REACTION AND HOST-PARASITE RELATIONS OF CERTAIN WOODY ORNAMENTAL PLANTS TO MELOIDOGYNE INCOGNITA

BY

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INTRODUCTION

The root-knot nematode Meloidogyne incognita (Kofoid and White 1919) Chitwood, 1949 occurs throughout the southern United States and is responsible for serious economic damage to many crop plants. While there have been many investigations dealing with the pathogenicity, host range, and control of this nematode, with vegetable and field crops, there has been very little work with woody ornamental plants. With the increasing economic importance of this latter group of plants, knowledge of the pathogenicity, host-parasite relations, and host range of root-knot nematodes on these plants becomes essential.

Recent advances in nematology have tended to invalidate, or at least make questionable, some of the earlier work with root-knot nematodes on ornamentals, as well as other plants. Prior to 1949 all root-knot nematodes were considered as being of a single species, Heterodera marioni (Cornu, 1879) Goodey, 1932. Chitwood (8), in 1949, removed the root-knot nematodes from the genus Heterodera and transferred them to the genus Meloidogyne. At that time he described 5 species and 1 subspecies in this latter genus. This, in effect, made it virtually impossible to determine which of the presently recognized root-knot species earlier workers had been dealing with when root-knot host lists had been compiled. More recently, evidence of physiological specialization within M. incognita (16, 25, 30) has further complicated interpretation of results from earlier work, particularly with respect to host range studies.

Certain other factors have complicated studies with woody ornamentals and nematodes. These plants are perennial and, as with any perennial

plant, longer periods of time and special techniques for inducing near optimum growth under greenhouse conditions may be necessary for proper evaluation of nematode-host relations. The continuing addition of new varieties and forms to an already long list of ornamentals has created a need for more information relative to response of these plants to nematodes.

The present work was initiated to determine the reaction to M. incognita, of certain woody ornamentals, whose response to this nematode had not been reported previously or reported as resistant, and to investigate host-parasite relationships in selected resistant species of these plants.

REVIEW OF LITERATURE

Early 20th century studies of root-knot nematodes and ornamental plants were generally gross in nature. Ornamentals showing root-knot symptoms were categorized as susceptible or resistant usually on the basis of severity of galling. Resistance or susceptibility has more recently been based mainly on the ability of the nematode to complete its life cycle within the plant. In addition to changes in criteria of resistance, the reclassification of root-knot nematodes in 1949 (8), as already mentioned, has further served to make questionable the reliability of older host lists. These host lists, however, still serve as practical guides for tracing ornamentals presumed to be hosts.

Marcinowski (24) published the first general host list of the root-knot nematode. Bessey (4), two years later added an enlarged host list to the literature. Buhrer, et al. (6), and Buhrer (5) published comprehensive more up-to-date host lists. T. Goodey (20) and Tyler (38) increased the host range literature with their publications. These host records depended on a multitude of different sources and as a result contain discrepancies in standardization of host names and differences relative to resistance. In 1956 J. B. Goodey and Mary T. Franklin updated T. Goodey's (21) catalogue of nematode parasites and their hosts, and in 1959 J. B. Goodey, et al. (19) supplemented this list with additional hosts. Franklin and Hooper (18), more recently published a list of plants recorded as resistant to Meloidogyne spp.

Whittle and Drain in 1935 (39) were among the first investigators to become concerned with root-knot damage to ornamentals. They grew de-

ciduous ornamentals in root-knot infested soil several seasons in order to determine their susceptibility. Later Altstatt (1) inoculated rose understocks with a larval and egg-mass suspension and grew them 9 months in order to rate their susceptibility. He showed the only understock free from infection was Rosa blanda X R. multiflora. Most of the information obtained from these early tests depended on gross examination of infected plants. Some proof of relative pathogenicity of various root-knot species on ornamentals and other plants has been undertaken subsequent to the taxonomic revision of this group of nematodes. Chitwood, et al. (9) tested 5 peach varieties and a hybrid to 2 species. The general trend was toward lowest root weight in pots having the largest quantity of inoculum and the peach varieties differed in their response to the 2 species. Chitwood's evidence also showed that moderate quantities of M. incognita and M. javanica sometimes caused significant increases in peach growth for which there seemed to be no explanation. Schindler (33) tested Albizzia julibrissin Durazz. to 4 species and 2 subspecies of Meloidogyne to discover whether root galls were actually caused by root-knot nematodes. After 5 months the plants inoculated with M. incognita and the subspecies acrita showed no galling. Those inoculated with M. javanica showed slight root proliferation, and those inoculated with M. arenaria, the subspecies thamesi and M. hapla showed various degrees of galling.

Since the reclassification of root-knot nematodes, some effort has been made toward determining distribution and general host range preferences of these and other plant-parasitic nematodes. Sasser (32) determined the susceptibility of a group of plants to 4 species of Meloidogyne.

He reported Nerium oleander L. susceptible to M. incognita but resistant to M. hapla and a Glen Dale Hybrid azalea resistant to 4 species and 1 subspecies. Three investigations more intimately associated with ornamentals were undertaken by McCoy(27), Stessel (36), and Sommerville, et al. (34). McCoy found Meloidogyne hapla in 3 out of 27 plant species tested. Stessel did not find Meloidogyne associated with nursery plants in Rhode Island. Sommerville, et al. found Meloidogyne species associated with boxwood, roses, trees, and turf from soil samples taken in 21 states.

More is known about the pathogenicity of Meloidogyne species in field and vegetable crops. Dropkin (16) tested soybean varieties to races of M. incognita acrita and noted that the relative amount of galling on the soybeans could not be correlated with egg mass production. Hare (22) tested pepper resistance to M. incognita acrita and reported only 4 varieties as being resistant as compared with 135 varieties being susceptible. McCracken (28) tested 90 varieties of agronomic and horticultural crops with 4 populations of M. incognita acrita and showed none of the varieties tested was resistant to invasion by the nematode.

Varietal responses vary with Meloidogyne species and races as the previous papers have indicated. Resistance in plants was originally thought to be (35) of a morphological type whereby the root tissue restricted entrance of the root-knot nematodes. Barrons (3) suggested that resistance was due to substances in plant tissues which neutralized the stimulating effect of the nematode saliva. Christie (12) agreed with Barrons on the nature of resistance but cautioned that other factors might be responsible for causing a plant to be an "unsuitable host".

Christie stated that failure of Meloidogyne larvae to complete their life cycles is common among highly unsuitable hosts. This type of host resistance is intimately linked with the inability of the larvae to stimulate normal giant cell formation. Where giant cells are not formed, larval development is retarded or prevented entirely. Another kind of resistance is a necrotic reaction associated with larval penetration and feeding. Dean and Struble (15) reported extensive necrosis in tomato and resistant varieties of sweetpotato and found that M. incognita larvae did not mature in these tissues. Dropkin and Nelson (17) also recognized a necrotic reaction in soybeans infected with M. incognita and M. incognita acrita. When necrosis developed in these soybeans very little cell enlargment occurred and larvae matured poorly. Resistance to root-knot nematodes in plants is still only partly understood.

