Susceptibility of Japanese Holly to Criconemoides xenoplax, Tylenchorhynchus claytoni, and Certain Other Plant-Parasitic Nematodes[1]

R. AYCOCK, K. R. BARKER and D. M. BENSON[2]

Abstract: Three cultivars of *Ilex crenata:* 'Helleri', 'Convexa', and 'Rotundifolia' were inoculated with either *Criconemoides xenoplax, Helicotylenchus dihystera, Hoplolaimus galeatus, Trichodorus christiei,* or *Tylenchorhynchus claytoni* at 0, 200 (low), or 2,000 (high) nematodes/ 15-cm diam pot. Plants were kept in the greenhouse 10 mo prior to transplanting into 2.25-m^2 field plots. Helleri was severely stunted by *C. xenoplax. Criconemoides xenoplax* and *T. claytoni* caused lower plant vigor and top weights of Rotundifolia after 3 years. Above-ground symptoms included stunting, chlorosis, and leaf drop. Convexa was not susceptible to the nematodes tested. Low and high initial populations of the five nematodes tended to reach equilibria over the 3-year sampling period. *Helicotylenchus dihystera* and *C. xenoplax* occurred in the greatest densities regardless of host. None of the test plants were damaged by *H. dihystera.* Convexa was the least suitable host for nematode reproduction. *Hoplolaimus galeatus,* which was originally isolated from cotton, failed to reproduce or survive on any plant tested. Nematode densities over the 3-year sampling period did not always fit a linear regression model.

Japanese holly (*Ilex crenata* Thunb.) is widely used for landscaping in North Carolina and other of the southeastern United States. Growth decline of these ornamentals is often associated with the presence of parasitic nematodes (2, 3, 10, 11). *Pratylenchus penetrans* (Cobb) Filip. & Schuum.-Stekh. causes stunting and root lesions on hollies (*I. crenata* 'Convexa' Makino and *I. crenata* 'Rotundifolia' Hort.) (5). Nursery plantings of *I. crenata* naturally infested with several different nematode species recovered from growth decline in 2-4 mo when treated with dibromochloropropane (DBCP) (2). DBCP was not phytotoxic to *I. crenata* at rates up to 27.4 liters (a. i.)/ hectare (4).

Although polyspecific communities of nematodes often are associated with hollies, the effects of many given species of these pests on the various hollies are unknown. Hence, this study was initiated to determine the host susceptibility and suitability of Japanese holly to individual nematode species commonly found in their root zones.

MATERIALS AND METHODS

Three cultivars of *Ilex crenata* were inoculated with five different nematode species. *Hoplolaimus galeatus* Cobb was isolated from cotton (*Gossypium hirsutum* L.) and increased on corn (*Zea mays* L.), and *Criconemoides xenoplax* Raski was isolated from peach [*Prunus persica* (L.) Batsch] and increased on peach. The following species were isolated from rotation plots and increased on the hosts indicated: *Tylenchorhynchus claytoni* Steiner—corn; *Helicotylenchus dihystera* (Cobb) Sher—soybean [*Glycine max* (L.) Merr. 'Lee']; *Trichodorus christiei* Allen—corn. Nematodes were extracted by the rapid centrifugation-flotation method (6). Rooted cuttings of *Ilex crenata* Bailey 'Helleri', 'Convexa', and 'Rotundifolia' were transplanted into a sand-soil-peat potting mix (1:1:1 v/v) 30 days prior to nematode introduction. Nematode inoculum for each pot was distributed into six, 10-cm-deep holes adjacent to the plant crown and four, 10-cm-deep holes equidistant from the plant crown and pot edge. Either 200 (low initial population or P_i) or 2,000 (high P_i) nematodes were pipetted into the soil around each plant in a 15-cm diam clay pot. Noninfested plants served as controls. Five replications for each nematode-host combination (total = 180 pots) were arranged in a randomized complete block design on greenhouse benches. Plants were watered and fertilized as needed for the next 10 mo. Nematode populations were determined after 5 mo by taking four soil tube cores per pot and extracting.

