

PARASITISM OF HOLLY CULTIVARS BY THREE ROOT-KNOT NEMATODE ISOLATES

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Meloidogyne hapla, the northern root-knot nematode, and *M. incognita*, the southern root-knot nematode, are common parasites of ornamentals. In the nursery-growing region of middle Tennessee, *M. hapla* is the more common of the two, occurring in about twenty-five percent of all nursery blocks.

Meloidogyne incognita is recognized as a parasite of hollies (3, 5), although few studies have substantiated this supposition experimentally (4). The ability of *M. hapla* to gall and reproduce on holly roots has been studied more frequently, but evidence of susceptibility is controversial and conflicting. Hassis et al. (2) found root-knot nematodes, including *M. hapla*, to be severe pathogens of *Ilex crenata*, causing severe galling of liner-size plants, but Sasser et al. (5) reported that *M. hapla* caused no galling of *I. crenata* 'Convexa', 'Helleri', or 'Latifolia', nor of *I. cornuta* 'Burfordii'. Heald (3) attempted to resolve the question of the susceptibility of *I. crenata* 'Helleri'. In his study, *M. hapla* galled roots and induced stunting and chlorosis. He concluded that different races of the nematode or different selections of the holly had been used in the various experiments. Bernard and Witte (1) reported little or no *M. hapla* galling on *I. × attenuata* 'Foster #2', *I. crenata* 'Hetzii', or *I. (cornuta × aquifolium)* 'Nellie R. Stevens'.

The objective of this study was to determine the ability of two geographically separate *M. hapla* isolates and one *M. incognita* isolate to gall the roots of seventeen cultivars of ornamental hollies, to determine whether reported differences in galling by *M. hapla* are due to different nematode isolates, and to correlate gall rating with the formation of giant cells.

Materials and Methods Two isolates of *M. hapla*, one from Tennessee (TN) and the other from North Carolina (NC), were maintained in the greenhouse on 'Florunner' peanut. The Tennessee isolate was collected originally in Warren County; the North Carolina isolate was of unknown provenance. A stock culture of *M. incognita* (MI), originally collected from Warren County, was maintained on 'Rutgers' tomato. Cuttings of 17 holly cultivars (Table 1) were collected in autumn and rooted under intermittent mist on a greenhouse propagation bed. Root balls were shaken free of propagation media prior to planting into 10 cm. pots filled with sandy loam soil. Plants were greenhouse grown for two months prior to inoculation. Nematode eggs were gathered from stock cultures with a sodium hypochlorite technique. Six holes were punched into the soil of each holly pot and 10,000 eggs in 20 ml. of water were poured over the surface. Plants were then thoroughly watered. Pots were arranged in a split-plot design, with five replicates

of each nematode isolate-holly combination. Two months later root systems were harvested and rated for galling as follows: 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls per root system.

Following gall ratings, galled root segments were fixed in FAA. After fixation was complete, the segments were dehydrated in an ethanol series, then embedded in paraffin. Embedded galls were sectioned (10 μm thick) on a microtome. Sections were placed on microscope slides and stained with a toluidine blue - aniline blue solution. Development of giant cells (Fig. 2A-C) was rated on a scale of 1-3: 1 = giant cells aborted, non-functional; little or no nematode development; 2 = giant cells moderately developed but functional, cell wall ingrowths poorly developed or absent; females maturing but with

Table 1. Susceptibility of holly cultivars to root galling by isolates of *Meloidogyne hapla* (TN, NC) and *M. incognita* (MI).

Cultivar	Nematode Isolate Gall Index*		
	MI	TN	NC
<i>I. × attenuata</i> 'Foster #2'	3.0	2.8	0.6
<i>I.</i> 'Calina' (<i>aquifolium</i> × <i>cornuta</i>)	2.4	4.6	3.2
<i>I. cassine</i> 'Loweii'	2.0	3.2	3.4
<i>I. cornuta</i> 'Needlepoint'	2.6	3.2	0
<i>I. crenata</i> 'Bennett's Compacta'	4.5	4.2	2.2
<i>I. crenata</i> 'Convexa'	4.8	4.4	5.0
<i>I. crenata</i> 'Green Lustre'	4.0	4.4	4.8
<i>I. crenata</i> 'Rotundifolia'	4.8	5.0	5.0
<i>I. glabra</i> 'Compacta'	3.2	3.6	0.8
<i>I. glabra</i> 'Nordic'	3.8	3.0	0
<i>I.</i> 'Elegance' (<i>integra</i> × <i>pernyi</i>)	4.2	4.0	3.8
<i>I.</i> 'Lydia Morris' (<i>cornuta</i> × <i>pernyi</i>)	3.5	3.4	2.4
<i>I. × meserveae</i> 'Blue Boy'	3.5	2.8	3.6
<i>I. × meserveae</i> 'Blue Girl'	2.5	4.4	3.6
<i>I.</i> 'Nellie R. Stevens' (<i>cornuta</i> × <i>aquifolium</i>)	3.5	3.0	1.8
<i>I. opaca</i> 'Jersey Princess'	†	2.2	0.4
<i>I. vomitoria</i> 'Stokes Dwarf'	2.0	0	0

*0 = no galls per root system, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = greater than 100 galls per root system.

