Incidence of Arthropods Infested with Conidia of the Dogwood Anthracnose Fungus, *Discula destructiva* Redlin, on Flowering Dogwoods in the Natural Environment[1]

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Abstract Arthropods were collected from flowering dogwoods infected with *Discula destructiva* Redlin, causal agent of dogwood anthracnose, to evaluate their ability to transport viable conidia. During 1994, 7.2% of all arthropods collected (n = 375) from diseased trees at three sites in the Great Smoky Mountains National Park were carrying viable conidia of *D. destructiva.* Most [92.6% (25/27)] of the conidia-infested arthropods were collected during June when sporulation and spread of dogwood anthracnose were increasing. From July through September, the expansion of disease severity and incidence of sporulation diminished and the frequency of conidia-infested arthropods decreased. Several species of arthropods with viable conidia of *D. destructiva* were collected lending support to the hypothesis that insects and other arthropods may play a role in the spread of dogwood anthracnose.

Key Words Dogwood, *Discula destructiva*, plant diseases, cultivars, phenology, plant resistance, dogwood anthracnose, insect vectors

The flowering dogwood, *Cornus florida* L., is one of the most popular ornamental trees, especially in residential neighborhoods in the eastern United States, where it is the focus of many springtime festivals (Chellemi et al. 1992). Dogwoods contribute to the aesthetics and economy of communities throughout this region. For example, dogwood sales in Tennessee alone were estimated at $30 million in 1989 (Witte 1995). Dogwoods also play an important role in forest ecology where their leaves provide high levels of calcium (Boring et al. 1981) and lipid-rich berries provide food for more than 80 species of birds and mammals (Eyde 1988, Whitmore 1992).

Since the late 1970s, dogwood populations have declined in the northeastern United States. This decline has been attributed primarily to dogwood anthracnose, a fungal disease caused by *Discula destructiva* Redlin (Redlin 1991). Dogwood anthracnose has spread throughout much of the northeastern United States and southerly through the Appalachian Mountain region into Tennessee and south central Alabama (Carey and Kelley 1991, Chellemi et al. 1992, Knighten and Anderson 1993, Daughtrey et al. 1996).

Wind and rain have been cited as possible factors in the rapid spread of dogwood anthracnose (Daughtrey and Hibben 1983). Additionally, Colby et al. (1995) suggested that insects contacting infected leaves could become infested and transport conidia of *D. destructiva.* Insects have been shown to vector more than 200 plant

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pathogens of which 23 are fungi (Borror et al. 1989, Webber and Gibbs 1989). Coleopterans, especially bark beetles and weevils, are responsible for dissemination of 80% of these fungal pathogens (Webber and Gibbs 1989).

Until recently, little information has been available on the association of insects and dogwood anthracnose. Colby et al. (1996) demonstrated that the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), could transport viable conidia of *D. destructiva* externally for as many as 16 d under laboratory conditions. In a related study, peak insect densities coincided with increased levels of dogwood anthracnose in the Great Smoky Mountains National Park (Neitch et al. 1994). The abundance of insects during peak periods of disease severity, coupled with their ability to accumulate and transport viable conidia, suggest that insects may play a role in the spread of dogwood anthracnose.

No information, however, is available on the incidence of conidia of *D. destructiva* on field-collected arthropods, specifically insects. This information would further define the potential role, if any, of insects and other arthropods in the epidemiology of dogwood anthracnose. Accordingly, the objectives of this research were to: (1) determine the incidence and seasonality of conidia-infested arthropods occurring on infected understory dogwoods and (2) identify *D. destructiva*-infested arthropods.

Materials and Methods

Arthropods were sampled from dogwoods infected with *D. destructiva* at three sites in the Great Smoky Mountains National Park. Sites were selected because of high incidence and severity of dogwood anthracnose. Trees with leaves exhibiting severe symptoms of dogwood anthracnose were selected and sampled. Locations were: (1) a designated "quiet walkway" near Sugarlands Visitor Center, (2) Jakes Creek, near Elkmont Campground, and (3) Rich Mountain, overlooking Cades Cove. Symptomatic foliage was collected at each site; and infection with *D. destructiva* was confirmed microscopically. On each sampling date, the Horsfal-Barratt (Horsfal and Barratt 1945) scale was used to rate disease severity because it provided a logarithmic function necessary for further scientific analysis (Campbell and Madden 1990).

Arthropods were collected from each site about every 3 to 4 wks from early June to late September 1994. During each collection, six dogwoods (1 m to 7 m tall) were sampled at each site, and dogwoods sampled were alternated on each collection date to allow for a larger sampling base. The site, date and number of arthropods collected from each dogwood were recorded.

Two sampling methods (hand picking and canopy agitation) were used. First, hand picking involved the placement of a clear plastic bag (20.3 × 10.2 × 45.7 cm) on the hand. After an arthropod was grasped, the bag was inverted and a culture tube (17 × 100 mm) was placed into the bag. The specimen was channeled into the tube, which was then capped and labeled with appropriate information (e.g., tree, location and date). Samples were placed into coolers and transported to the laboratory. To minimize cross contamination, one collection bag was used for each arthropod and then discarded. Each tree was sampled using this procedure for approximately 6 to 7 min. Although hand picking was time consuming, labor intensive, and yielded fewer specimens than more intensive sampling, this method permitted behavioral observations and ensured that the collected specimens had contacted dogwood foliage. Hand picking equally collected those arthropods with firm and weak grasps of foliage.

