SECTION 5
PATHOLOGY AND NEMATOLOGY

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Stem Water Potential of Root-Knot Nematode Infected Japanese Holly in the Georgia Piedmont

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Nature of Work: Japanese hollies (Ilex crenata Thumb.) are prominent landscape plants with few disease or insect problems, but occasionally their growth declines or is disappointing in the presence of root-knot nematodes, particularly in sandy soils (Barker and Benson; Jones and Benson). The question of what impact root-feeding nematodes have on the stem water potential of woody plants under field conditions has not been frequently addressed, although considerable attention has been given to water potential in nonwoody plants such as tomato (Meon et al, Wudiri & Henderson) and snap bean (Wilcox-Lee and Loria).

The objectives of this research were to determine if root-knot nematodes have an effect on the growth of Japanese holly in clay soil, and if stem water potential would be affected by the presence of nematodes.

Thirty 30 in. (76 cm) dia microplots were established at the Georgia Station in a Davidson clay loam soil which has a clay content of 27 to 40%. Each plot was lined with fiberglass to a depth of 24 in. (65 cm), providing a total soil volume of 9.7 ft^3 (0.27m^3). Following methyl bromide treatments (1 lb) of each plot, one Ilex crenata convexa and one I. crenata rotundifolia were planted in each plot in the fall of 1985. In the spring of 1986 root-knot nematodes, Meloido~yne arenaria, were added to the randomized plots at 0, 700 or 1400 nematodes per 6 $in.^3$ (100 cc) soil. There were 10 plots at each population level.

The average stem water potential was determined with a Scholander type pressure bomb by noting the pressure at which the exudate was first noticed from the cut surface of two severed stems from each plant on 6 dates. Measurements taken on June 11, 12 and September 25, 1987 were considered “dry” because natural rain had not occurred within 6 days, whereas those taken on June 29, July 11, and October 1 were considered ‘moist’ because rain had occurred 6 days prior to measurements.

Plant height and width was recorded on February 1 0, and June 1 1, 1 987 and March 1 4, 1 988. The sum was divided by 2 for a growth index. The holly plants were dug in March 1988 and the diameter (caliper) of the main stems measured. Soil was washed from the roots, and the root system scored for root-knot nematode infection on a 0 to 4 scale, with 0 = no visible knots, 1 = 1 to 25% of the roots with knots, 2 = 26 to 50% of the roots with knots, 3 = 51 to 75% and 4 = greater than 76% of the roots with knots. Plant dry weights were determined. Data were analyzed by analysis of variance.

Results and Discussion: Two years after infestation root-knot nematodes had no significant effect (ANOVA) on the stem water potential of Japanese hollies at two infestation levels in the clay soils of the Georgia piedmont. There was no significant (P = 0.05) differences between the water potential of the two cultivars of holly under similar soil moisture conditions. Lower pressures, indicative of higher plant moisture, occurred under moist soil conditions for both convexa and rotundifolia than under drier soil conditions (Table 1). It is known that certain diseases (Beagle-Ristaini and Duniway, Crist and Schoeneweiss), including nematodes (Wilcox-Lee and Loria), as well as environmental changes can affect the stem water potential in plants. However, utilizing stem water potential measurements in field grown plants as an indicator of nematode infection under clay soil conditions is open to question (Fortnum and Sadler).

The nematode population levels used in this experiment did not significantiy affect the holly growth indices, stem diameter or dry weights, over the 2 year period. The root-knot index at harvest was greatest on plants at the higher infestation level and on susceptible tomato plants planted after the holly plants were harvested. More than 42,000 nematode eggs were later recovered from one tomato plant, indicating sufficient nematodes were present at the time of holly harvest to initiate an infection on tomato (data not presented).

