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LIMB DIEBACK OF FLOWERING DOGWOOD CAUSED BY *COLLETOTRICHUM ACUTATUM*

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Abstract. **In 1991 and 1992, an array of symptoms consisting of leaf spots, defoliation, limb and twig dieback, canker-like deformities, and mortality of flowering dogwood trees (*Cornus florida* L.) was observed in several nurseries in north and central Florida. Older affected trees produced flower buds, but the buds did not open. Many trees were destroyed because of unsightly dead limbs and deformed trunks. In 1998-99 the disease re-appeared in north Florida nurseries and once again caused extensive damage. The disease is currently present in nurseries and landscapes and represents a serious threat to nursery production of flowering dogwood in Florida. *Colletotrichum acutatum* J. H. Simmonds was consistently isolated from diseased leaf, pith, and wood samples, from acervuli produced in abundance on diseased leaves, twigs, branches of small and large trees, and from lesions on diseased, rooted cuttings. A modified bud grafting technique was used to insert small pieces of agar containing mycelia of the pathogen beneath the bark of small trees. Typical symptoms developed within 5 months on inoculated trees from which the suspected pathogen was consistently re-isolated. In greenhouse experiments, conidia of the isolated pathogen were used to inoculate small dogwood trees. Within 2 to 3 weeks, a slowly-developing leaf spot was produced on inoculated plants. After 3 months, numerous acervuli were produced on inoculated leaves and on adjacent small twigs from which the same pathogen was consistently re-isolated. Inoculated trees died within 8 months after inoculation. Based upon the size and morphology of conidia and growth characteristics of the fungus on agar medium, the pathogen was identified as a *Colletotrichum acutatum*. Analysis of fungal DNA by the polymerase chain reaction and comparison of PCR products with those from other *Colletotrichum* species confirmed the fungus as a pathotype of *C. acutatum.***

In 1991 and 1992, a damaging disorder of flowering dogwood (*Cornus florida* L.) was frequently observed in north and central Florida where nursery production of dogwood is concentrated. Symptoms consisted of leaf spots, leaf necrosis, and twig and limb dieback. In nurseries and in landscapes, some affected trees died. Others were destroyed because of unsightly dead limbs and deformed trunks. Nurserymen reported that the problem had not been encountered prior to 1991.

Symptoms of this presumptive disease were strikingly similar to those of dogwood anthracnose caused by *Discula destructiva* Redlin (Anderson et al., 1994; Redlin, 1991). Following the 1991-92 episode, Chellemi et al. (1993), investigated the leaf spot and twig blight aspects of the disorder. They isolated and identified the responsible pathogen as *Colletotrichum acutatum* J. H. Simmonds. Concurrent, but independent studies were initiated by Strandberg (2001) to identify factors responsible for trunk damage and mortality phases of the disease on large container-grown trees; initial efforts to implicate a causal agent in diseased trunks and cankers were unsuccessful. Work by Chellemi et al. (1993) provided the first report of *C. acutatum* as a pathogen of flowering dogwood in the United States. However, there are earlier mentions of dogwood anthracnose caused by *C. gleosporioides* (Alfieri et al., 1994; Horst, 1990). Because of the complexities of Colletotrichum taxonomy, it is unclear if these reports might refer to the disease described here. Smith (1993) reported, in Connecticut, the infection of mature fruits of cultivated and wild dogwood trees by *C. acutatum*. Two years later, Britton and Redlin (1995) reported that seed-borne *C. acutatum* caused damping-off and seedling mortality of dogwood grown from field-collected seeds.

After 1994, the incidence of the disease subsided, but in 1998-99, it re-appeared in north Florida, and once again caused serious economic damage and losses for nurserymen. During 1999, in a cooperating nursery in north Florida where severe disease losses were occurring, symptoms were observed on flowering dogwood plants from propagation through several stages of nursery production including trees growing in large (300 L) containers. A *Colletotrichum* species was consistently isolated from acervuli produced on symptomatic, rooted cuttings, small plants, and from affected leaves, twigs, and trunks of larger plants and small trees. This pathogen was also identified as *Colletotrichum acutatum* by morphological and cultural characteristics and molecular-based techniques (Strandberg, 2001).

Brief, preliminary reports by Britton and Redlin (1995 a), Chellemi et al. (1993), Smith (1993), and Strandberg (2001) appear to be the only published reports pertaining to *C. acutatum* as a pathogen of dogwood. This paper summarizes current knowledge about an apparently new dogwood disease incited by *C. acutatum* and describes symptoms and damage as they occur in Florida. Methods used to isolate, culture and identify the pathogen are also presented.

