Japanese Hollies: Intolerant Hosts of Meloidogyne arenaria in Microplots

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Abstract: Japanese hollies were intolerant of *Meloidogyne arenaria* in field microplot experiments. *Ilex crenata* var. *rotundifolia* was relatively more tolerant than *I. crenata* var. *convexa* or *I. crenata* var. *helleri*. When *M. arenaria* was added at various initial population densities to soil containing plants of "Helleri," "Convexa," and "Rotundifolia," respectively, 91, 75, and 25% were killed by the end of the third growing season. No control plants died during the same period. Initial numbers of *M. arenaria* larvae and eggs were the only population densities that were correlated (negatively), regardless of cultivar, with plant growth over the three growing seasons. A linear relation was found for initial density of *M. arenaria* and growth of *I. crenata rotundifolia*. Increasing nematode density by 10-fold suppressed the growth of this cultivar by 23%.
Key Words: Ilex crenata var. *convexa, I. crenata* var. *helleri, I. crenata* var. *rotundifolia,* population dynamics, root-knot.

Meloidogyne species have been recognized as serious pathogens on many woody ornamentals, including Japanese holly (*Ilex crenata* Thumb.) in the southeastern USA (5, 6, 8, 9). In a greenhouse test, Sasser et al. (9) showed that many types of *Ilex* were resistant to *M. hapla* but susceptible to *M. arenaria, M. incognita,* and *M. javanica. Ilex crenata* var. *burfordi* was resistant also to *M. arenaria*. Heald (6) reported *I. crenata* var. *helleri* to be susceptible to all four nematode species.

General symptoms associated with plant-parasitic nematode attack usually include poor growth, low vigor, yellowing or bronzing of foliage, dieback of branches, and restricted and galled root systems (5). Although survey work has demonstrated *Meloidogyne* spp. on *Ilex crenata,* most of the data on pathogenicity were obtained in greenhouse tests. Host tolerance may be even lower in the field than the greenhouse. Grower advisory services need such information for each major nematode-plant combination.

We report herein the intolerance of three Japanese hollies, *Ilex crenata* ("Rotundifolia," "Convexa," and "Hel-

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leri"), toward *Meloidogyne arenaria* in field microplots.

MATERIALS AND METHODS

The installation of microplots, soil preparation, and fumigation rates have been described (2). The loamy sand had 91% sand, 3.3% clay, and 5.7% silt. *Meloidogyne arenaria* inoculum was obtained from Rotundifolia and increased on tomato (*Lycopersicon esculentum* Mill 'Floradel') for 67 days. Tomato plants were grown in a 1:1 (v/v) mixture of sandy loam soil and #35 sand in 14-cm deep flats on greenhouse benches. Inoculum (chopped tomato roots plus soil) and soil from nematode-free tomato plants were added in various proportions to establish a high, medium, or low nematode population density in each microplot. Control microplots received nematode-free tomato soil. Inoculum was mixed uniformly into the top 15 cm of soil in each microplot. Approximate total numbers of eggs + larvae added per 500 cm³ of plot soil were: high, 120,000 eggs + 1,650 larvae; medium, 60,000 eggs + 825 larvae; and low, 30,000 eggs + 412 larvae. Four replications of each inoculum density were used in a randomized complete block design.

Uniformly rooted plants of either Rotundifolia, Convexa, or Helleri growing in a sand:soil:peat mixture (1:1:1, v:v:v) were planted singly into each microplot on 27 April 1973, the day after the plots were infested. The faster growing Rotundifolia plants were pruned at transplanting.

Five days after soil infestation, 10 soil cores/microplot were collected, and the eggs and larvae were extracted by the procedures previously described (3, 4, 7). Nematode assays of plot soil made 1 week after infestation yielded much lower densities (Table 1). The statistical analyses included the nematode population data in Table 1. The final nematode extractions, however, involved a combination of a semi-automatic elutriator and centrifugation (3), whereas previous larval extractions were by centrifugation (7).

Techniques and methods for nematode sampling, fertilizing, insect control, rating, and measuring plant vigor and growth have been described (2). Surface area was computed by a planimeter from a 10.2- x 12.6-cm photograph of the plant printed with a 135-mm lens at 0.56 m. A camera utilizing a 50-mm lens and 35-mm film was positioned 0.87 m above the ground at a distance of 2.13 m from the plant to take the photograph.

TABLE 1. Effects of population density of *Meloidogyne arenaria* on growth of three cultivars of *Ilex crenata* in microplots.

