 days. Controls consisted of plants that were inoculated with *M. hapla* only on day 0, 5, 10, 20, or 30 after start of inoculations. Nematode inoculum density for all treatments was 5,000 J2/500-cm³ pot. Each treatment was replicated five times, and the experiment was repeated once. Influence of population density of the resistance inducing-inoculum was determined by applying *M. incognita* at 0, 1,000, 5,000, or 10,000 J2 per pot, 10 days before challenge-inoculation with 5,000 J2/plant of *M. hapla*. Each treatment was replicated 5 times, and the experiment was repeated once. In calculating the reproduction ratio of *M. hapla* after induction of resistance with *M. incognita*, possible reproduction of the induction inoculum was assumed negligible.

Induction of resistance in pyrethrum: Preliminary studies on the relative infectivity of *Meloidogyne hapla*, *M. incognita*, and *M.*



javanica on pyrethrum clones 223 and 487 showed that both clones were highly susceptible to *M. hapla* and moderately susceptible to *M. incognita*, but highly resistant to *M. javanica* (data not included). On that basis, clone 223 and *M. javanica* were selected for testing induction of resistance to *M. hapla*. Experiments were conducted in pots and in field plots. The pot experiment consisted of treatments in which the plants were initially inoculated with *M. javanica*, followed by challenge inoculation with *M. hapla* on day 0, 5, 10, 20, or 30 after start of inoculations. Control treatments were inoculated with either *M. javanica* or *M. hapla* only. Nematode inoculum was applied at 5,000 J2 per potted plant. Each treatment was replicated 10 times.

The field experiment was conducted at Egerton University, Njoro, Kenya, in a plot where pyrethrum had been grown for 4 years previously and was known to be heavily infested with *M. hapla*. Pre-plant soil sampling and nematode extraction by the Baermann's filter and pan method (1) indicated average infestation of 80 J2/250-cm³ of soil. On the basis that roots of pyrethrum seedlings could extend to about 1,000-cm³ within 30 days, according to casual observation, an initial inoculum density of 320 J2/plant was assumed for calculation of nematode reproduction ratios. Possible reproduction of other plant-parasitic nematodes was assumed negligible. Pyrethrum seedlings derived from tis-

FIG. 1. Reproduction ratios (Pf/Pi) of *Meloidogyne hapla* (Mh), *M. incognita* (Mi), *M. javanica* (Mj), or *M. arenaria* (Ma) as single, concurrent, or sequential inocula 60 days after infecting potted tomato plants. Pf/Pi is the final egg count per root system divided by initial inoculum density (5,000 second-stage juveniles (J2) per plant). Each treatment consisted of five replications, and the data are pooled means of two experiments. 1A: Pf/Pi of the 4 *Meloidogyne* species in tomato cultivars Celebrity (Cel), Lemonboy (Lem), Quickpick (Qui), and Betterboy (Bet). 1B: Pf/Pi of *M. hapla* in tomato 'Celebrity' applied on day 0, 5, 10, 15, or 20 either alone (control) or as challenge after *M. javanica* (applied on day 0). 1C: Pf/Pi of *M. hapla* in tomato 'Celebrity' applied on day 10 after *M. incognita* that was applied at inoculum density of 0, 1,000, 5,000, or 10,000 J2 per plant.

sue culture stock were grown in pots for 4 months before transplanting to the field. Three treatments consisting of seedlings that were inoculated with either *M. javanica* or *M. hapla* 10 before transplanting, and those that did not receive any advance inoculum, were transplanted to the field plot. Each treatment, consisting of a single seedling, was replicated 20 times in a completely randomized block design. The plot was irrigated every evening. Effect of the nematode treatments on growth of the pyrethrum plants was measured at intervals of 0, 30, 60, and 90 days after transplanting. Because pyrethrum has a tufted growth habit with little stem elongation during the seedling stage, the rate of growth was measured based on number of expanded leaves. Nematode reproduction ratios in roots were determined 90 days after transplanting.

Statistical analysis: Data were compared by the factorial analysis of variance (ANOVA), and differences among treatment means were determined at a probability level of 5% (17). Pairwise comparisons were done with the Duncan's multiple-range test. Means of repeated experiments were pooled and the standard errors assessed.