†Not tested.

reduced egg production; 3 = giant cells large, well-formed, with abundant cell wall in-growths; females large, highly fecund. Correlation of gall rating and giant cell rating was calculated.

Results and Discussion Four cultivars were heavily galled (rating ≥ 3.5) by all three nematode isolates (Table 1). Cultivars of *I. crenata* generally were highly susceptible to all three nematodes. Nine of 17 cultivars were heavily galled by MI (Table 1), and all cultivars were capable of supporting at least limited galling and growth of MI. The results with the two *M. hapla* isolates were variable. TN was more pathogenic than NC, with significantly higher gall ratings (difference ≥ 1.0) on nine cultivars. Six cultivars appeared to be highly resistant (rating < 2.0) to galling by the NC isolate, while only one cultivar was highly resistant to TN. Gall size varied among the cultivars, but gall morphology for each nematode species was typical. Galls of MI were smooth (Fig. 1A), whereas galls caused by the *M. hapla* isolates had adventitious roots extending from them (Fig. 1B).

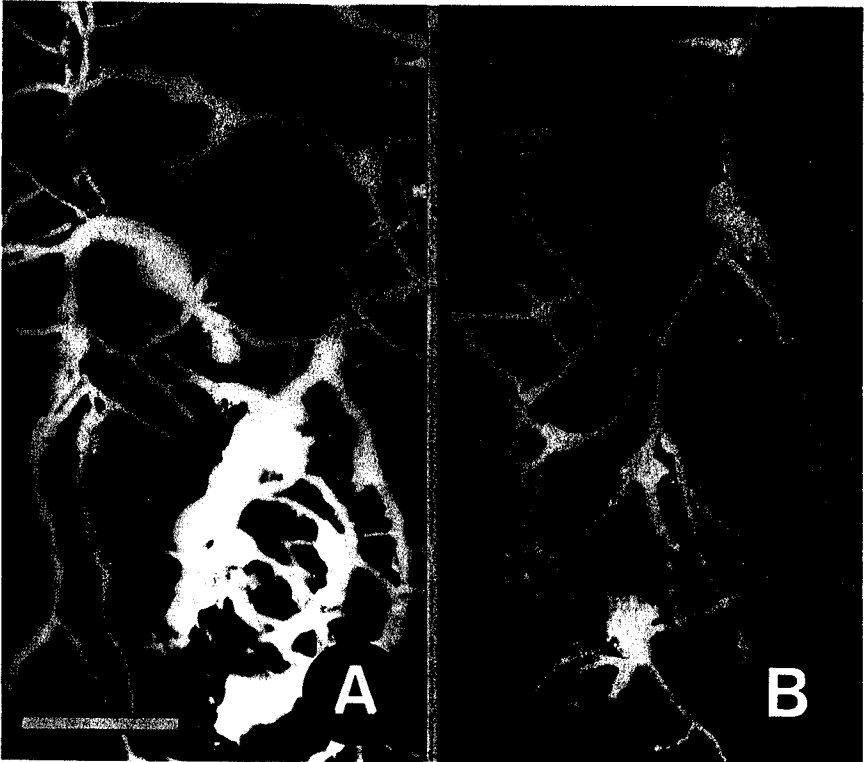


Figure 1. Galling of *Ilex crenata* 'Rotundifolia' by root-knot nematodes. A) Galls produced by *Meloidogyne incognita*. B) Galls produced by *M. hapla*. Scale line = 5 mm.