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The second method involved canopy agitation in which a polypropylene tarpaulin (tarp) (3.6 × 3.6 m) was placed under the canopy of each dogwood. The main stem was vigorously shaken for approximately 15 to 30 sec. Arthropods that fell onto the tarp were collected with forceps and placed separately into culture tubes (17 × 100 mm). After each individual was collected, forceps were surface-sterilized with a 0.525% sodium hypochlorite solution (Clorox Co., Oakland, CA) to prevent cross contamination. Vials were capped, labeled with appropriate information, placed into coolers, and transported to the laboratory.

In the laboratory, arthropods collected from infected dogwoods were individually placed into culture tubes (17 × 100 mm) containing 10 ml of sterile water. Tubes were capped and agitated for approximately 8 to 10 sec using a vortex machine (Baxter-Scientific Products, McGraw, IL). This solution was serially diluted, and 1 ml of each dilution was drop-plated onto potato dextrose agar (PDA) amended with 30 mg/L each of chlortetracycline and streptomycin sulfate.

Dilution cultures were incubated at 20°C and observed for approximately 2 wks for sporulation of *D. destructiva.* Subculturing was often necessary to produce pure isolates of *D. destructiva* because many other fungi and bacteria were isolated from the specimens. Wet mounts of sporulating mycelia of *Discula* spp. were examined for conidia as described by Redlin (1991) and recorded when present. When colonies of *D. destructiva* were confirmed, infested arthropods were maintained in a freezer and later identified; location and date of collection were recorded.

Results and Discussion

About 7% of all the arthropods (n = 375) collected from diseased dogwoods at the study sites were infested with viable conidia of *D. destructiva*, and conidia-infested arthropods were found at all three sites in the Great Smoky Mountains National Park during 1994 (Table 1). The percent of conidia-infested arthropods collected throughout this study was highest (11.4%; 14/123) at Rich Mountain while conidial infestations were lower at Jakes Creek (5.9%; 7/119) and Sugarlands (4.5%; 6/133).

Most [92.6% (25/27)] of the conidia-infested arthropods at all three sites were collected during June (Table 1) when epidemics of dogwood anthracnose were rapidly expanding (Fig. 1). In fact, the amount of diseased foliage increased about five- to six-fold during June at all sites. This rapid expansion of dogwood anthracnose correlates with production of conidia of *D. destructiva.*

When environmental conditions are conducive for disease development (such as high humidity and cool temperatures), dogwood foliage becomes symptomatic rapidly. Thus, the relatively high incidence of arthropods infested with conidia in June was not surprising since environmental conditions associated with periods of rapid increase in severity of dogwood anthracnose were conducive for production of conidia of *D. destructiva* during May and early June in the Great Smoky Mountains National Park. About 22% of the arthropods collected at Rich Mountain during June were infested with conidia; percent infestation was lower at Jakes Creek and Sugarlands (about 11% and 9%, respectively). The increase in sporulation of the dogwood anthracnose fungus during June (Fig. 1) (M. T. W., unpubl. data) enhanced the likelihood that arthropods could contact and transport viable conidia of *D. destructiva.* In fact, about 13.9% (25 of 180) of the arthropods collected during June were infested with conidia of *D. destructiva*, while only about 1% (2 of 195) of those collected after June were infested. The low incidence of infested arthropods collected after June

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Table 1. Arthropods infested with viable conidia of *Discula destructiva* collected each month from three sites in the Great Smoky Mountains National Park, 1994

Date	Site	Number collected	Number infested	Percent infested
June	Jakes Creek	63	7	11.1
	Rich Mountain	59	13	22.0
	Sugarlands	58	5	8.6
		180	25	13.9
July	Jakes Creek	15	0	0.0
	Rich Mountain	21	1	4.8
	Sugarlands	20	0	0.0
		56	1	1.8
August	Jakes Creek	16	0	0.0
	Rich Mountain	18	0	0.0
	Sugarlands	19	1	5.3
		53	1	1.9
September	Jakes Creek	25	0	0.0
	Rich Mountain	25	0	0.0
	Sugarlands	36	0	0.0
		86	0	0.0
Total	All Sites	375	27	7.2

(Table 1) is consistent with Neitch et al. (1994) who observed that peak arthropod densities coincided with rapid increases in levels of dogwood anthracnose.

The severity of dogwood anthracnose (rated as percent diseased foliage) was 91.0, 62.5, and 81.5% at Rich Mountain, Jakes Creek, and Sugarlands, respectively. The high level of disease severity at Rich Mountain may partially explain the greater numbers of infested arthropods that were found at that site. Severity levels of dogwood anthracnose increased at all locations from the second week of May until late June to early July (Fig. 1). Most arthropods infested with viable conidia were collected during this period (June 10 to 15) (Table 1). After disease severity had leveled off by early July, only one conidia-infested arthropod was collected.