In an attempt to resolve some of the aspects of resistance, certain investigators have taken a histological approach. Christie's (10) description of gall morphology in tomato serves as a classic guide for present day studies. He suggested that all evidence points to the substance secreted through the stylet of the nematode as the stimulating factor causing abnormal root developmental changes.

Typical susceptible and resistant host reactions have been noted in some ornamentals. A few of the susceptible reactions have been characterized by pronounced galling of roots and growth reduction as in Forsythia intermedia Zabel infected with M. incognita acrita (29), and sizable populations of M. hapla larvae reaching maturity in rose roots (13). Similar root-knot responses occurred in gardenia infected with 3 species of Meloidogyne (14). Some typical resistant reactions have been

observed in the same plants. M. hapla larvae that entered rose roots undergoing secondary growth stimulated fewer and smaller giant cells with wound cork developing in the areas of invasion (13). M. hapla was not capable of maturing and reproducing in roots of Forsythia intermedia (29).

MATERIALS AND METHODS

The nematode used in these studies has been maintained in the greenhouse since 1956 on tomato. This population originated from 5 selected single egg masses from cotton grown in the nematode-wilt nursery near Hollis, Oklahoma. This nematode had originally been identified as Meloidogyne incognita acrita. In line with the suggestion of Triantaphyllou and Sasser (37) that the subspecies no longer be recognized, this nematode is now designated as M. incognita.

The nematode was increased on Rutgers tomato grown in 6 x 8 x 36-in. wooden boxes and in 4-in. clay pots, both contained steam sterilized soil and were recessed in metal trays to avoid contamination.

The ornamental plants were acquired primarily from Athens Nursery Company, Athens, Alabama as 2½-in. pot-grown liners. The remaining ornamentals were obtained from the Oklahoma State University Department of Horticulture as liners, cuttings or seed. Plants used in the tests are listed in Table I.

Each test on an ornamental species included 5 replicates with nematodes and 2 uninoculated controls. All plants were grown in a sterilized soil mixture of 3 parts loam to 1 part sand. Each replicate was inoculated with 2 g of chopped root-knot infected tomato roots. Each plant was knocked out of the pot in which it was growing and washed free of soil under a stream of tap water. The plant was then placed in a 4-in. pot to which soil and inoculum were evenly added.

These potted plants were placed on inverted 4-in. pots in metal trays in the greenhouse and allowed to grow for a 6 to 8 week period. At the

TABLE I

A LIST OF WOODY ORNAMENTALS TESTED
FOR THEIR REACTION TO MELOIDOGYNE INCOGNITA

Botanical Name	Common Name
Camellia sasanqua Thunb.	Sasanqua Camellia
Cedrus deodara Loud.	Deodar Cedar
Chamaecyparis pisifera Sieb. & Zucc. squarrosa Beissn. & Hochst.	Cyano-virdis Sawara Flasecypress
Cotoneaster horizontalis Decne.	Rock Cotoneaster
Elaeagnus pungens Thunb.	Thorny Elaeagnus
Hedera helix L.	English Ivy
Ilex cassine L. angustifolia Ait.	Alabama Dahoon
Ilex cornuta Lindl.	Burford Chinese Holly
Ilex crenata Thunb.	Hetz Japanese Holly
Jasminum nudiflorum Lindl.	Winter Jasmine
Juniperus chinensis L.	Hetz Chinese Juniper
Juniperus horizontalis Moench douglasi Rehd.	Waukegan Creeping Juniper
Juniperus horizontalis Moench plumosa Rehd.	Andorra Creeping Juniper
Juniperus procumbens Miq.	Japgarden Juniper
Loropetalum chinense Oliv.	Loropetalum
Poncirus trifoliata Raf.	Trifoliate - Orange
Prunus laurocerasus L. zabeliana	Zabel Common Laurelcherry
Osmanthus fortunei Carr.	Fortunes Osmanthus
Syringa persica L.	Persian Lilac

Table I (Continued)

Botanical Name	Common Name
Thuja occidentalis L.	Woodward Eastern Arborvitae
Thuja orientalis L.	Berckmanns Oriental Arborvitae
Thuja orientalis L.	Dwarf Greenspike Oriental Arborvitae
Vitex agnus-castus L.	Lilac Chastetree
Zizyphus jujuba Mill.	Common Jujube

termination of each test, soil samples were taken from each inoculated plant and control then processed by Seinhorst's inverted-flask technique as modified by Chapman (7). It was determined from controlled experiments that the nematodes recovered with this technique represented principally those resulting from reproduction on the test plant rather than those surviving from the original inoculum added.

All root systems were washed free of soil and the extent of galling was rated on a scale of 1 - 5: 1 denoting no galls, 2 a trace, 3 moderate, 4 severe and 5 very severe galling. Since only young, actively growing roots are attacked by the nematode, a root sample for staining and observing nematode development was taken only from that part of the root system which had grown since inoculation. These roots were finely chopped, mixed thoroughly, and a 200 mg subsample was taken for staining. The remainder of chopped roots was mixed with steamed soil in a 4-in. pot and in this a Rutgers tomato plant was grown for 30 days. At the end of this time the tomato roots were washed free of soil and the amount of galling was rated on the same scale used for the ornamentals. The purpose of this bioassay test was to supplement other data on reproduction by the nematode in the ornamental test plant.

The staining procedure used for studying nematodes in whole roots was that developed by McBeth, et al. (26). The 200 mg portion of roots was boiled a few seconds in lactophenol-acid fuchsin and washed in tap water to remove excess stain. These roots were then cleared in lactophenol for 1 to 3 days.

The cleared root pieces were crushed between 2 glass slides, and with a binocular dissecting microscope counts were made of the various

developmental stages of the nematodes. Developmental stages were determined using the method of Christie (11) as illustrated in Fig. 1. A description of these stages follows. Group A includes larvae that have begun to grow to the stage where they still retain a somewhat conical tail. Group B includes larvae that have a more or less hemispherical posterior end terminated by a spike to the stage where they are nearing the final molts. Group C includes females that had completed the final molts to the stage where they were almost fully grown. Group D includes females that are fully grown but had not laid eggs. Group E includes egg-laying females.

In order to study in more detail host-parasite relations of some of the apparently more resistant plants, infected roots of these were prepared for sectioning and staining. Plants used were Juniperus horizontalis douglasi, J. horizontalis plumosa, Thuja orientalis Dwarf Greenspike, and Poncirus trifoliata. The first 3 species were grown for 75 days and the fourth species for 42 days after inoculation. Root pieces were killed in FAA, dehydrated in tertiary butyl alcohol, and infiltrated with a paraffin, beeswax, Tissuemat mixture for 2 weeks. Sections were cut on a rotary microtome at 15 μ and stained with safranin and fast green (31).

Bailey's Manual of Cultivated Plants (2) and Standardized Plant Names (23) served as guides for standard horticultural nomenclature.