RESULTS

Reproduction of nematodes in tomato with or without induced resistance: Preliminary experiments showed that *M. hapla* reproduced well on all four test tomato cultivars, whereas *M. incognita*, *M. javanica*, or *M. arenaria* reproduced poorly, as was expected (Fig. 1A). Sixty days after inoculation, the reproduction ratios of *M. hapla* ranged from 80 on 'Celebrity' to 23 on 'Lemonboy', whereas the reproduction ratios of the other three *Meloidogyne* species averaged 5 on the four cultivars. Following advance inoculation with *M. incognita* on 'Celebrity' tomato, reproduction of challenge *M. hapla* in the plants dropped significantly ($P \leq 0.05$). An inoculation interval of 5 or 10 days caused a 37% or 82% drop (Fig. 1B). Longer intervals did not increase ($P \leq 0.05$) the drop any further.

M. hapla inoculum that was applied on younger seedlings attained higher ($P \leq 0.05$) reproduction ratios than those applied to older seedlings, indicating an age-induced resistance. Control plants that were inoculated on day 0, 5, 10, 15, or 20 after advance inoculation supported nematode reproduction ratios of 77, 59, 37, 10, 7, and 6, respectively (Fig. 1B). Increasing advance inoculum density from 0 to 1,000, or from 1,000 to 5,000 J2 per plant, decreased reproduction ratios of challenge inoculum by 57% or 65%, respectively (Fig. 1C). Larger inoculum densities did not decrease ($P \leq 0.05$) the reproduction ratio any further.

Effects of induced resistance on reproduction of M. hapla and on plant growth in pyrethrum: In greenhouse experiments, reproduction of *M. hapla* was suppressed 31% or 72% by *M. javanica* inoculum that was applied to the pyrethrum plants 5 or 10 days in advance, relative to controls (Fig. 2). Longer intervals between the inoculations did not increase ($P \leq 0.05$) the magnitude of induced resistance any further. Concurrent advance and challenge inoculations enhanced nematode reproduction ratio by 17%. In the field plot experiment, pyrethrum seedlings that received advance inoculation with *M. javanica* 10 days before transplanting had a mean nematode reproduction ratio of 21, which was 45% less than the mean reproduction ratio in the unprotected control plants. In pot experiments, seedlings that were inoculated with *M. javanica* 10 days before challenge with *M. hapla* produced 34% more leaves 90 days after the infection, relative to those without the protective inoculum. Seedlings that received either *M. javanica* alone, or no nematode inoculum, grew at similar rates. In field experiments, seedlings inoculated with *M. javanica* 10 days before transplanting produced twice the number of leaves as were on unprotected controls 90 days after transplanting (Fig. 3).

DISCUSSION

Our results indicated that infection of tomato or pyrethrum plants with incom-

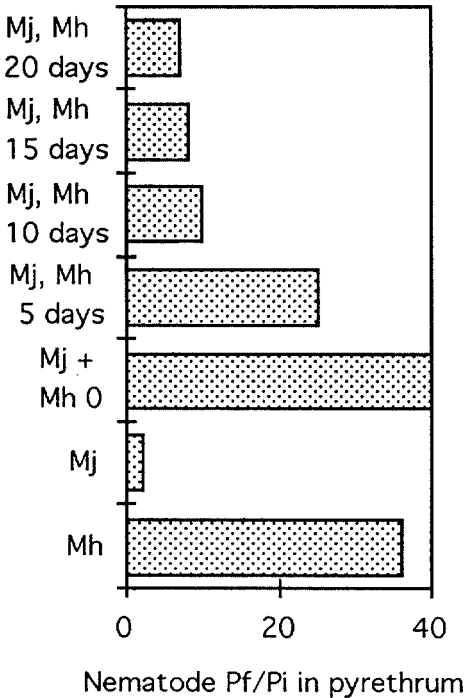


FIG. 2. Reproduction ratios (Pf/Pi) of *Meloidogyne javanica* (Mj), *M. hapla* (Mh), or *M. hapla* applied on day 0, 5, 10, 15, or 20 after *M. javanica* (Mj, Mh 0 day, ... Mj, Mh 20 days) 60 days after infection in potted pyrethrum clone 223. Pf/Pi is the final egg count per root system divided by initial inoculum density (5,000 second-stage juveniles per plant). Data are means of 10 replicated plants.