Ten months after inoculation, these plants were transplanted singly, with roots

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[2] Professors and Assistant Professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27607. The authors gratefully acknowledge the technical assistance of Billy I. Daughtry, Donald W. Corbett, DeWitt Byrd, Jr., and Mrs. Margaret G. Gouge, as well as personnel at the Central Crops Research Station.

and soil intact, into 2.25-m^2 plots that had been fumigated (1.0 kg/10 m^2 methyl bromide) 30 days earlier at the Central Crops Research Station, Clayton, North Carolina. The same statistical design was used. Sterilized peat moss at the rate of 7,938 cm^3/plot was mixed into the Appling loamy sand with a rototiller prior to planting. For nematode assays, ten soil cores were collected around the drip line of each plant 2 wk after they were transplanted to the field in May, and then in the fall and spring for the next 2 years. Plants were fertilized and irrigated as needed. Each summer, plants were rated for vigor (rating scale: 0 = dead, 1 = most stunted plants, and 10 = most vigorous plants and pruning weights were recorded for each plant. The experiment was terminated the third fall after field transplanting. Final plant vigor rating, nematode count, and top weight for each plant were taken. For statistical analysis of the nematode population data, the log of nematode density (X) plus one [ND_t = log (X + 1)] was used.

RESULTS

Criconemoides xenoplax was pathogenic on cultivars Helleri and Rotundifolia. Symptoms commonly observed were interveinal chlorosis, leaf drop, and stunted plants. Plant vigor ratings and total top weights (which included accumulated pruning weights) were lower ($P < 0.05$) than controls (Table 1). Effects of low or high P_i on plant vigor or top weight were not different, so values in Table 1 are combined population means. Compared to controls, *T. claytoni* caused stunting in Rotundifolia and Helleri. However, Rotundifolia plants inoculated with *T. christiei* and *H. dihystera* had lower initial plant vigor, but not final top weight which did not differ from controls. Although *C. xenoplax* was associated with low vigor of Convexa at 27 mo, the plants recovered after 36 mo. Cultivar Convexa was damaged least, regardless of nematode species, and *H. dihystera* and *H. galeatus* were the least damaging of the nematodes, regardless of cultivar.

Helicotylenchus dihystera and *C. xenoplax* densities were generally high over the 39-mo period, regardless of cultivar (Fig. 1-3). Since curves for low and high P_i were similar, only the data for high P_i are presented. The population curves of the other three nematodes were significantly different, and the nematodes ranked in declining P_f order, *T. claytoni*, *T. christiei*, and *H. galeatus*, on all cultivars of *I. crenata*. Also, the density of a given nematode was similar for each cultivar except that *H. dihystera* was lower on Convexa than on Helleri or Rotundifolia (Fig. 1-3).

In regression analyses, *C. xenoplax* populations were not correlated with poor growth as measured by plant vigor or top weights of Helleri at any sampling date. Although top weight of Rotundifolia was not correlated with *T. claytoni* densities, plant vigor at 36 mo was correlated negatively with the nematode population 15 mo earlier (Fig. 4-A). Rotundifolia plant vigor at 36 mo was correlated negatively with *C. xenoplax* density at 10 mo, but top weight and vigor after 39 mo were correlated positively with nematode density 21 and 19 mo after plant inoculation, respectively (Fig. 4-B). *Criconemoides xenoplax* density at 39 mo was not correlated with Rotundifolia vigor or top weight.

