Isolation of *Discula destructiva* and Other Fungi from Seeds of Dogwood Trees

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ABSTRACT

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Dogwood fruit were collected from trees with symptoms of dogwood anthracnose in 11 locations in western North Carolina in September 1989–1991. Fruit from each location were divided into four groups based on symptoms: completely necrotic, discrete necrotic lesions, shriveled without necrosis, and symptomless. Fruit and extracted seeds were surface-disinfested and placed on acidified potato-dextrose agar. *Discula destructiva* was isolated more often from seeds than from whole fruit, particularly from seeds extracted from completely necrotic fruit. The mean isolation frequency from extracted seeds was 0.12 in 1989, following a wet summer. *D. destructiva* was isolated from only 0.008 and 0.05 of extracted seeds in 1990 and 1991, respectively, when spring and early-summer rains were followed by midsummer dry periods. Infected seeds could provide a mechanism for long-distance dispersal of *D. destructiva* by animals or birds.

Additional keywords: *Botryosphaeria, Colletotrichum, Cornus florida,* dieback, *Phomopsis*

Dogwood anthracnose, caused by *Discula destructiva* Red. (11) on *Cornus florida* L. and *C. nuttallii* Audubon, has spread rapidly since it was first reported in the Pacific Northwest in 1976 by Byther and Davidson (3), and in New York in 1978 by Pirone (10). It now affects an estimated 3.89 million ha in 163 counties in the southeastern United States alone (7). Yet information about the dispersal and dissemination of *D. destructiva* is very limited.

The presence of a mucilaginous matrix in the cirrhi suggests that conidia are mainly dispersed by rain splash. However, we observed sporulation of the fungus on dogwood fruit attached to infected twigs in moist chambers. Thus, humans and other animals or birds may disseminate infected fruit and seeds. The present studies were designed to determine whether *D. destructiva* was present in fruit and seeds of dogwood in the field, and to compare the incidence of *D. destructiva* with that of other fungi.

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MATERIALS AND METHODS

1989 Survey—North Carolina. Eleven collections of at least 200 fruit each were taken from dogwood trees with symptoms of anthracnose. The collection sites were severely infected individuals or groups of trees located along roadsides on high ridges in the Nantahala Mountains of western North Carolina where anthracnose is epidemic. Fruit were separated into four groups based on symptoms: 1) completely necrotic, 2) discrete necrotic lesions, 3) shriveled without necrosis, and 4) symptomless. Twenty-five fruit representing each symptom type from each collection were surface-disinfested for 1 min in a 10% EtOH-0.525% NaOCl solution and placed whole on fresh potato-dextrose agar acidified with 1 ml/L of 85% lactic acid (APDA).

Seeds were extracted from another 25 fruit of each symptom type from each collection by soaking the fruit overnight and processing them in a blender with about 200 ml of water for approximately 3 min. The blender was modified by replacing the blades with short segments of plastic tubing to prevent destruction of the seeds. Extracted seeds were rinsed free of fruit pulp, surface-disinfested, and treated as above.

The incidence of *D. destructiva* and other suspected pathogens was recorded after 2 wk incubation at room temperature under fluorescent lights. Most fruit and seeds contained several fungi, and some contained none. The isolation frequency of each fungal genus was expressed as: number of plates containing genus X/number of plates in sample, regardless of other genera cohabiting the tissue. The arcsine square root of the isolation frequency of each genus within each symptom type per collection was used in an analysis of variance, and means were separated with Duncan's multiple range test (12).

1989 Survey—north Georgia. A total of 2,670 fruit from four symptomless and five diseased trees at nine different locations in northern Georgia were collected in 1989. Seeds were extracted from half of each sample, and both fruit and seeds were plated as above. Most fungi were identified after 2 wk incubation. Isolation frequencies of all identified genera were recorded as above for fruit and seeds from each location. Data were analyzed by a general linear models procedure (12).

1990 And 1991 surveys—North Carolina. Fruit were collected from the same 11 collection sites as in 1989 and subdivided into groups representing symptom types as above. Because fewer *Discula* isolates were obtained from fruit than from seeds in 1989, no intact fruit were plated. All samples were processed for seed and treated as above.

RESULTS

1989 Survey—North Carolina. The incidence of *D. destructiva* in seeds and fruit of infected trees averaged 0.12 over all symptom types. *D. destructiva* was isolated more frequently from seeds than from whole fruit, and most often from seeds extracted from completely necrotic fruit (Table 1). However, the fungus was also detected in seeds of symptomless fruit.

Unlike *D. destructiva,* other dogwood pathogens, including *Phomopsis* spp., *Colletotrichum* spp., and *Botryosphaeria* spp., were isolated more frequently from whole fruit than from seeds. Furthermore, the incidence of *Colletotrichum* spp. was greatest in symptomless fruit in 1989. The frequencies of *Phomopsis* and *Botryosphaeria* spp. were unaffected by fruit symptoms.