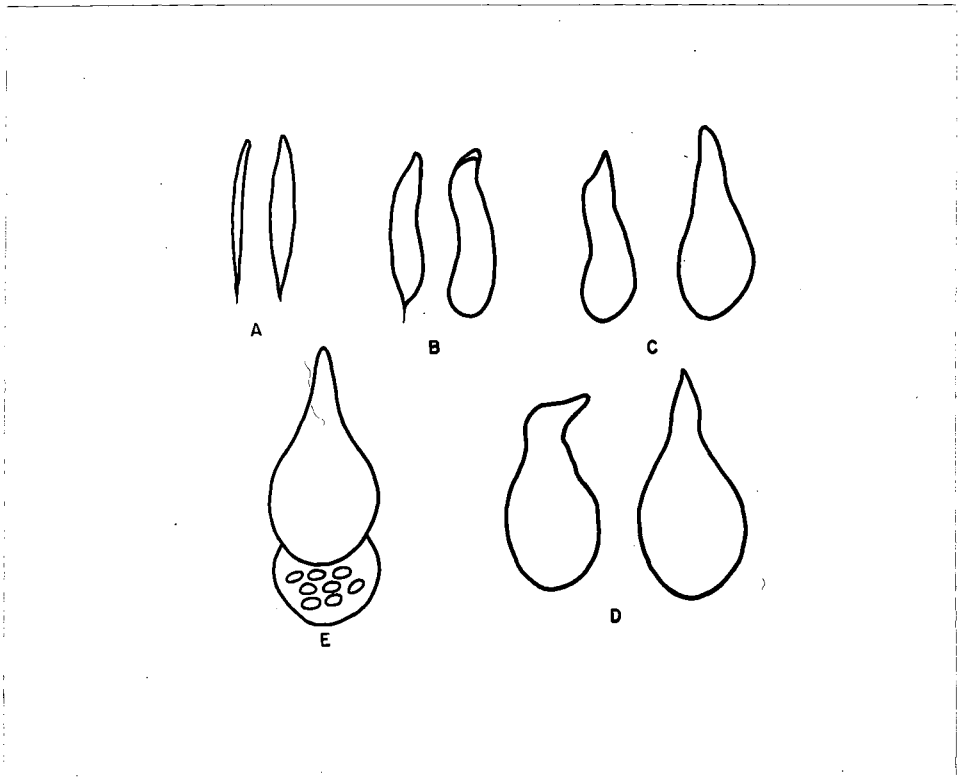

Fig. 1. Classes into which nematodes were grouped according to the amount of development they had undergone. (After Christie, 1946)

RESULTS

Host Response and Host Suitability for Nematode Development and Reproduction

Test plants used in this investigation were studied to determine their susceptibility to *M. incognita*. Relative susceptibility was based on data from galling and bioassay indexes and also from observations on nematode development in these test plants. Results appear in Table II; all data presented are means. Low values for the galling and bioassay indexes indicate a species may be a non-suitable host whereas high values for these catagories may indicate a suitable host. Supplementary evidence for determining host-suitability is provided by data from nematode development. Generally failure of nematodes to reach the adult and egg-laying stage in a species indicate the species is a non-suitable host. Moderate to large numbers of nematodes that do become adults and lay eggs indicate the species is a suitable host.

Larvae entered all species but failed to reproduce in 14 species, and reproduction was high in only 3 species. The majority of nematodes in 17 species did not develop beyond stage B, and in 6 species nematodes were noted not to develop beyond stage C. Gall index values were higher in those plants supporting the nematode through its complete life cycle. Egg mass size ranged from 10-25 eggs per egg mass in *J. horizontalis douglasi* to 100-300 eggs per egg mass in *O. fortunei*. Few eggs per egg mass suggest that maturation was somehow unfavorably affected. This reduction in egg mass size, if consistent may be associated with some degree of host resistance to normal larval development. The bioassay index test verified the presence of adult egg-laying females in the

TABLE II

REACTION OF WOODY ORNAMENTAL PLANTS
TO MELOIDOGYNE INCOGNITA

Plant	Gall Index[a]	Bioassay Index[b]	Nematodes in each Developmental Stage[c]				
			A	B	C	D	E
	No.	No.	No.	No.	No.	No.	No.
Osmanthus fortunei	4.1	5.0	14.7	4.9	23.0	52.8	30.2
Jasminum nudiflorum	4.0	4.8	18.8	31.2	24.2	25.8	12.8
Vitex agnus-castus	4.0	1.7	9.1	19.8	1.7	0.0	0.0
Syringa persica	3.8	-	29.6	17.0	1.4	0.0	0.0
Ilex cornuta burfordi	3.4	2.0	2.4	15.7	9.5	4.0	0.5
Ilex crenata hetzi	3.1	3.5	2.0	4.0	4.3	6.0	12.1
Ilex cassine angustifolia	3.1	1.0	3.0	3.3	0.1	0.0	0.0
Poncirus trifoliata	2.6	1.2	2.6	0.0	0.0	0.1	0.0
Thuja orientalis Dwarf Greenspike	2.0	1.0	1.8	57.2	7.4	1.0	0.0
Camellia sasanqua	1.8	1.4	1.9	3.0	0.2	0.1	0.0
Loropetalum chinense	1.6	2.0	3.6	1.2	0.6	0.0	0.0
Cotoneaster horizontalis	1.2	1.8	0.0	13.0	1.0	0.0	0.0
Juniperus horizontalis douglasi	1.2	3.2	1.0	2.6	4.4	1.2	1.4
Thuja orientalis Berckmanns	1.2	1.0	1.8	4.5	0.0	0.0	0.0
Hedera helix	1.2	1.1	0.2	0.0	0.0	0.0	0.1
Prunus laurocerasus zabeliana	1.2	1.0	0.2	0.0	0.0	0.0	0.0

Table II (Continued)

Plant	Gall Index[a]	Bioassay Index[b]	Nematodes in each Developmental Stage[c]				
			A	B	C	D	E
	No.	No.	No.	No.	No.	No.	No.
Thuja occidentalis woodwardi	1.1	1.2	1.0	23.2	0.0	0.0	0.0
Juniperus horizontalis plumosa	1.0	2.0	0.0	1.6	2.0	0.0	0.2
Chamaecyparis pisifera squarrosa	1.0	1.0	0.4	11.2	0.0	0.0	0.0
Elaeagnus pungens	1.0	1.0	0.3	0.0	0.0	0.0	0.0
Cedrus deodara	1.0	-	1.4	5.8	0.8	0.0	0.0

[a] 1 indicates no galling; 2 slight or trace amounts of galling; 3 moderate; 4 severe; and 5 very severe galling.

[b] From tomato roots in soil to which chopped roots of test plant had been added to determine if nematode reproduction had occurred in test plant. Index values as in footnote [a].