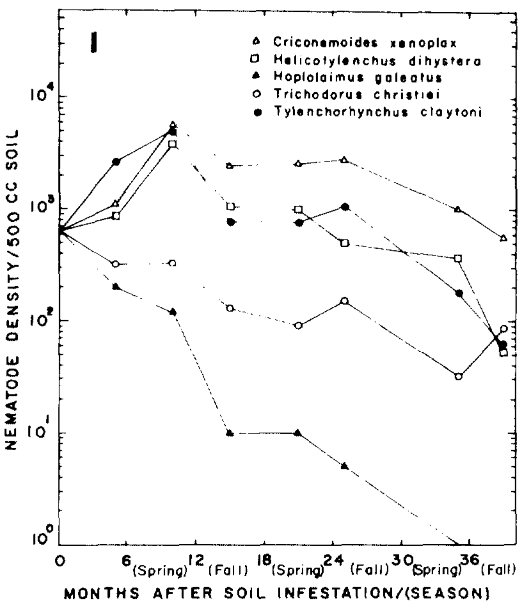
As a measure of host suitability for nematode reproduction and survival, the rate of increase, R, ($R = P_f/P_i$; where P_f is the final population density, and P_i equals the initial population density) was calculated according to Oostenbrink (8) for each nematode 5, 25, or 39 mo after soil infestation. A nematode population reproduces on its host at a faster rate than it dies in soil if the R value is greater than 1.0. Values of R ranged from 0.1 for *H. galeatus* to 142.6 for *C. xenoplax* on Rotundifolia (Table 2). All nematodes except *H. galeatus* increased at a greater rate from the low P_i than from the high P_i. For instance, the low P_i of *C. xenoplax* increased 6-fold after 5 mo on Helleri, and by 39 mo it had increased to an R value of 93.6. A similar increase was measured on Rotundifolia (R= 142.6 at 39 mo). However, *C. xenoplax* did not increase on Convexa. *Hoplolaimus galeatus* reproduced very little on any plant as R values were 0.8 or less. *T. christiei* and *T. claytoni* were the only nematodes that reproduced on Convexa, and this occurred during the time plants were in the greenhouse (Table 2). *T. claytoni* damaged Rotundifolia, but the low P_i of this pest increased equally well on Helleri where little damage was

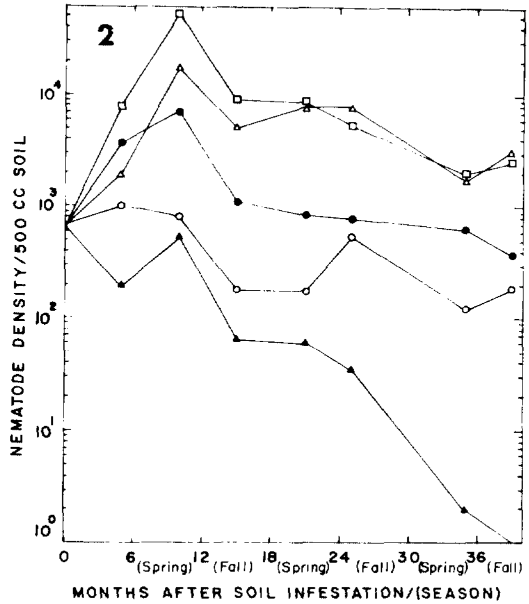
TABLE 1. Growth responses of three cultivars of *Ilex crenata* inoculated with five different nematodes.

Nematode	Helleri: Postinoculation plant vigor rating[a] after: 16 mo	27 mo	36 mo	39 mo	Top wt (g)	Convexa: Plant vigor rating after: 16 mo	27 mo	36 mo	39 mo	Top wt (g)	Rotundifolia: Plant vigor rating after: 16 mo	27 mo	36 mo	39 mo	Top wt (g)
Trichodorus christiei	7.5	8.2	8.8	8.8	1875	6.8	6.7	8.1	9.0	1055	6.4**	6.1*	8.1	8.4	1254
Tylenchorhynchus claytoni	5.4**[b]	5.9	6.3	6.0	1080*	7.1	5.8	7.2	8.1	904	6.7**	5.7**	7.5	7.8*	1104*
Helicotylenchus dihystera	7.8	8.0	8.6	8.3	1770	7.2	5.9	7.9	8.2	977	7.0*	7.0	8.4	8.6	1384
Criconemoides xenoplax	5.1**	4.1**	5.2*	3.9**	787**	5.6	5.3*	5.9	6.6	763	3.9**	3.4**	4.4**	4.8**	537**
Hoplolaimus galeatus	8.0	7.7	8.3	7.8	1579	8.5	7.0	8.4	9.1	1161	7.9	7.0	8.7	9.3	1455
Control	7.4	7.4	7.6	7.7	1629	7.0	7.2	6.5	7.4	937	8.9	7.8	8.7	9.5	1461
LSD .05	1.5	1.9	2.0	2.2	544	1.7	1.5	1.9	2.1	304	1.5	1.4	1.3	1.3	313
.01	2.0	2.6	2.7	3.0	726	2.3	2.0	2.6	2.8	406	2.0	1.8	1.8	1.8	418

[a]Rating scale: 0 = dead; 10 = vigorous growth.