Significance to Industry: In contrast to these findings, Barker and Benson found three cultivars of Japanese hollies (rotundifolia, convexa, and helleri) quite intolerant to M. arenaria in the loamy sand soils (91% sand) of North Carolina. Nematode populations declined markedly after 14 months before increasing again by 29 months. Although the initial populations we used were not the same as those in the North Carolina studies, their initial high density populations of 1,000 to 1,200 per $6in^3$ (100 cc's) 1 week after infestation were somewhat comparable to our 1,400 level. Therefore, under water stress conditions of sandy soils, nematode infected plants probably are less tolerant of nematodes than in soils with higher clay contents. Although root-knot nematodes are very serious pests of Japanese holly it appears these plants may tolerate moderate population levels in predominant clay soils with no significant effect on water potential.

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Table 1. Mean stem water potential (-MPa) of nematode infested Japanese holly (Ilex crenata convexa and Ilex crenata rotundifolia) under "dry" and "moist" soil conditions during 1987.

Inaial Nematode Infestation	Water potential (-MPa)											
	Ilex crenata convexa						Ilex crenata rotundifolia					
	"Moist"			"Dry"			"Moist"			"Dry"		
Level/6in^3	6/29	7/1	10/1	6/11	6/12	9/25	6/29	7/1	10/1	6/11	6/12	9/25
None	.68[a]	.68	.73	1.21	1.24	1.34	.87	.77	.83	1.18	1.24	1.35
700	.67	.57	.66	1.25	1.24	1.37	.74	.61	.80	1.21	1.27	1.26
1400	.68	.62	.69	1.30	1.34	1.30	.90	.72	.76	1.21	1.22	1.35

[a] Mean of 10 replicates; 2 measurements per plant. No significant differences by analysis of variance (P = 0,05) between infestation levels.

Table 2. Growth index (Gl), stem diameter (SD), plant dry weights (DW), and root-knot nematode index (R-KI) of Japanese hollies grown for 2 years in Georgia piedmont.

Initial Nematode Infestation	Ilex crenata 'convexa'				Ilex crenata 'rotundifolia'			
Level/6in³	Gl[a]	SD[b]	DW[c]	R-KI[d]	Gl[a]	SD[b]	DW[c]	R-KI[d]
None	55.3	21.2	255	0	55.9	26.8	610	0
700	61.2	22.7	320	1.5	55.6	27.4	466	1.1
1400	59.9	23.7	303	2.1	56.1	26.7	483	1.8

[a] Mean of 10 measurements taken on 2-10-87, 6-10~7, and 3-14-88 (width + height /2 in cm)

[b] Mean of 10 stem diameters, in mm.

[c] Mean of 10 plants, in grams.

[d] 0 = none, 1 = 1-25% roots with knots, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% roots with knots; Mean of 10 plants examined at harvest.

Nematode Distribution on an Ebb and Flow Bench System

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Nature of Work: Plant propagators and commercial growers in U.S. are beginning to utilize the ebb and flow tray or bench system used in Europe for growing plants. The system is similar in principle to hydroponics. The watering of plants or flooding of the tray is operated according to the crop being produced, media type, environmental conditions, etc. Atmatjidou et. al. recently studied the potential transmission of certain disease organisms, namely Xanthomonas (bacterium) and Pythium (fungus), using the Midwest Gro-Master ebb and flow benches. They reported low transmission of the Xanthomonas bacterium when the pathogen populations were high, but greater transmission with the root rotting fungus, Pythium aphanidermatum and to some extent with P. ultimium.

The question of whether nematode distribution/or transmission occurs in the ebb and flow circulating system has been raised, yet we knew of no data on which to base a conclusion. Therefore, we performed several experiments to determine the extent of nematode distribution in such a system.

The 4.080 in^3 reservoir of the ebb and flow system we used held 17.6 gallons (66.8 liters). Ninety eight thousand juvenile root-knot nematodes were added, agitated, and the pump operated for 15 minutes to fill the tray with water. When the tray (46 in. w x 72 in. I x 2 in. d.) was filled to its maximum level (12 gallons) the pump was turned off, the return valve closed and six 10 ml water samples were withdrawn from different locations in the tray; one sample from each corner, one from the middle, and one from the reservoir. The number of nematodes were counted in three 1 ml subsamples from each 10 ml sample. The average number of nematodes recovered was determined. Additional trials were made with higher populations. After each sampling the water from the tray was drained back into the reservoir and the tray was washed. During several trials the water in the reservoir was not agitated prior to pumping in order to determine if the nematodes would settle and remain only in the reservoir. Data are presented in Table 1. Trials with nematode eggs, infected and noninfected plants were conducted. Data are presented in Tables 2 and 3.