[c] A) Larvae still possess conical tail; B) larvae with hemispherical posterior end terminated by spike; C) females that have completed the final molts to the nearly fully grown stage; D) females that are fully grown but have not laid eggs; E) egg-laying females.

species with highest egg mass numbers. Typically, distinct galls or knots were not found on roots of coniferous species. Symptoms on these were uniform swellings or enlargements 2 to 3 times the normal root diameter and trace amounts of galling. Other damage to these plants was shown by reduced root growth and short irregularly formed roots. Uninoculated roots of this group of plants were consistently uniform, long and fibrous. In these tests there were no outstanding differences in top growth between inoculated and uninoculated plants.

Reproduction in I. crenata hetzi was high, relatively low in I. cornuta burfordi and not noted in I. cassine angustifolia. These levels of reproduction were verified by bioassay indexes which were respectively high, low and zero. Respective gall indexes did not closely substantiate the foregoing values. It has been shown (11), however, that extent of galling and rate of reproduction are not always related. Galls on the latter species were smaller than galls on the other 2 hollies.

The roots of J. nudiflorum and O. fortunei, the 2 most susceptible species, were heavily galled and many infected roots of both species ceased growing. Individual galls on O. fortunei predominately contained more than one larva; occasionally 20 or more larvae were counted from one gall. P. trifoliata was moderately galled, but larvae were found in only a few galls, and many of these larvae when extracted were highly vacuolated. H. helix was not galled; root growth was, however, stunted in comparison to uninoculated controls. Generally uninoculated root systems of all species tested were better developed than those of inoculated plants.

Host-Parasite Relations with Certain Resistant Plant Species

No information relative to pathological histology of Meloidogyne sp. and ornamental conifers has been found in the literature. This group of plants is generally considered resistant, probably because little or no galling occurs. Since varied root responses developed as a consequence of larval penetration in these plants an attempt was made to determine the causes of these responses. A histopathological study was undertaken to study larval penetration, development, reproduction, and host response in 3 conifers and 1 dicotyledonous plant.

Larvae penetrated active J. horizontalis plumosa roots within 12 hours after inoculation. Larvae moved intercellularly through the cortex into the vascular region where they settled to feed. Established larvae stimulated giant cell formation but there was no noticeable necrosis. Giant cells were few, thin-walled, and asymmetrical. They contained many large nuclei and were usually aligned along the main axis of the root. Moderate hypertrophy occurred in cortical and vascular tissues occupied by larvae. Xylem element formation in these vascular regions was disrupted. Vascular disorganization eventually resulted from hypertrophied parenchymatous tissue. Few larvae developed beyond stage B and none were observed to reach stage E. J. horizontalis douglasi reacted in a similar manner to invasion by larvae. Effects of larval penetration in these 2 junipers is shown in Fig. 2.

Histopathological studies showed the reaction of T. orientalis Dwarf Greenspike was similar to that of the junipers. Differences were nevertheless present. A distinct resistant reaction resulted when some infections became necrotic. Necrosis spread from cells in feeding areas

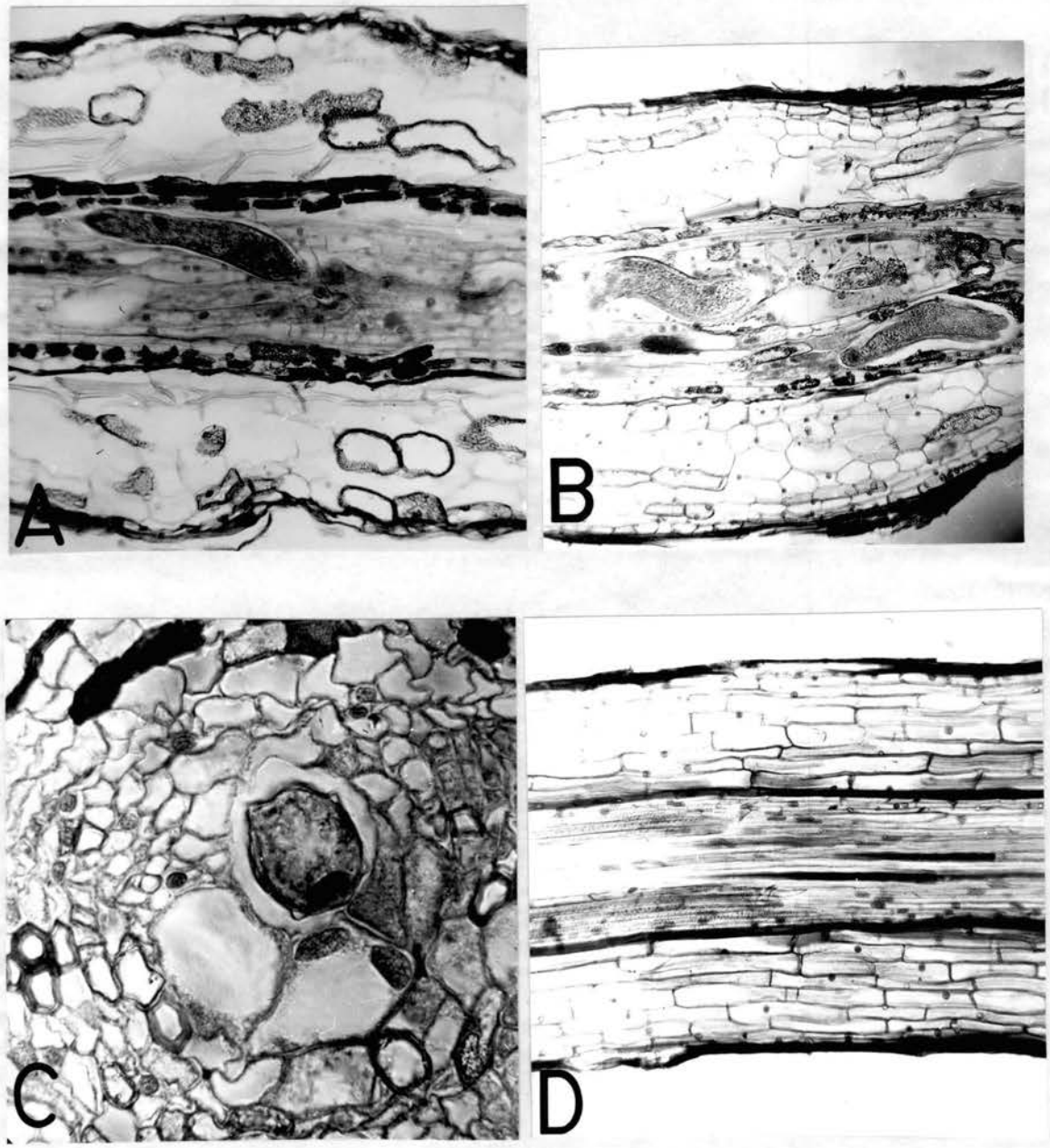

Fig. 2. Sections through Juniperus horizontalis plumosa and J. horizontalis douglasi roots showing Meloidogyne incognita feeding in vascular tissues. A,B) Longitudinal sections of infected J. horizontalis plumosa and J. horizontalis douglasi roots respectively. C) X-section of J. horizontalis plumosa root showing M. incognita and adjacent giant cells. D) Uninfected longitudinal section of J. horizontalis plumosa root.

to adjacent cells in all directions. Nematodes seldom reached the adult egg-laying stage. When egg masses were found, they were always in the cortical area and they contained fewer eggs per egg mass than was normally present in susceptible species such as O. fortunei. Males were produced from more eggs than usual which may be an indication of resistance because it is a situation uncommon to susceptible species as noted by Dropkin (16). Larval development and giant cell formation are shown in Figs. 3 and 4.