1989 Survey—north Georgia. Fungi isolated from 2% or more of fruit or seeds are listed in Table 2. Seed and fruit mycoflora of symptomless and anthracnose-infected trees were essentially similar, except that *Xylaria* spp. were isolated more frequently from the fruit and seeds of symptomless trees. More than twice as many fungal isolates per sample were obtained from fruit as from seeds (1.5 versus 0.6 isolates per sample, respectively). *D. destructiva* and *Chaetomium* spp. were isolated more frequently from seeds than from fruit. In contrast, all other fungi listed were isolated more frequently from fruit than from seeds. Fungi isolated from less than 2% of fruit or seeds included: *Aspergillus* spp., *Botrytis* spp., *Cladosporium* spp., *Nigrospora* spp., *Penicillium* spp., *Phyllosticta* spp., *Aureobasidium* spp., and several unidentified Ascomycetes. A total of 26% of the fungi did not sporulate and could not be identified.

1990 Survey—North Carolina. *D. destructiva* was isolated less frequently from seeds in 1990 (Table 3) than in 1989. Incidence averaged only 0.008 over all symptom types and was greatest in seeds extracted from fruit with lesions. *Colletotrichum* spp. were the most frequently isolated fungi. In contrast to 1989, both *Colletotrichum* and *Botryosphaeria* spp. were more common in seeds extracted from necrotic fruit than from healthy fruit.

1991 Survey—North Carolina. Incidence of *D. destructiva* averaged 0.05 over all symptom types and did not vary significantly due to fruit condition in 1991 (Table 3). The fungus was isolated

Table 1. Isolation frequency of fungi from fruit and seeds of dogwood trees with symptoms of anthracnose in 1989

Fruit symptom	Isolation frequency from whole fruit/extracted seeds[y]: *Discula destructiva*	*Phomopsis* spp.	*Colletotrichum* spp.	*Botryosphaeria* spp.
Necrotic	0.10A[z]/0.56a	0.13A/0.02a	0.10B/0.01b	0.12A/0.00a
Lesions	0.06AB/0.13b	0.13A/0.06a	0.20A/0.04a	0.14A/0.02a
Shriveled	0.02B/0.04c	0.09A/0.00a	0.16AB/0.01ab	0.10A/0.01a
Symptomless	0.02B/0.05bc	0.08A/0.02a	0.22A/0.00b	0.19A/0.05a

[y] Based on isolations from 25 fruit and 25 seeds of each symptom type from each of 11 locations.
[z] Analysis of variance and Duncan's multiple range test were performed on the arcsine square root transformation of isolation frequencies. Numerators represent isolation frequencies from fruit; means in columns followed by the same uppercase letter do not differ significantly at $P = 0.05$. Denominators represent isolation frequencies from extracted seeds; means in columns followed by the same lowercase letter do not differ significantly at $P = 0.05$.

Table 2. Isolation frequency of the most common fungal genera obtained from dogwood fruit and seed collected from healthy and diseased trees in northern Georgia in 1989

Genus	Tree condition[y]: Diseased		Healthy	Tissue type: Whole fruit		Extracted seed
Alternaria	0.23		0.13	0.245	*[z]	0.089
Botryosphaeria	0.04		0.03	0.057	*	0.024
Chaetomium	0.03		0.02	0.005	*	0.047
Colletotrichum	0.11		0.07	0.145	*	0.043
Discula	0.02		0.01	0.010	*	0.023
Epicoccum	0.03		0.04	0.050	*	0.017
Fusarium	0.04		0.02	0.053	*	0.009
Pestalotia	0.09		0.04	0.124	*	0.019
Phoma	0.07		0.02	0.060		0.038
Phomopsis	0.10		0.11	0.156	*	0.060
Xylaria	0.09	*	0.20	0.232	*	0.039

[y] Anthracnose symptoms were present on diseased trees; healthy trees were symptomless.
[z] Based on *t* test results of 2,670 isolations, * denotes significant differences between adjacent values at $P \leq 0.05$.

Table 3. Isolation frequency of fungi from extracted seed collected from dogwood trees with symptoms of anthracnose in 1990 and 1991

Fruit symptom	Frequency of isolation[y]: *Discula destructiva* 1990	1991	*Colletotrichum* spp. 1990	1991	*Phomopsis* spp. 1990	1991	*Botryosphaeria* spp. 1990	1991
Necrotic	0.003ab[z]	0.090a	0.283a	0.430a	0.140a	0	0.030a	0.130a
Lesions	0.027a	0.067a	0.177ab	0.310a	0.070ab	0	0.003b	0.030ab
Shriveled	0.003ab	0.020a	0.083b	0.150a	0.040b	0	0.007b	0.000b
Symptomless	0.000b	0.023a	0.083b	0.100a	0.070ab	0	0.000b	0.010b

[y] Based on isolations from 25 seeds of each symptom type from each of 11 locations.
[z] Means in columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

from a low percentage of seeds from symptomless fruit. As in 1990, *Colletotrichum* spp. were the most frequently isolated fungi, and both *Colletotrichum* and *Botryosphaeria* spp. were more frequently isolated from seeds from necrotic fruit than from healthy fruit.