Results and Discussions: Root-knot nematode juvenile and eggs were recovered from artificially infested water in an ebb and flow bench system. Numbers of nematode larvae and eggs decreased dramatically when the nematode/egg suspensions in the reservoir were not agitated prior to operating the irrigation pump. No eggs were detected on the tray samples 96 hrs after the initial infestation when the reservoir contents had not been agitated for 72 hrs., and only 3 dead nematode larvae were found in the tray samples 72 hours after the last agitation.

Root-knot juveniles and adult males were recovered from the irrigation system when heavily infected plants were placed on the tray. When 4 infected tomato plants, in 4 in. pots, were placed in 5 locations, and watered for 15 min. daily for 8 days, 56 nematodes (53 juveniles and 3 adult males) were recovered from the system. Thus, nematodes from infected plants were capable of entering the system.

In a contrasting experiment, healthy tomato plants were placed on the ebb and flow tray then the water system infested with 550,000 root- knot eggs (54 eggs/10ml). The plants were harvested and roots examined for root knot development after 28 days of watering. Results are presented in Table 3.

Significance to Industry: Monitoring for pathogens in the ebb and flow systems should be a standard procedure for early detection of organisms, including plant pathogenic nematodes. One alternative would be to add chemicals to the system that would inhibit or kill the pathogens, but each material would have to be tested for its efficacy on a variety of pathogens, phytotoxicity, safety, and an approved label issued prior to making any recommendations. Perhaps the best alternative is to maintain strict sanitation of the system and avoid potential contamination by any pathogen. If nematodes are detected in the system, avoid agitation of the water reservior.

Literature Cited

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Table 1. Distribution of juvenile root-knot nematodes in an ebb and flow svstem.

	Average number recovered / 10 ml sample						
Sample location / TRIAL	1	2	3[a]	4	5[a]	6[a]	7[a]
Left front	0	13	16	63	0	6	0
Right front	23	6	0	30	0	3	0
Center	3	20	6	43	0	0	3
Left rear	13	30	26	36	0	0	0
Right rear	10	10	23	16	0	0	0
Reservoir	53	26	70	56	60	136	76
Nematode population per 10 ml:	15	26	26	72	72	72	72
Total x 10^3	98	174	174	— — — 478 — —			

[a] Reservoir not agitated before running the pump for 15 minutes.

Table 2. Distribution of root-knot nematode eaas in ebb and flow svstem.

Location	Average number of eggs recovered/10 ml.		
	Initial	24 hrs. Iater	96 hrs. Iater
Left Front	63	3	0
Right Front	63	40	0
Center	63	50	0
Left Rear	10	13	0
Right Rear	43	86	0
Reservoir	66	406	83
Calculated values:[a]			
Total in tray	323,000	257,000	0
Total in reservoir	217,000	283,000	540,000

[a] Based on original concentration of 80 eggs/10 mls.

Table 3. Infection of tomatoes (Rutgers) in the ebb and flow system 28 days following infestation with root-knot nematode eggs.

Location in Tray	Avg. number of root knots/plant[a]
LF	0.0
RF	1.3
C	0.0
LR	40
RR	4.5

[a] Average of 8 plants per location.

Chemical and Cultural Control of Dogwood Anthracnose

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Nature of Work: Dogwood anthracnose, caused by Discula sp., was detected in a field of two year old flowering dogwood seedlings at a nursery in Middle Tennessee in 1990. The field consisted of 22 rows and each row was 300 ft long. Nearly 100% of the trees (12 to 18 trees/ft of row) had anthracnose symptoms, but most of the lesions were located in the lower 25% of the tree's canopy. The trees in the field were condemned by the nurseryman and the field was obtained by university personnel for experimental purposes.