[b]Asterisks (* and **) indicate a significant difference $P = 0.05$ and $P = 0.01$, respectively, as compared to control.





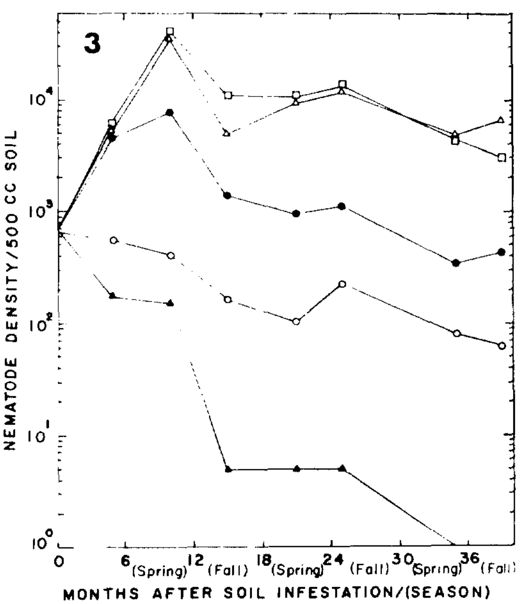


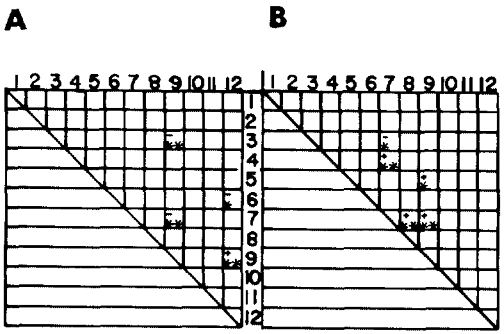
FIG. 1-3. Population curves for five nematode species on three hosts sampled Spring and Fall over a 39-month period. *1) Ilex crenata* 'Convexa'; *2) I. crenata* 'Helleri'; *3) I. crenata* 'Rotundifolia'. Initial population was 2,000 nematodes.

observed. Although *H. dihystera* did not damage any of the three cultivars of *I. crenata* which were tested, both Helleri (R = 3.8-40.7) and Rotundifolia (R = 5.9-29.4) were suitable hosts for reproduction.

Nematode densities did not decline in a linear way. When the semilogarithmic transformation (1) was applied to either curve of low or high P_i for each nematode-plant combination, only 50% of the linear regression coefficients were significant. Values of significant slopes ranged from −0.019 for low P_i of *T. christiei* on Convexa to −0.104 for high P_i of *H. galeatus* on Rotundifolia. Seasonal variation in nematode density between spring and fall each year accounted for the poor linear regressions.

DISCUSSION

The pathogenicity of *C. xenoplax* to Helleri and Rotundifolia in these studies is the first report of this association. Heald and Jenkins (5) used a 5-fold greater nematode density of *C. curvatum* Raski on Convexa and Rotundifolia, but they did not



LEGEND

1. Plant vigor	16 mo	7. Nematode density	10 mo
2. Plant vigor	27 mo	8. Nematode density	15 mo
3. Plant vigor	36 mo	9. Nematode density	21 mo
4. Plant vigor	39 mo	10. Nematode density	25 mo
5. Top weight		11. Nematode density	35 mo
6. Nematode density	5 mo	12. Nematode density	39 mo

FIG. 4-(A, B). Correlation matrices for two growth indices and nematode density over a 39-month period. *A) Ilex crenata* 'Rotundifolia' and *Tylenchorhynchus claytoni; B)* Rotundifolia and *Criconemoides xenoplax.* Asterisks (*, **) indicate significant correlation [negative (—), or postive (+)] P = 0.05 and 0.01, respectively.

find a pathogenic relation. *Criconemoides xenoplax* may be better adapted to Japanese holly in North Carolina than *C. curvatum,* even though *C. curvatum* has been frequently encountered on *I. crenata* (10).