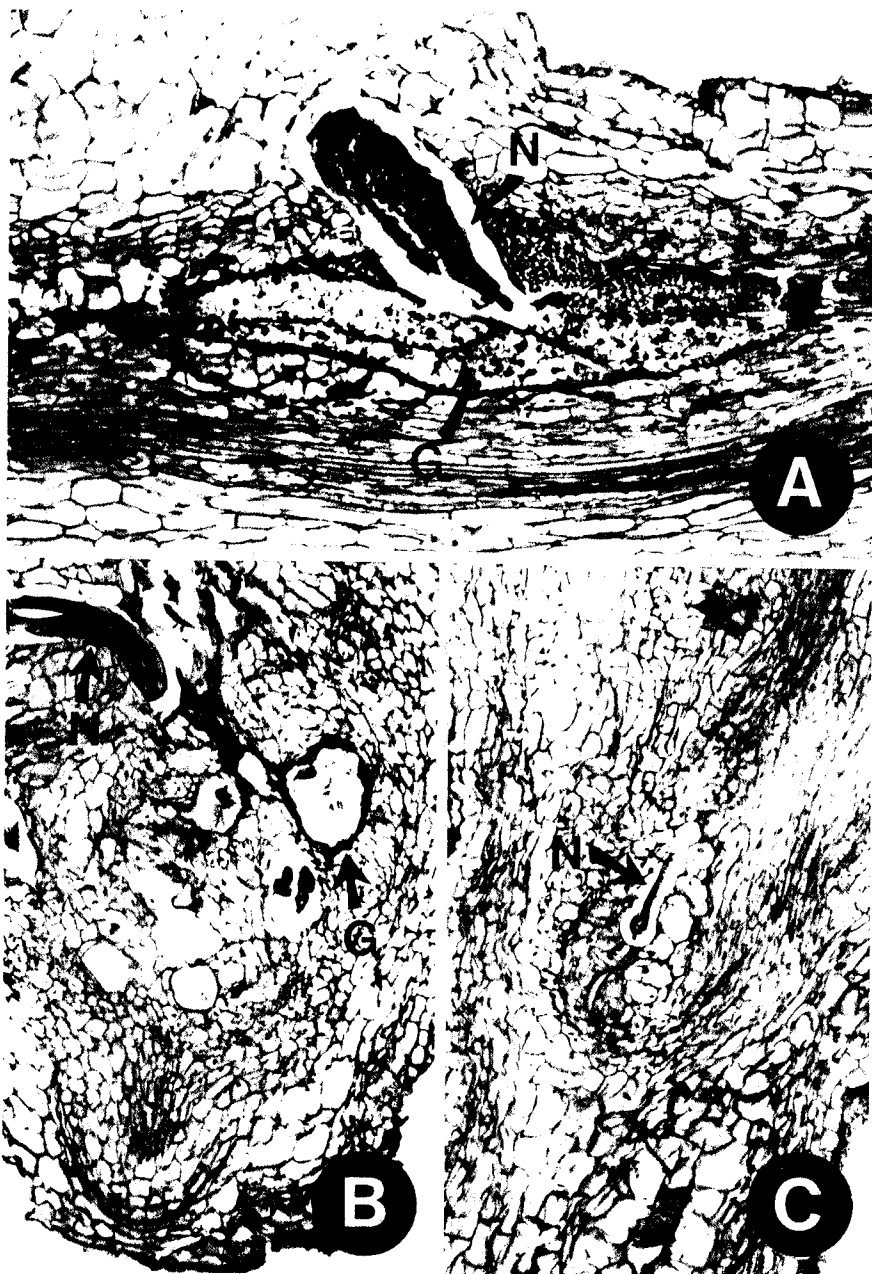


Figure 2. Differences in root-knot nematode giant cell formation on hollies. A) Fully developed giant cells. B) Moderately developed giant cells. C) Lack of giant cell development. (G: giant cells; N: nematode.)

Giant cell rating and gall rating were positively correlated ($P < 0.05$), but giant cell rating accounted for only forty-eight percent of the variability in gall rating (Fig. 3). This variability was due primarily to the formation of numerous galls on the root systems of some cultivars, but little subsequent giant cell formation. In most sections with a giant cell rating of one, a hypersensitive reaction was observed. Hypersensitivity is a form of resistance in which cells around the invading nematode lignify and die, thus preventing the nematode from successfully establishing a feeding site.

Root-knot nematode species commonly are recognized as having widely varying host preferences, depending on location, crop history, and nematode isolate. Variability inherent in many root-knot species often has led to the reporting of contradictory results. In this research, the variability of the host response to *M. bapla* was demonstrated. The TN isolate was collected in Warren County from commercial woody ornamental nursery beds, and may have become selectively adapted to hollies through their frequent cultivation on *M. bapla*-infested land. Geographic variability in nematode virulence to hollies complicates the problem of nematode management, since results reported from one state may not be completely valid in another state. Growers should be aware of the presence of nematodes in their blocks and should check root systems for nematode symptoms.

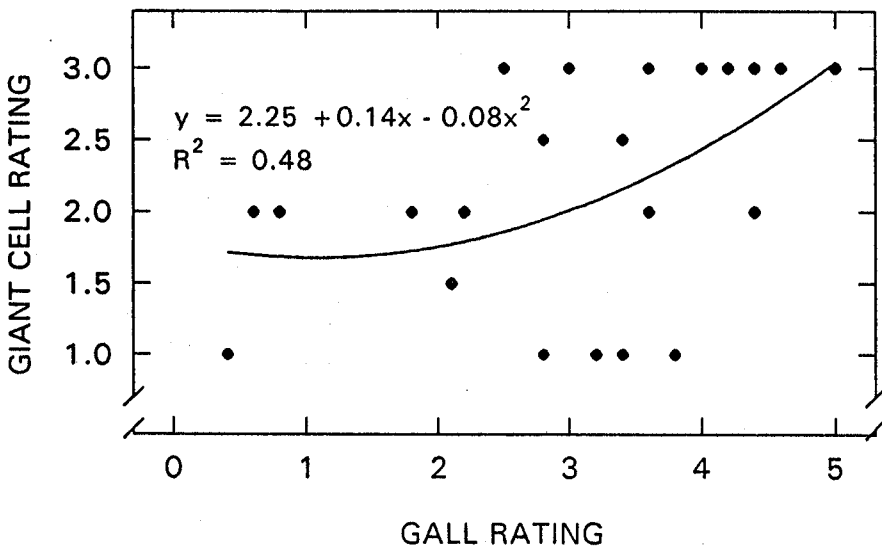


Figure 3. Correlation of gall rating with giant cell development rating.

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