Nine fungicide treatments were evaluated for disease control. These included: ASC 66791 (0.32 oz/gal), Banner 1.1 E (0.02 fl oz/gal), Benlate 50 DF (0.16 oz/gal), Bravo 720 (0.3 fl oz/gal), Dithane DF (0.24 oz/gal), Hydroclear's Ionic Solution (5 fl oz/gal), Lynx 2 F (0.07 fl oz/gal), Systhane 2 EC (0.04 fl oz/gal), and SAN 619 (0.04 oz/gal). In addition to fungicide treatments, symptomatic leaves were pruned out of one half of the plots that were to be sprayed with fungicides. Each pruning combination (18 total) and a fungicide untreated control (with and without pruning) were compared using one row plots (20 ft long) arranged in a randomized complete block design with each treatment combination replicated six times. Fungicides were applied at 2 week intervals. Disease severity was rated after 10 weeks using a canopy scale given in Table 1. Trees in only the center 14 ft of each row were rated with the scale.

Results and Discussion: All fungicide treatments were effective in reducing the level of anthracnose in the tree canopy when compared to the control (Table 2). Pruning was effective in reducing the amount of disease in the canopy when compared to controls without pruning. However, the combination of pruning and fungicide treatment was no more effective in reducing anthracnose incidence in the canopy than the use of the fungicide alone.

Dogwood anthracnose severity was reduced in trees that had been treated with fungicides and/or had symptomatic leaves removed. However, no treatment eliminated the disease completely. Further research needs to be conducted to determine if multiple pruning dates in combination with fungicide treatments can eliminate this disease in infected trees.

Significance to Industry: Dogwood anthracnose can be controlled in nursery fields using several different fungicides (Table 2). Pruning out diseased tissue in combination with fungicidal sprays was no more effective in disease control than fungicidal sprays alone.

Table 1. Canopy scale for rating dogwood anthracnose severity.

Score	Portion of canopy (from base of tree) with disease symptoms
0	no symptoms
1	1 - 20%
2	21 - 40%
3	41 - 60%
4	61 - 80%
5	81 - 100%

Table 2. The effectiveness of fungicidal treatments with and without pruning for decreasing dogwood anthracnose severity.

Fungicidal Treatment	Pruned Yes or No	Canopy Scale
ASC 66719	No	1.6 ab
	Yes	1.2 a
Banner 1.1 E	No	1.6 ab
	Yes	1.2 a
Benlate 50 DF	No	1.0 a
	Yes	1.0 a
Bravo 7 2 0	No	1.4 a
	Yes	1.4 a
Control	No	3.8 d
	Yes	2.6 c
Dithane DF	No	1.4 a
	Yes	1.2 a
HydroClear's Ionic	No	1.8 ab
Solution	Yes	2.2 bc
Lynx 2 F	No	1.0 a
	Yes	1.2 a
Systhane 2 EC	No	1.8 ab
	Yes	1.6 ab
SAN 619	No	1.8 ab
	Yes	1.6 ab

A Simple Assay For Separating Fungi Associated With Dogwood Anthracnose

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Nature of Work: Discula spp. has been isolated from necrotic lesions on leaves and from blighted twigs and berries of C. florida L. and C. kousa Hance (Chinese dogwood). Fungal isolates grown on agar media produce conidia of similar size and shape, but have two morphologically distinct mycelia. The mycelia of the first, Type I, sectors readily, has cottony growth and characteristically does not produce abundant spores. The mycelia of the second type, Type II, has more uniform radial growth, is more appressed to the medium and produces copious amounts of spores.

Little physiological research on the causal organism of dogwood anthracnose has been published. The objective of this research was to determine production of extracellular enzymes by the two Types of Discula mycelia with emphasis on phenol oxidase (laccase).