The association of *T. claytoni* along with other nematodes on *I. crenata* has been reported (2, 10, 11), but apparently this is the first report of a pathogenic relationship between this nematode and Rotundifolia. Both vigor and top weight reflected the damage caused by this nematode. Compared to controls, *T. christiei* and *H. dihystera* resulted in lower plant vigor of Rotundifolia initially, but plant vigor and top weight at 39 mo were not significantly affected. No plants tested were damaged by *H. galeatus,* and none were suitable hosts. The common frequency with which *H. galeatus* has been encountered on *I. crenata* (2, 10) suggested that our population from cotton was not adapted to this ornamental.

Host suitability was correlated with host susceptibility for *C. xenoplax* and *T. claytoni,* but not for *H. dihystera.* Helleri and Rotundifolia supported high populations of *H. dihystera* without decline of plant vigor or loss of top weight. Convexa was the least suitable Japanese holly tested.

No difference in plant vigor or top weight was observed between the low and high initial P_i. This may be explained by the fact that, whether low or high initially, nematode densities tended to level out over the sampling period. This tendency of initially different densities to reach an equilibrium has been reported for *Heterodera schachtii* Schmidt on cabbage (7). This leveling of population densities apparently was due to a given species reaching the equilibrium density as described by Seinhorst (9). This equilibrium occurs when available food is just sufficient to maintain the population. The merging of nematode densities was evident also for all nematode species on Convexa even though it was an unsuitable host.

TABLE 2. Rate of population change (R) of five nematodes on three varieties of Japanese holly at 5 and 39 months after inoculation.

		R[b]					
		Helleri		Rotundifolia		Convexa	
Nematode	P_i[a]	5 mo	39 mo	5 mo	39 mo	5 mo	39 mo
Trichodorus christiei	low	48.0	3.7	24.7	3.8	5.4	1.1
	high	1.5	0.3	0.8	0.1	0.5	0.1
Tylenchorhynchus claytoni	low	52.0	6.1	42.5	16.2	22.3	0.3
	high	5.5	0.6	6.9	0.7	4.0	0.1
Helicotylenchus dihystera	low	19.5	40.7	9.8	29.4	0.6	0.7
	high	11.9	3.8	8.9	5.9	1.3	0.1
Criconemoides xenoplax	low	6.1	93.6	48.7	142.6	1.9	0.8
	high	2.9	4.6	8.0	10.3	1.6	0.9
Hoplolaimus galeatus	low	0.8	0	0.1	0	0.1	0
	high	0.3	0	0.3	0	0.3	0

[a]Initial low population was 200 nematodes/pot, and the initial high population was 2,000 nematodes/pot.
[b]R equals P_f/P_i, where R is the rate of increase or decrease, P_f is the final population and P_i is the initial low or high population (based on number of nematodes in 500 cm^3 of soil).

The lack of correlation of a simple linear regression model for the nematode density data over the 3-year sampling period reflects the dynamic influence of the host in the population cycle. Apparently, a cyclic population shift occurs each growing season as new root growth in the spring stimulates nematode reproduction. If the host is susceptible to the nematode (i. e., stunting and lower vigor in this study), increased densities mean lower available food and a declining population in the fall. Additional levels of P_i with more frequent sampling may be necessary to demonstrate this relationship. When the host is not susceptible to damage but is suitable for nematode reproduction, densities will continue to increase as food becomes available as with *H. dihystera*. However, this population increase is limited to the available space for root growth per unit volume of soil. Of course, the carry-over population the following spring is influenced by the ability of a given nematode species to survive semi-dormancy of the host during winter.

The susceptibilities of cultivars Helleri and Rotundifolia to *C. xenoplax* and *T. claytoni* are useful diagnostic data for recommending whether or not control measures are warranted. Why a host is tolerant to given nematode species remains an important unanswered question.

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