patible or mildly virulent *Meloidogyne* species induced resistance in the plants such that the reproduction of challenge inoculum of normally compatible *M. hapla* was highly suppressed. The findings are comparable to several reported cases of 'cross-protection' in which related but mildly virulent strains of viruses have been tested and used commercially in the control of some viral diseases such as Tobacco Mosaic Virus in tomatoes, Citrus Tristeza Virus, and Papaya Ringspot Virus (22,23,25). Similar results have been experimentally observed with several fungal and bacterial pathogens as well (16,17,25,26,29). On the basis of our literature search, this may be the first report of direct induction of resistance in a host plant against a nematode species with another nematode.

The increase in magnitude of induced resistance that corresponded to longer in-

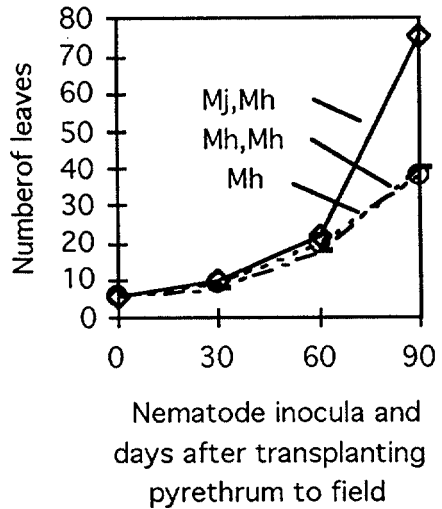


FIG. 3. Number of leaves produced by pyrethrum seedlings within 90 days in a field naturally infested with *Meloidogyne hapla*. Treatments consisted of inoculation with 5,000 juveniles per plant of either *M. javanica* or *M. hapla*, or none (Mj,Mh, Mh,Mh, or Mh, respectively) 10 days before transplanting. Data are means of 20 replications.

tervals between advance and challenge inoculations within the first 10 days apparently was dependent on the post-infection accumulations of antimicrobial substances. Zacheo et al. (31) observed that post-infection accumulation of peroxidase enzymes in tomato plants resistant to *M. incognita* reached maximum levels about 10 days after inoculation with the incompatible nematodes. The intensity of induced resistance was also found to initially increase with increasing population density of advance inoculum to about 5,000 J2 per potted plant. This result paralleled observations by Caruso and Kuc (3), wherein the extent of resistance induced to anthracnose fungus, *Colletotrichum lagenarium*, in cucumber by the fungus or other fungi was directly related to the concentration of induction inoculum until a saturation point was reached, after which there was no further increase in resistance. An age-induced resistance to *M. hapla* was observed among control treatments such that seedlings inoculated at 30 days of age supported nematode reproduction ratios that were about 30% higher than those inocu-

lated at 40 days of age. Greater resistance to nematodes in older seedlings has been severally reported (13,19) and could be due to the fact that older seedlings have most of their roots much more differentiated and therefore less susceptible to infection, whereas the converse is the case on younger seedlings.

In addition to suppression of nematode reproduction rates, pyrethrum seedlings with induced resistance also grew more rapidly compared to control treatments that were not similarly protected. Similar positive response in plant growth following induction of resistance has been observed by several workers as well (2,3,15, 16). Cruickshank and Mandryk (5), however, reported severe stunting of tobacco plants following stem inoculation with the blue mold fungus, *Peronospora tabacina*, in order to protect leaves against the same fungus. Such conflicts within experimental results will hopefully be soon resolved as we get to understand better the mechanisms of induced resistance.

Although the mechanisms of induced resistance response in plants are not yet well understood, the phenomenon is similar to immunization in human and veterinary medicine (9). Just as intensive research in immunology has led to great advances in prevention of some important diseases of humans and livestock, similar efforts in this area could lead to development of plant genotypes with an enhanced physiological ability to suppress infection and damage by pathogens, including nematodes.

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