Type I and II fungi were isolated from anthracnose lesions on C. florida and C. kousa in Tennessee and additional isolates were obtained from investigators in different geographical regions. All stock cultures were maintained on clarified PDV8 agar at 18 C with 60-75 µmol $-m^{-2}s^{-1}$ of light provided by cool-white fluorescent tubes for 16 hr day^{-1}.

Laccase activity was detected using an agar assay medium and by isolating the enzyme from filtrates of liquid cultures. Inoculum for both tests was grown on PDV8 agar. For the agar assay, 5 mm diam. mycelial pluys were cut from the periphery of 7-10 day old colonies and placed mycelium side down onto gallic acid medium (GA) (malt extract, 15 g; gallic acid [3,4,5-trihydroxybenzoic acid], 5 g; and agar, 20 g) contained in 60 mm petri dishes (1). At least 5 replications were made for each of 42 Type I and 10 Type II isolates. GA medium was inoculated with agar plugs of PDV8 and served as controls. Dishes were sealed with parafilm and wrapped in aluminum foil to prevent photooxidization of the gallic acid. Cultures were incubated in the environmental conditions described previously and examined daily for 10 days for condensed phenols.

For protein isolation studies, 30 ml of YME liquid medium (yeast extract, 1 g; malt extract, 20 g) contained in 125 Erlenmeyer flasks were inoculated with two 5 mm diam. mycelial plugs. Cultures were incubated without shaking in the environmental conditions previously described. After 2 weeks, mycelia were collected on filter papers and the extracellular enzymes precipitated by adding 66 g $(NH_4)_2SO_4$ • 100 m^{-1} culture filtrate at 4 C for 1 hr. Proteins were pelleted in 50 ml centrifuge tubes at 3,000 x g for 15 min and then redissolved in distilled water. Proteins from 2

cultures for each of 8 isolates (4 Type I and 4 Type II) were combined and dialyzed against distilled water overnight at 4 C.

Laccase activity was assessed using three substrates: 0.02 M gallic acid, 0.005 M 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ATBS) (2) and 0.25 mg ml1 gum guaiac each dissolved in 0.05 M succinate buffer at pH 4.5. Three subsamples for each fungal isolate were prepared by adding 0.5 ml of crude protein to 1.5 ml of each substrate. Reaction mixtures were incubated at room temperature overnight. The changes in absorbances were monitored at 382 and 436 nm for gallic acid and ATBS substrates respectively; whereas, changes in the color of the gum guaiac substrate were determined visually. Laccase enzyme (Sigma) at 1 mg •ml^{-1} served as a control.

Results and Discussion: Forty-one (98%) of the Type I isolates produced laccase which caused browning (condensed phenols) of the gallic acid agar medium within 1 day after inoculation. The magnitude and intensity of the browning reaction depended on the isolate; some isolates (e.g. TN 13) strongly discolored a very large zone; whereas, others (e.g. TN 14) only turned the agar plug brown. Type II isolates did not produced laccase and therefore the medium or the agar plug did not turn brown for the entire experimental period of 10 days.

Since gallic acid is toxic to many fungi (3) and may inhibit production of extracellular enzymes, isolates were grown in liquid YME. Crude protein preparations from all Type I isolate filtrates were capable of oxidizing gallic acid in the liquid substrate; whereas, none of the Type I isolates produced the enzyme. The reaction required at least 16 hr to produce maximum absorbance values (Table 1). The laccase from Sigma rapidly catalyzed ATBS and gum guaiac to typical green and blue products respectively; whereas, the laccase from Type I Discula sp. cultures did not demonstrate specificity for these substrates (Table 1).

Although the role of laccase in pathogenesis or fungal nutrition is not known, the ability to produce the extracellular enzyme is strongly correlated to the morphology of the mycelium and spore production. The apparent substrate specificity of the laccase enzyme from Type I Discula sp. is interesting and warrants further investigation.

Significance to Industry: This work provides a simple and rapid method to evaluate fungi associated with dogwood anthracnose. Two Discula spp. that are isolated from necrotic lesions can be distinguished from each other on the basis of discoloration of gallic acid medium within 24 hr.