Poncirus trifoliata was chosen for a histological analysis because of its habit of developing galls that seldom contained larvae. Larvae penetrated the root-tip region and eventually migrated into vascular tissues to feed where giant cell formation was initiated. Giant cell formation was rare and those initiated were small, thin-walled, and asymmetrical. The nuclei of giant cells and all parenchymatous cells in their vicinity contained large dark staining nucleoli. These nucleoli were characteristic of root meristem tissue of this plant. Necrotic reactions commonly occurred in cortical and vascular tissues occupied by larvae. This necrosis, in severe cases, sometimes spread filling entire root tips. Galling was a direct result of extensive cortical and vascular hypertrophy and hyperplasia. This growth response consequently disrupted all xylem and phloem organization. Larvae never entered roots in abundant numbers and were never noted to develop beyond stage A. Growth responses and damage associated with larvae are illustrated in Fig. 5.

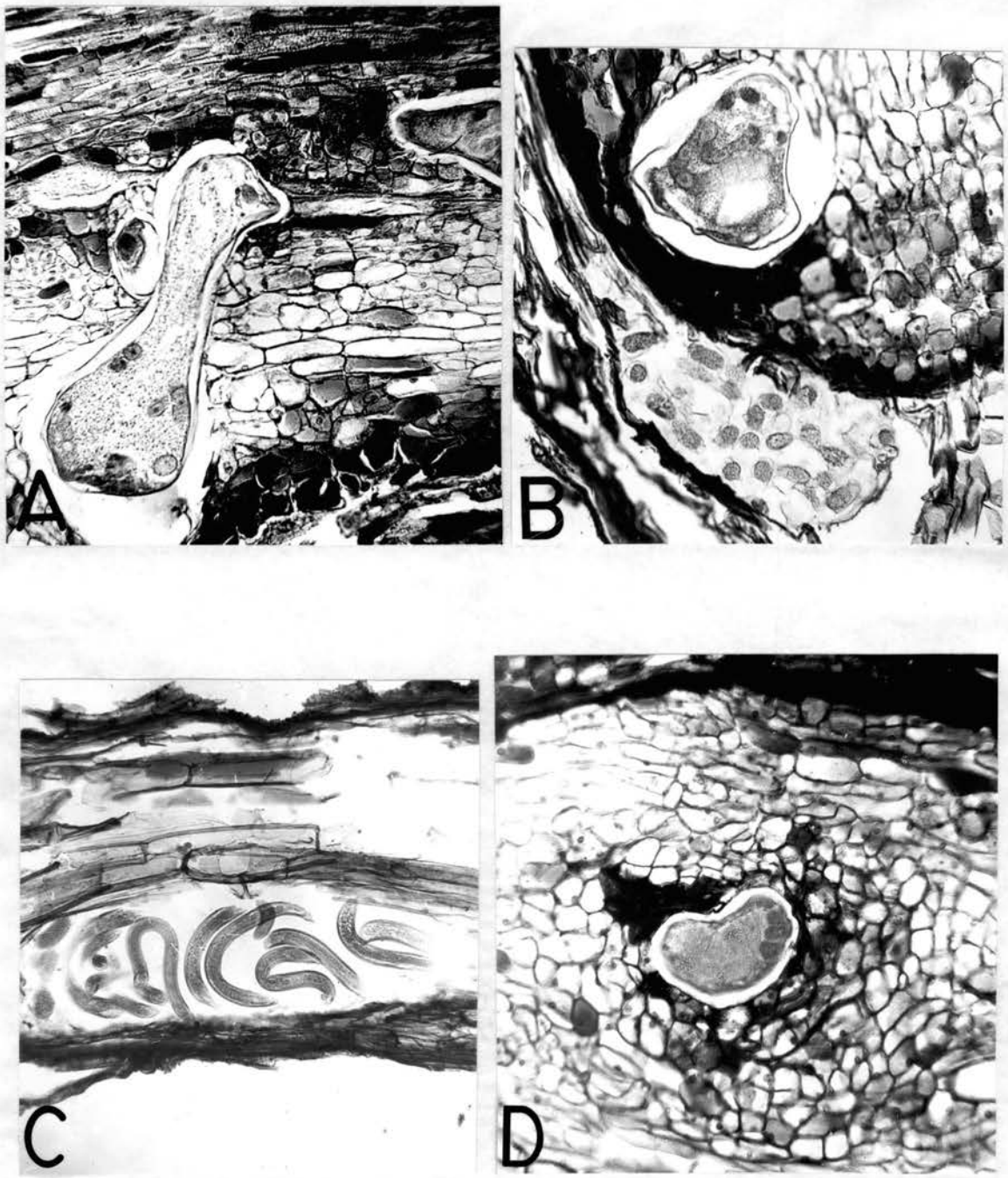

Fig. 3. Longitudinal sections of Thuja orientalis Dwarf Greenspike showing development of and damage caused by Meloidogyne incognita. A) Larvae in vascular and cortical tissues. B) Adult with egg mass deposited in cortex. C) Larvae in cortical tissue. D) Necrotic phloem surrounding nematode.