It is unclear if the level of conidia-infested arthropods was merely an index of disease severity or if disease severity was influenced by transportation of conidia of *D. destructiva* by arthropods. Most of the infested arthropods collected in this study (Table 2) were able to move to other dogwoods in close proximity. However, some of the organisms, such as arachnids and immature lepidopterans, may not influence the level of disease severity within a localized area because of their behavior. For example, spiders remain close to their webs to capture prey while many species of

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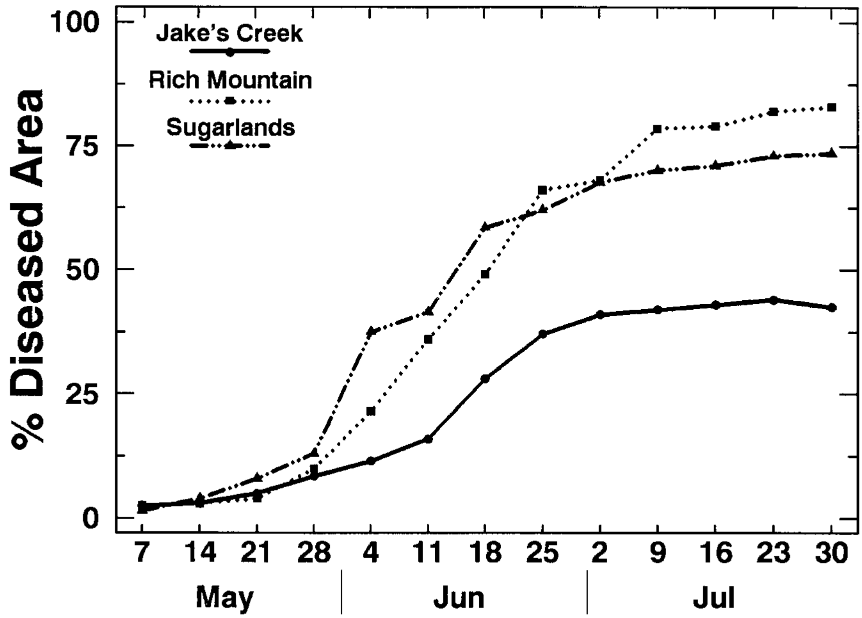

Fig. 1. Severity of dogwood anthracnose at three locations in the Great Smoky Mountains National Park, 1994. Disease severity was rated using a modified Horsfal-Barratt disease rating scale.

immature lepidopterans usually remain on one tree as long as there is a constant food source. Other groups, such as curculionids, formicids, hemipterans, homopterans, and immature and adult orthopterans, are mobile enough to move from tree to tree. Because of potential movement among dogwoods, this agile group of arthropods may intensify the severity of dogwood anthracnose in a localized area but would probably not substantially affect the spread of dogwood anthracnose to healthy stands.

Of the infested insects collected in this study, insects capable of longer-range flight, especially coleopterans, dipterans, and hymenopterans, were found in low numbers (approximately 7%). In a related study, these three orders comprised more than 60% of all insects collected during a two-year diversity study on dogwoods in a forest environment (Neitch et al. 1994). However, our sampling scheme was unable to adequately collect these quick-flying insects; thus, we were unable to fully evaluate the incidence of conidia infestation on these groups.

More precise and efficient sampling methods would enable researchers to better understand the role of arthropods in the epidemiology of dogwood anthracnose. One possible method, as described by Neitch et al. (1994), involves the application of chemical insecticides to the tree canopy. Arthropods capable of avoiding capture by previous sampling methods could then be easily collected and evaluated for conidia. Preliminary studies, however, would be necessary to determine the effect of the insecticide, if any, upon conidial viability before this method could be used successfully.

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Table 2. Percent of different taxonomic groups of field-collected arthropods infested with conidia of *Discula destructiva,* Great Smoky Mountains National Park, 1994*

Taxonomic group	Number infested	Percent of total infested**
Arachnida	2	7.4
Coleoptera	1	3.7
Hemiptera	1	3.7
Homoptera	1	3.7
Hymenoptera	1	3.7
Lepidoptera (immatures)	7	25.9
Orthoptera (immatures)	8	29.6
Orthoptera (adults)	2	7.4
Psocoptera	3	11.1
Unidentified (damaged)	1	3.7
TOTAL	27	100

* Of the 375 arthropods collected, 27 (7.2%) were infested with viable conidia.
** Percent breakdown of conidia-infested arthropods (n = 27).

Our results demonstrated that about 7% of the arthropods collected from dogwoods in a natural environment were infested with viable conidia of *D. destructiva.* Most infested arthropods were collected during the spring when conditions favored conidial production. However, their precise role, if any, in transportation and deposition of conidia, as well as in the epidemiology of dogwood anthracnose, remains uncertain. Further research is necessary to document conidial infestation levels of "mobile" taxa to determine if these arthropods serve as effective dispersal agents of *D. destructiva.*

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