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Table 1. Detection of phenol oxidase activity of eight isolates of Type I and Type II fungi.

		Substrate			
Isolate	Colony type	Gallic acid (agar)[z]	Gallic acid[y]	ATBS[y]	Gum Guaiac
GA-1	I	25	0.53	0.01	nc[x]
MA-11	I	24	0.32	0.00	nc
NC-2	II	0	0.02	0.00	nc
NY-326	II	0	0.00	0.01	nc
S6	II	0	0.02	0.01	nc
SC-101	I	14	0.26	0.00	nc
TN-13	I	27	0.41	0.00	nc
VA-17b	II	0	0.03	0.00	nc
Laccase	- -	- -	0.80	0.45	blue

[z] Values represent the mean diameter of the brown zone after 24 hr.

[y] Values represent the mean absorbance of 3 subsamples after 16 hr.

[x] no change in color.

Laboratory Assays For Use In Dogwood Anthracnose Research

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Nature of Work: In the southeastern United States, dogwood anthracnose (caused by the fungal pathogen, Discula sp.) poses a significant threat to native and ornamental flowering dogwoods, Cornus florida L. (2). In areas of increased disease pressure, the Chinese dogwood, C. kousa Berger ex Hance, may also become anthracnose-infected (1). Laboratory research progress to date has been limited to the extent that suitable assays for the quantification of pathogen virulence and host disease resistance have not been available. The purpose of this study was to develop bioassays for these purposes.

Significance to Industry: Laboratory tests which may be used to accurately predict or evaluate the pathogenicity of Discula isolates and for host resistance screening would be useful in the identification of promising Cornus germplasm and anthracnose research. As this information becomes available, it may be used in the identification and marketing of nursery materials throughout the region.

Discula growth in apples: Previous work has demonstrated that Cryphonectria parasitica [(Murr.) Barr] isolates which cause Chestnut blight could be differentiated by their ability to grow in apple fruit (3). The ability of the pathogen to elicit necrotic lesions on 'Golden Delicious' apples could be correlated with the ability of individual isolates to cause annual cankers on laboratory-inoculated chestnut trees. Apples were washed with detergent and rinsed several times in tap and finally with sterile distilled water. Seven mm plugs were aseptically cut from the apples at each of three inoculation points to a depth of approximately five mm. Nine mm plugs were aseptically cut from the margin of Discula colonies growing on potato-dextrose agar (PDA). These plugs were transferred to the inoculation sites on the 'Golden Delicious' apples and the site sealed with transparent tape to prevent dessication. Larger plugs of fungal thallus were used to ensure inoculum contact with the apple fruit in all directions. PDA plugs were used as controls in this experiment. Apples were labelled, placed in plastic bags (which were cut to allow gas exchange), and placed in the growth chamber for one month at 17 C in constant darkness. Necrotic lesions evident on the apple fruit were measured and mean lesion diameters determined.

Dogwood twig assay: Twigs were removed from dormant Cornus species (C. florida, C. mas, C. kousa, C. controversa, and C. sericea L.) and cut into 9 cm segments. These segments were autoclaved for 20 minutes prior to being "stuck" into experimental chambers (Magenta 'GA-7' boxes containing 50 ml of PDA, autoclaved for 20 minutes). Twigs, in groups of three, were inserted approximately 1 cm into the agar, and chambers were inoculated with both Discula colony types (I and ll,

GA-1 and NC-2, respectively). Inoculated chambers were placed in the growth chamber at 18 C with a 16/8 photoperiod. One month later, the bottom 0.5 cm of each twig was removed prior to assessment and sticks were examined for presence of characteristic Discula sporulation and the growth of the pathogen within the woody material. The distance to which the fungus had colonized the twig and/or to which it has sporulated determined by visual assessment and laboratory culture of 2 cm segments aseptically cut from each twig .