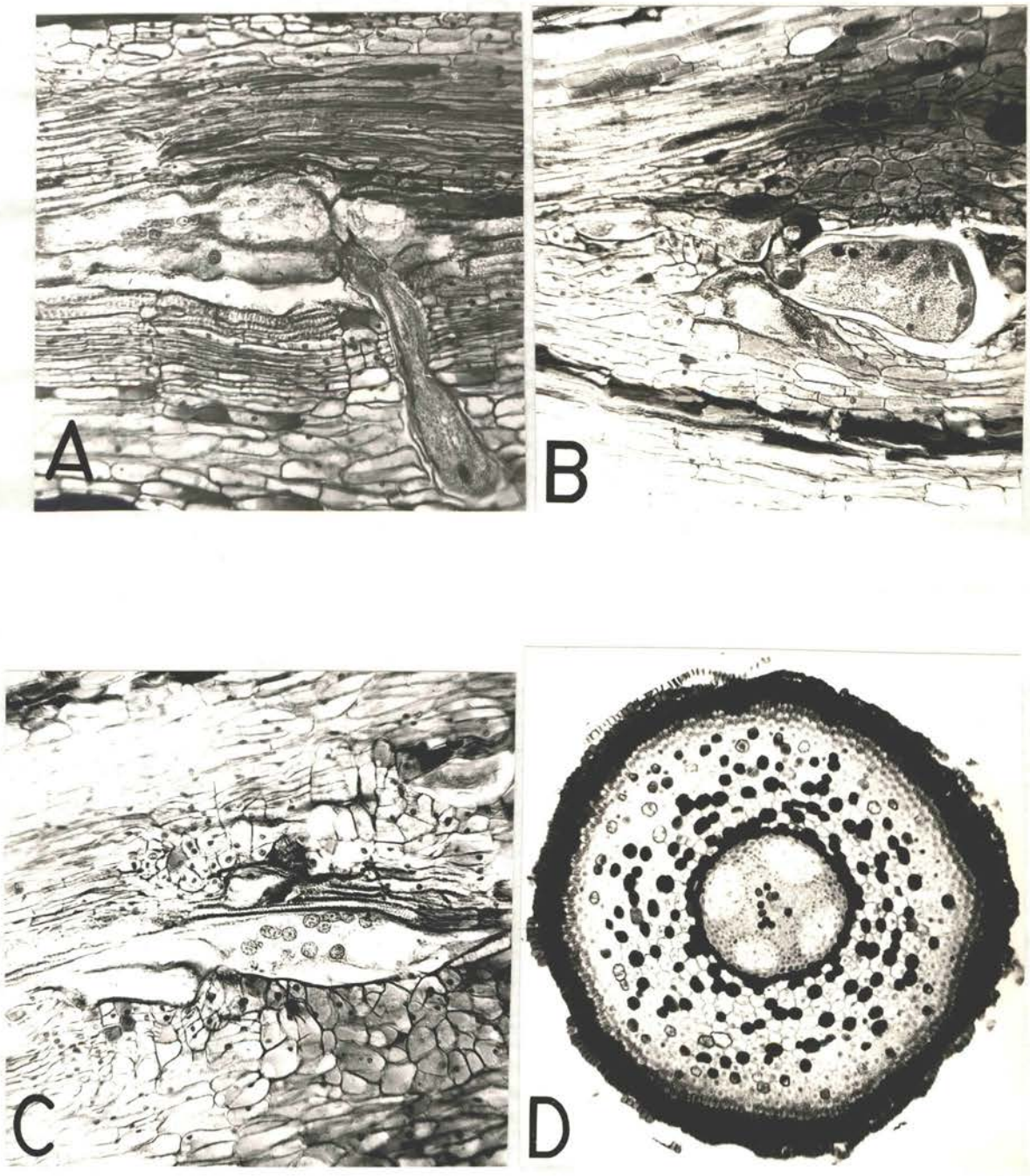

Fig. 4. Sections through Thuja orientalis Dwarf Greenspike roots showing Meloidogyne incognita and associated giant cells. A,B) Longitudinal sections. C) Longitudinal section showing giant cell. D) X-section of uninfected root.

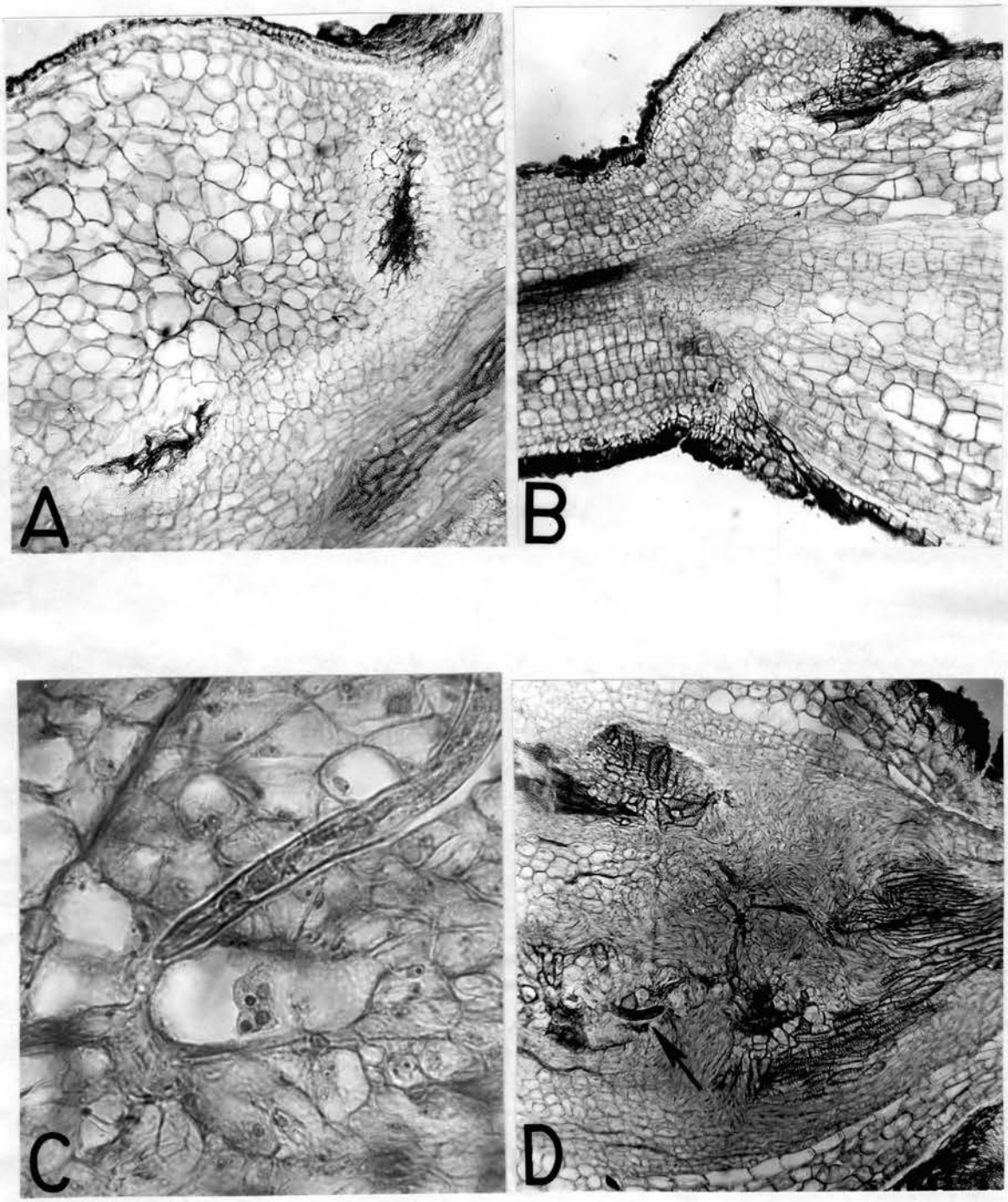

Fig. 5. Longitudinal sections through Poncirus trifoliata roots showing damage caused by Meloidogyne incognita. A) Necrotic areas in hypertrophied cortex. B) Gall development on portion of root. C) Larva and adjacent giant cell containing nucleus with larger than usual nucleoli. D) Larva (arrow) in disorganized vascular tissue.

Host Response - Top Growth Reduction

An experiment was set up utilizing 4 juniper species with 2 types of inoculation, over a 6 month period, to determine if larval penetration and feeding had an effect on top growth.