DISCUSSION

This study indicates that a widespread concern regarding the hazard of introducing dogwood anthracnose to new areas through the movement of seeds may be justified in wet years. *D. destructiva* was isolated from both fruit and seeds of infected trees. In 1988, Hibben and Daughtrey (6) described dogwood anthracnose infection of *C. florida* leaves, flowers, and stems. The studies reported here support the addition of fruit and seeds to the list of susceptible tissues.

D. destructiva incidence in seeds from North Carolina was much lower in 1990 and 1991 than in 1989. Britton (2) documented the occurrence of secondary cycles of foliar infection in these same 3 yr. In southwestern North Carolina, 1989 was a wet year with secondary cycles of anthracnose continuing throughout the summer. In contrast, late spring rains in 1990 and 1991 initiated secondary foliar infections in May and June, but midsummer dry periods resulted in few secondary cycles later in the season (2). The results reported here suggest that high levels of infection of dogwood fruit and seed by *D. destructiva* may also depend on favorable infection conditions throughout the season.

Isolation frequency of *D. destructiva* was greater from extracted seeds than from fruit. *D. destructiva* may colonize seeds rather than fruit. Alternatively, *D. destructiva* may have been suppressed by competitors in the fruit tissue, since a higher number of competitors were isolated from whole fruit than from extracted seeds. These may have reduced either *D. destructiva* fruit colonization or isolation recovery success. Growth of *D. destructiva* on APDA is slower than that of most other fungi recovered in this study.

Several genera of fungi reported pathogenic to dogwood were isolated, in addition to saprophytic competitors. Waterman (15) listed a *Phomopsis* sp. as causing dieback in Tennessee in 1941. Toole and Filer (14) demonstrated the pathogenicity of *Colletotrichum gloeosporioides,* which causes necrotic leaf spots and dieback on *C. florida.* In 1993, Chellemi and Knox (4) reported that *Colletotrichum acutatum* causes foliar necrosis and limb dieback in Florida; and Smith (13) demonstrated that the same fungus causes necrotic lesions on dogwood fruit. The pathogenicity of *Botryosphaeria rhodina* has also been demonstrated (9). It is not known whether this species is the only incitant of a disease commonly called dogwood canker. Three *Botryosphaeria* spp. are listed on *Cornus* spp. in the host index compiled by Farr et al (5), but the pathogenicity of *B. obtusa* and *B. dothidea* have not been determined. Alfieri et al (1) included *Botryosphaeria* sp. as causal agents for dieback of dogwood in Florida. The *Botryosphaeria* spp. isolated in this study consisted mostly of *B. obtusa,* with occasional *B. dothidea.* These are ubiquitous fungi, but their role in the ecology of dogwood fruit and seed is unknown.

The isolation of *D. destructiva* from fruit and seed, even in low frequency, is relevant because this fungus may be exotic. The eastern range of dogwood anthracnose is presently limited to an area which comprises about one-fourth of the natural range of Eastern flowering dogwood. Movement of infected fruit and seeds may result in the establishment of the pathogen in locations not adjacent to the present disease range. If infected seed remain viable, seed transmission of *D. destructiva* might contribute directly to the spread of anthracnose. Nonviable infected seed might contribute indirectly by contaminating healthy seed.

Dissemination of infected seeds by wildlife is also possible. Many animals utilize dogwood fruit as an important source of winter nutrients. Small mammals generally digest the seed, and thus probably do not contribute to the spread of *D. destructiva.* However, with the exception of wild turkey and other birds with functional gizzards, most avian species ingest whole fruit and either regurgitate or pass the seed undigested (8). These studies suggest that removal of the fruit pulp increases the competitive advantage of *D. destructiva* and thus might enhance the spread of the fungus by migratory or other birds.

We conclude from these studies that *D. destructiva* is present in the seeds or seed coats of infected dogwood fruit. Incidence is variable, depending upon climatic conditions, and perhaps to some degree on the amount of colonization of fruit tissues by other fungal species. Although the major method of dispersal is probably the movement of conidia by rain splash, the threat remains that movement of infected seed may contribute to the long-distance spread of *D. destructiva.* It is therefore advisable to avoid collecting any seeds for nursery production from trees in areas affected by anthracnose.

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