Mean initial nematode density/500 cm³ soil	Vigor rating[a] (Months after soil infestation) 5	Vigor rating[a] 29	Plant height x width (cm^2) 29	Plant surface area (cm^2) 29
Ilex crenata var. *convexa*				
High density—6,320	1.7	0	0	0
Medium density—3,184	3.6	1.8	882	3.17
Low density—1,025	3.0	0.3	246	1.19
Control—0	7.3	9.4	4,292	23.86
LSD: $P = 0.05$	2.7	1.8	1,077	4.12
$P = 0.01$	3.7	2.5	1,547	5.91
Ilex crenata var. *helleri*				
High density—5,154	3.0	0.3	200	0.97
Medium density—2,071	2.9	0	0	0
Low density—1,041	3.5	0	0	0
Control—0	7.9	9.9	2,508	15.30
LSD: $P = 0.05$	2.7	0.4	355	2.47
$P = 0.01$	3.8	0.5	510	3.54
Ilex crenata var. *rotundifolia*				
High density—4,977	3.1	1.8	900	3.30
Medium density—2,746	4.3	3.1	1,713	6.87
Low density—456	4.8	2.9	1,323	5.25
Control—0	7.4	9.6	4,900	20.17
LSD: $P = 0.05$	2.1	2.0	1,181	4.73
$P = 0.01$	2.9	2.8	1,647	6.59

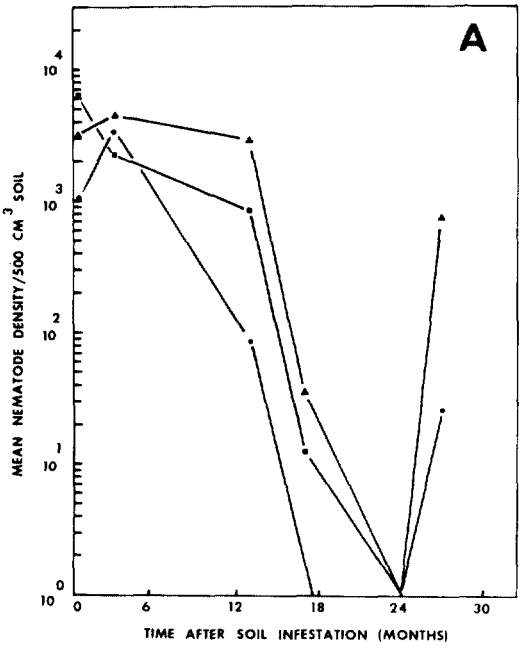
[a]Vigor rating based on scale: 10 = most vigorous plant, 0 = dead plant. For statistical analysis, 0.1 was added to all dead plant values.

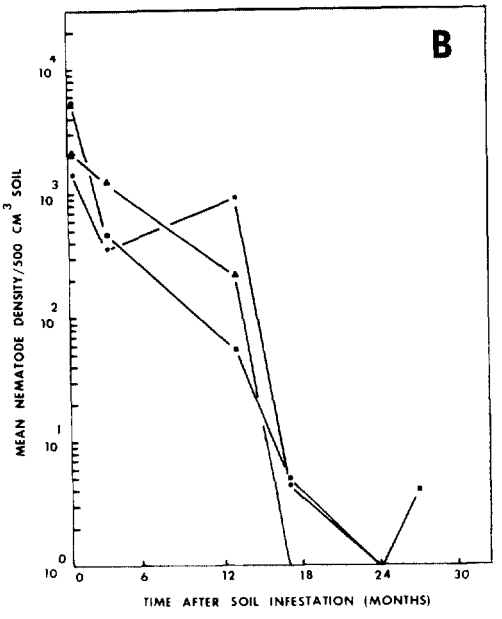
RESULTS

Within 5 months, plants inoculated with *Meloidogyne arenaria* were less vigorous, regardless of cultivar, than control plants (Table 1). Aboveground symptoms of inoculated plants included: minimal new growth, a stunted appearance, leaf chlorosis, and some defoliation. Many inoculated plants of Convexa (9 of 12) and Helleri (11 of 12) died by the second growing season so that population densities for 14 months (Fig. 1-A, B) and thereafter are

based on the few remaining replicates. No control plants died during the experiment. The death rate of Convexa was similar for each inoculum density of *M. arenaria* tested, although the medium level had higher densities at each sampling date. Growth of Convexa and Helleri was inversely correlated ($P \leq 0.05$) with initial, but not later, nematode population densities. Because of the death of numerous plants of these cultivars, no regression analyses were done.

Densities of *M. arenaria* declined rapidly after 14 months on roots of Convexa and Helleri (Fig. 1-A, B). Nematode population densities in Fig. 1-A and 1-B represent populations for three Convexa plants and one Helleri after 14 months.





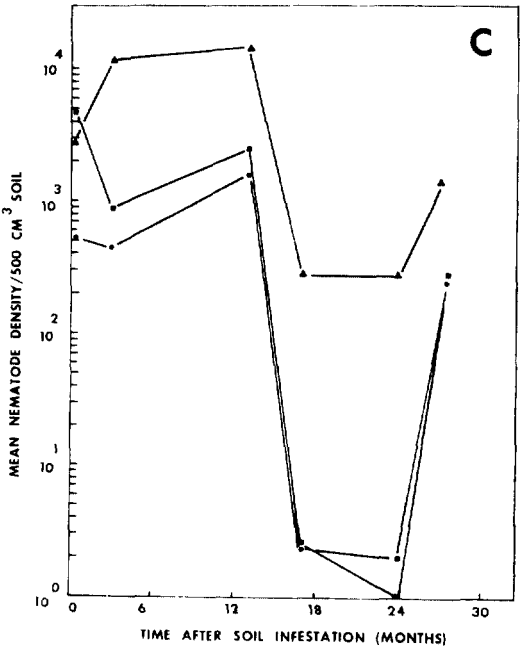


FIG. 1-(A-C). Density of *Meloidogyne arenaria* over a 29-month period on: *A) Ilex crenata* var. *convexa; B) I. crenata* var. *helleri;* and *C) I. crenata* var. *rotundifolia.* Symbol solid square box denotes high density initially, solid triangle = medium density, and solid circle = low density.