The first inoculation, inoculation I, utilized 4 replicates each of J. horizontalis plumosa, J. procumbens, J. horizontalis douglasi and J. chinensis hetzi. These plants were inoculated once with 2 g of chopped, galled Rutgers tomato roots added to soil in 6-in. pots.

The second inoculation, inoculation II, utilized 4 replicates each of the same 4 junipers. Again 2 g of chopped galled Rutgers tomato roots were added to soil in 6-in. pots containing these plants. This time the junipers were inoculated initially and again at 4 monthly intervals. Four uninoculated plants of each species served as controls. At the end of 6 months top and root growth were measured.

All plants with either inoculation were slower to form new leaves and laterals when compared with controls. There was an increase in growth with inoculation I as compared with inoculation II (Fig. 7). From averages of 4 replicates, top growth of controls was greater than that of inoculation II for 3 out of the 4 species. Growth of J. chinensis hetzi, with inoculation I, was slightly greater than the growth of the controls. Growth of J. horizontalis plumosa and J. horizontalis douglasi controls averaged 21.8 cm and 12.2 cm respectively over those of inoculation II and 18.2 cm and 11.8 cm respectively compared to those of inoculation I. Noticeable differences were present in amount of root growth produced. There was a decrease in amount of new root formation from inoculation I to II respectively. These differences in top and root

growth are illustrated in Figs. 6 and 7.

Samples of root systems, from all 4 junipers, were stained with lactophenol-acid fuchsin to survey the range of nematode development. A few nematodes were found to have matured and reproduced in J. procumbens and J. horizontalis douglasi. The presence of egg masses proves M. incognita is capable of reproducing in some juniper species. The remaining portions of all plant root systems were added to soil with a Rutgers tomato plant and grown for 30 days. At the end of this period moderate to severe galling was recorded from the bioassay tomato plants planted with root systems of the above 2 junipers, another indication that substantial reproduction can occur in some junipers. No galling was recorded from the tomatoes planted with root systems of the other 2 junipers.

Fig. 6. Juniperus horizontalis plumosa showing relative top and root growth 6 months after 2 types of inoculation with Meloidogyne incognita. Left to right, 5 monthly inoculations, single initial inoculation and uninoculated control.

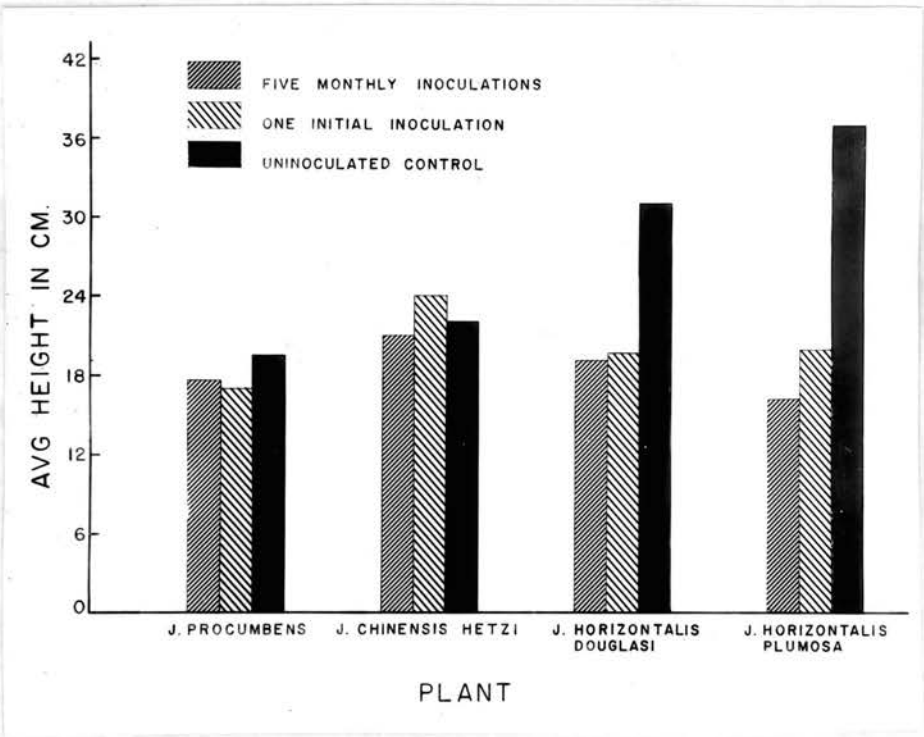

Fig. 7. Effect of Meloidogyne incognita on top growth of 4 Juniperus spp. after 6 months with single and multiple inoculations.

Determination of Time of Entry by Root-knot Larvae in Certain Ornamentals

Root observation boxes were used to determine more exactly the time of entry of root-knot larvae into roots of certain of the more resistant ornamental plants. Each box measured 14 x 8 x 2-in. and was fitted with a removable 11 3/8 x 7 3/8-in. glass plate and plywood front. Plants used in this study were J. horizontalis plumosa, P. trifoliata, Zizyphus jujuba and Hedera helix. Rooted plants of these were transferred to vermiculite in the observation boxes. New, white roots were inoculated with 1 ml of a larval suspension containing approximately 2000 root-knot larvae per ml. Larvae were injected around the root-tip with a 10 ml glass syringe fitted with a thin glass tube. Inoculated roots were marked on the glass plate and observed several times under the binocular dissecting microscope. Most inoculated root-tips were removed 48 hr after inoculation, stained as previously described in lactophenol-acid fuchsin, and observed for numbers of larvae present.

Roots of junipers when disturbed by removing the plant from a propagation bed or during transplanting operations went into a state of dormancy. During this dormant period root-tips appeared suberized and were brown in color. Larvae were never found to enter dormant roots as shown by controlled inoculations. When root growth resumed and a new white root-tip broke through the old epidermis the root was again subject to larval penetration. Root growth periodicity, which is an inherent normal character, occurred independently among roots of a single root system and was probably a result of biochemical changes in the plant as suggested by Wilcox (40).

Roots of J. horizontalis plumosa grown in these boxes were found to contain numerous larvae. As many as 120 larvae have been counted in one root-tip. Roots of this species were removed at intervals of 12, 24 and 36 hr to determine about how soon after inoculation larvae entered roots. Larvae in large numbers were always seen in the region behind the root-cap when roots were examined 12 hr after inoculation. Numbers of larvae were consistently low in P. trifoliata roots with 21 larvae counted in 5 root-tips during one test. H. helix in the test plant reactions test showed indications of being resistant, but its root system was seriously stunted. Seven roots of this species, inoculated in an observation box, contained 64 larvae which showed roots were not resistant to larval invasion. Z. jujuba, another plant tested in these boxes was easily entered by larvae with as many as 175 counted in one root-tip.

DISCUSSION

The overall objective of this experiment was to evaluate reactions of certain woody ornamentals to M. incognita. The plants used had previously not been listed as hosts or had been reported as resistant to M. incognita.