Results and Discussion: Results of the apple growth bioassay (Table1) indicate that Discula isolates may be differentiated on the basis of their growth within 'Golden Delicious' fruit. Necrotic lesions developing on the fruit are easily measured and no lesions developed within the fruit of agar-inoculated controls. Care should be taken to avoid damaging fruit to be used in this assay and inoculated apples should be spaced carefully within the growth chamber to avoid contact. Dissection of lesions developing on the fruit indicated that Discula isolates do not sporulate within this tissue; however, the pathogen was easily reisolated following the transfer of small portions of the necrotic lesion to potato-dextrose agar supplemented with 25 mg I -1streptomycin sulfate and chlortetracycline. Further work will be required to determine whether Discula growth in apples may be correlated with pathogenicity and/or virulence.

Initial results of the dogwood twig assay (Figures 1 and 2) indicate that Discula isolates differ in their ability to colonize autoclaved Cornus woody materials. Both isolates GA-1 and NC-2 colonized and sporulated on twigs of C. kousa and C. mas. Colonization of C. florida and C. controversa did not appear to be limited, although sporulation developed predominantly on the lower portions of the twigs. Both colonization and sporulation on C. sericea were limited when compared to the other species tested. Preliminary field evidence (Brown and Windham, unpublished) indicates, however, that the results of this assay do not correspond with results of host anthracnose resistarice tests currently in progress. This assay does provide a mechanism by which further research on the ability of Discula isolates to grow within woody dogwood tissues and elicit the development of annual cankers may proceed.

Literature Cited:

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Table 1. Growth of Discula isolates In 'Golden Delicious' apple fruit

Isolate	Colony Type	Isolated From	Mean Lesion Diameter (a)
NY-326	ll	C. florida	10 a
VA-17b	ll	C. florida	13 ab
MA-11	I	C. kousa	13 ab
MA-12	I	C. kousa	14 ab
NC-2	ll	C. florida	14 ab
TN-3	I	C. florida	16 bc
GA-1	I	C. florida	17 bcd
TN-14	1	C. kousa	19 cde
TN-8	I	C. florida	20 cde
TN-13	I	C. kousa	20 de
TN-133	I	C. florida	21 e
TN-1	I	C. florida	21 e

(a) Numbers followed by common letters do not significantly differ at 0.05% confidence level according to Duncan's Multiple Range Test.

Figure 1. Sporulation of Discula isolates in twig assay (a).

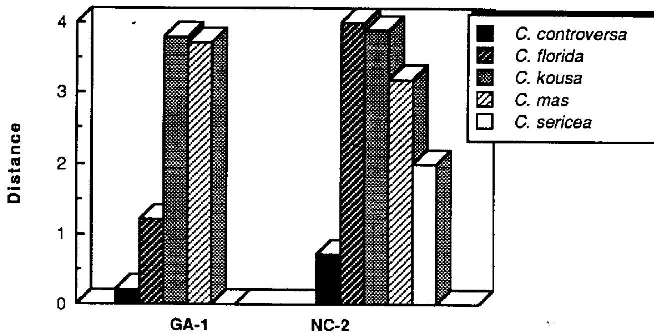
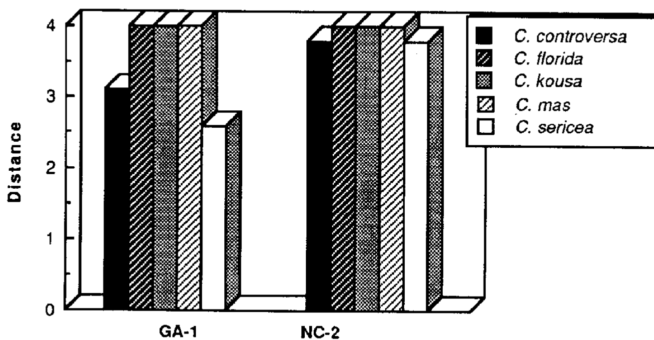


Figure 2. Growth of Discula isolates in dogwood twig assay (a).



(a) Distance scale: **1** = 2 cm, **2** = 4 cm, **3** = 6 cm, **4** = 8 cm.