Rotundifolia was relatively more tolerant of *M. arenaria* than were Convexa and Helleri, but all inoculated plants grew significantly less than control plants (Table 1). Only one of the 12 Rotundifolia plants inoculated with *M. arenaria* died within 14 months; two more died between 24 and 29 months. This variety changed very little in vigor after 14 months, whereas Convexa and Helleri continued to decline (Table 1).

Population densities of *M. arenaria* on Rotundifolia decreased markedly after 14 months (Fig. 1-C) but, by 29 months, had climbed again almost to the levels found prior to 14 months. After the initial sampling date, plots with the initial medium density had consistently greater nematode numbers than either the high or low initial density plots for the remainder of the experiment.

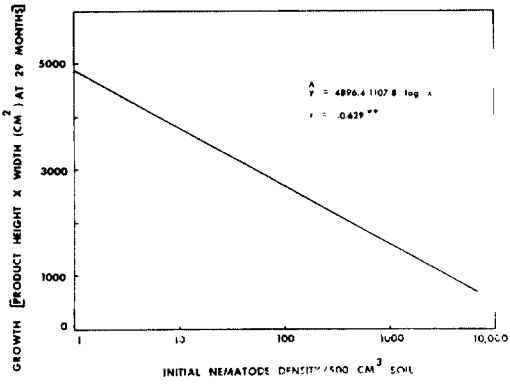


FIG. 2. Regression line for the effect of growth of *Ilex crenata* var. *rotundifolia* at 29 months on the initial density of *Meloidogyne arenaria* in microplots $X = \log_{10}(P_i + 1)$.

A stepwise regression model was used to compare the effects of nematode population density at the various sampling times with each growth variable for Rotundifolia. Only the initial nematode density and plant growth were significantly correlated. For instance, the regression model for growth (the product of plant height and width) and initial nematode density (X) was $y = 4{,}896.4 - 1{,}107.8 \ (\log x + 1)$ $P \leq 0.01$ (Fig. 2). Therefore, a 10-fold increase in density of *M. arenaria*/500 cm³ of soil would result in a 23% growth depression of Rotundifolia.

DISCUSSION

Convexa, Helleri, and Rotundifolia (varieties of *Ilex crenata*) were all susceptible to *M. arenaria* under field conditions. Although Rotundifolia appeared more tolerant than Convexa or Helleri, plant size at inoculation may partially explain this difference. Plants of the most rapidly growing Rotundifolia were pruned at transplanting as they were much larger initially than those of the other two cultivars. *Macroposthonia xenoplax* may not damage older plants of *I. crenata* (unpublished data, Barker et al.), although damage was previously reported for young plants (1). Field experiments that compare nematode density and plant age are needed.

Plants of *I. crenata* never recovered once symptoms of nematode decline had appeared. The stress of supporting *M. arenaria* on the plant-root system became clear when inoculated plants died after semi-drought periods. Although microplots were irrigated during the dry periods, the additional stress of nematode populations killed inoculated plants of Convexa and Helleri.

Nematode densities on intolerant hosts often decline as a linear function of time once the plant is severely damaged and cannot support the high densities (2). This response was observed for *M. arenaria* in these studies until the final sampling period when density increased. Since only a few plants of Convexa and Helleri were alive at the final sampling period, variation in sampling from relatively small plants may account for this density increase. The semi-automatic elutriator (3) used for the final extractions may be responsible for the higher nematode numbers.

Rotundifolia growth was related linearly to log initial density of *M. arenaria*. Although linear regressions usually adequately describe the effect of nematodes on growth of field crops, little work has been done to determine this relationship on perennial ornamentals (2). Correlation between plant growth and nematode population density at the time of planting, and lack of such correlation after this time, is a common phenomenon with perennials. The effects of previous damage to the root system, and the time required for relatively slow-growing woody ornamental plants to express decline contribute to this result. Because of this problem, the presence or

absence of quantitative correlations determined after planting often cannot be used to ascertain whether nematodes are the cause of poor growth in the field. Knowledge of tolerance limits for different cultivars to their principal nematode pathogens can be useful in a control program, but should not be used by nurseries as a substitute for production of disease-free stock.

Experiments at lower initial densities of *M. arenaria* on Convexa and Helleri are needed to determine whether a minimum threshold density exists and if growth is linearly related with very low densities.

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