The plants were evaluated for their reaction to root-knot in several ways and these techniques together supplied adequate information with which to measure host suitability or unsuitability. Evaluating a test plant with one technique alone, however, was not a consistently true measure of host resistance. Data from unhealthy Loropetalum chinense and inadequate root samples from Cedrus deodara were used so that tests with these plants may not have provided a true estimate of their response.

No growth differences were noted between the controls or treatments of any of the plants tested for 6 to 8 weeks. This measure of host response may have been more useful with a longer inoculation period. The gall index showed that response among all species varied from no galling to very severe galling. As a rule more severe galling occurred among those plants with a high nematode population. The gall index was useful as a quick means of surveying host damage, but did not provide a means of determining nature of host resistance. The bioassay index provided supplemental information relative to amount of reproduction in the test plants and appeared to give the best estimate of nematode reproduction of the various techniques used. Counts of nematodes in each developmental stage showed larvae were capable of penetrating species in which they did not mature as easily as they penetrated species in which they did mature. This freedom of entry in resistant and susceptible species makes question-

able the presence of a morphological barrier in the roots of most resistant species tested here. Plants supporting a high population of adult nematodes and a high level of reproduction were considered to be very susceptible. The susceptibility of these plants was also verified by high bioassay and gall index values.

Some of the bases for explaining host resistance are founded on the interaction between the host and parasite. Host resistance could be 1) of a morphological type (35) where the root tissues restrict penetration of larvae, 2) an antagonistic type (3) in which plant substances neutralize the giant-cell-inducing effect of the nematodes, 3) a necrotic type (17) that results from nematode feeding or disruption of tissues ultimately preventing the larvae from utilizing live tissues, and 4) a type which involves a tolerance of the host to normal larval development. Resistance then in a broad sense of the term could include all the above types of resistant reactions, for as Christie (12) has stated "We must recognize the possibility that all resistant plants are not resistant for the same reason".

Observations from the histopathological study using 2 junipers, 1 arborvitae, and P. trifoliata have shown these plants to be more or less resistant for one or more reasons listed above. M. incognita in T. orientalis Dwarf Greenspike was capable of maturing, but only slowly. However, often after invasion of the root, necrosis developed near the head region of the larvae; then maturation stopped or proceeded so slowly that nematodes never reached maturity. Other investigators (15, 17) have observed similar necrotic reactions. Root-knot resistance in J. horizontalis douglasi, J. horizontalis plumosa and T. orientalis

Dwarf Greenspike was also manifest in the inability of the larvae to stimulate normal giant cells with accompanying extreme cortical hypertrophy. This insufficiency of cortical hypertrophy is probably the reason roots of these plants are not characteristically galled. In a similar instance Osborne and Jenkins (29) suggested that mild cortical hypertrophy in galls initiated by M. hapla was the reason the galls were small. In comparison with normal giant cell development (10) in susceptible plants, giant cells in the junipers and arborvitae were different in that they were smaller, fewer, thin-walled, very irregular and usually aligned along the main axis of the root. Alignment of this kind suggests the stelar root tissues, especially the endodermis and pericycle, may have a restrictive effect on radial growth of these giant cells.

Galls on P. trifoliata were characteristically the size of normal galls that occur on species susceptible to root-knot. However, normal giant cell formation and nematode development were never observed in P. trifoliata. This particular interaction, where in well-developed galls nematode development was limited, suggests the nematode is capable of stimulating hypertrophy and hyperplasia without undergoing development. This type of gall and nematode association is so complex that few if any questions have been answered concerning the basis of this relationship. It is apparent, however, from root-observation-box tests that larvae can penetrate roots of this plant less freely than those of other resistant species. This resistance therefore is expressed by the host-cell interaction with the parasite.

Another objective in these studies was to determine if nematodes reduced the growth of some of the more resistant species. Results from

single and multiple inoculations with 4 junipers showed that after 6 months growth was reduced. Multiple inoculations were more effective in reducing growth because they provided a relatively constant source of available inoculum for infection. Nematode reproduction in junipers was found to be low; therefore, single inoculations did not afford the opportunity to study continual infection with a constant source of nematodes.

It is assumed that if the multiple inoculations closely approximated similar conditions of nematode inoculum available in the field, growers then could expect a reduction in growth of nematode infected junipers during a single season. The general opinion of investigators has been that nematode-infected plants, even if they are not killed, are less healthy and hardy than uninfected plants. A grower with nematode infected junipers could anticipate other problems such as increased winter damage.

Another experiment was set up using root observation boxes in order to observe and time inoculations with some of the more resistant species. Observations made while using these boxes have revealed a new type of resistance in certain conifers studied, particularly J. horizontalis plumosa. This resistance seems to be morphological in that larvae are incapable of penetrating dormant root-tips, but are able to invade actively growing root-tips. This periodic growth activity, responsible for dormant and active roots was observed by Wilcox (40) in Abies procera as a part of its normal physiology. No information, however, relative to the effect of root periodicity on plant resistance has been encountered in the literature. Root periodicity may eventually have a practical importance if dormancy could be regulated to control resistance. Because

most roots of conifers become dormant in the late summer there is a very short growing season when most roots are active. Control measures, such as applications of nematocides, to be most effective, should be applied during the period of active growth.

In light of evidence provided by other investigators (10, 14, 17) on giant cell and larval development in susceptible species, and of data presented here, resistance of arborvitae and juniper to root-knot is mainly expressed in host-cell response to the parasite. Certainly the basis for resistance with these plants includes all 4 host resistance types previously mentioned. Which type of resistance predominates or plays the most important role, is difficult to judge from a limited study of this kind.

SUMMARY

Twenty-one woody ornamental plants either previously not reported as hosts or suspected of being resistant were tested for their reaction to Meloidogyne incognita. Larvae entered all species but completed their life cycle in only 7 while 3 species supported a high level of reproduction. Larvae did not mature in 9 species but root growth in these was severely retarded.

Histopathological studies of Thuja orientalis Dwarf Greenspike and Juniperus horizontalis plumosa showed that larvae developed slowly through the stage where they were nearing the final molts but seldom completed the last molt. Egg masses were produced in the arborvitae but were not observed in the juniper. Root swellings, not typically root-knot symptoms, were common in this group of plants and resulted from mild cortical and vascular hypertrophy and small giant cell formation. Necrosis was apparent only in arborvitae. Larval development in Poncirus trifoliata was not observed beyond the stage where they were beginning to increase in size. Nevertheless, larvae were capable of stimulating normal gall formation, a consequence of extreme cortical and vascular hyperplasia and hypertrophy.

Top growth and root growth of 4 juniper species were reduced after 6 months from 2 types of inoculations. Growth of these junipers was reduced more by 5 monthly inoculations than with a single inoculation. At the end of 6 months a few egg masses were found in J. procumbens and J. horizontalis douglasi.

Root periodicity, a cyclic pattern of growth and dormancy, was observed in several coniferous plants. Timed inoculation tests showed

infection of J. horizontalis plumosa depended on actively growing roots. Periodic dormancy in roots appeared to inhibit invasion by larvae.

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