Materials and Methods

Isolation and inoculation. Small portions of diseased leaves or twigs bearing acervuli were excised and placed in 1 to 3 mL of sterile water to float the conidia out of acervuli. The resultant dilute spore suspension was streaked onto PDA [potato dextrose (glucose) agar] plates and incubated for 2 to 5 days at 22 °C. Colonies of the fungus were tentatively identified by their orange or salmon-colored spore masses and by their

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characteristic spore morphology (Adaskaveg and Hartin, 1997; Adaskaveg and Förster, 2000; Sutton, 1992; Walker, 1991) then isolated by hyphal-tip transfers to fresh PDA. When sporulating acervuli were not present, portions of diseased leaves, twigs, pith, or wood were excised, dipped in 70% ethanol, surface-sterilized for 2 to 3 min in 1% calcium hypochlorite, then rinsed in sterile water. Tissues were blotted dry and plated on PDA. Unsterilized cross sections of affected limbs and pieces of discolored pith and branch tissues were also plated on PDA. Fungi were re-isolated from colonies that grew from these tissues and maintained on PDA for further identification and pathogenicity tests. Similar methods were used to isolate and identify other fungi from diseased trees and to confirm the identification and pathogenicity of *C. acutatum* isolated from inoculated plants in pathogenicity tests.

Pathogenicity tests. Two methods were used to inoculate dogwood plants. In the first, (1992 experiments) an 8-mm agar plug was cut from 7-d old colonies of *C. acutatum, Epicoccum nigrum*, or *Pestalotiopsis* spp. (these species were routinely isolated from affected trees) growing on PDA. Agar plugs bearing mycelia were inserted beneath the bark of young dogwood plants; an adapted "T" bud grafting technique was used to cut the bark and insert the agar plug. Incisions were sealed with Parafilm® for 2 weeks after inoculation. Inoculated plants were kept in a warm greenhouse (approximately 28 °C day and 16 °C night temperature) until final evaluation at 50 d after inoculation. In the second method (1999 experiments), *C. acutatum* was grown on PDA for 4 d at 24 °C under 16 h light period (Cool-White fluorescent, 50 $\mu E \cdot sec^{-1} \cdot m^2$). Conidia were rinsed from a single 4-day-old PDA lawn plate with 100 mL of distilled water. The conidial suspension was diluted to contain approximately 10^6 conidia/mL and applied to young dogwood plants with a household spray bottle. Test plants were sprayed until leaves were wet (but not to runoff) then placed in a moist chamber for 48 hr (100% RH, 24 °C). Control plants were sprayed with water. Treated plants were placed in a warm greenhouse (approximately 28 °C day and 16 °C night temperature) and examined periodically for symptoms for up to 8 months after inoculation.

Identification of the pathogen. Colletotrichum acutatum was grown on PDA at 24% C under 16 hr light period (Cool-White fluorescent, 50 $\mu E \cdot sec^{-1} \cdot m^2$). Colony and conidium morphology was determined after 7-10 d. Conidia (50 per sample) were evaluated microscopically for shape and size. DNA was extracted from shake cultures of *C. acutatum* grown for 7 d at 22 °C in V-8 broth [V-8 juice 100 mL, $CaCO_3$, 2 g, water, 900 mL (Tuite, 1969)] which had been centrifuged and filtered to remove $CaCO_3$ and other particulate matter. Mycelia were recovered by filtration then stored at –70 °C. DNA was extracted from *C. acutatum* and other isolates of known *Colletotrichum spp.* by the method of Doyle and Doyle (1990).

Extracted DNA was analyzed by a polymerase chain reaction (PCR) protocol using the CaInt2 - ITS4 and CgInt - ITS4 primer pairs (Adaskaveg and Hartin, 1997; Brown, et al. 1996). Resulting PCR products were compared with those produced by *C. acutatum* pathotypes isolated from leatherleaf fern, lime, post-bloom fruit drop-affected navel orange fruit, and strawberry fruit, and with isolates of *C. gloesporioides* from citrus, Camellia, Nandina, holly, and strawberry.

Results

Symptoms of the disease. An array of symptoms occurred on containerized dogwood plants in the nursery and on trees planted in the landscape. Leaf damage ranged from irregular, brown, necrotic lesions (leaf spots) without prominent borders, to complete necrosis, and eventually, abscission (Fig. 1A). Defoliation of entire limbs, twig and branch dieback, canker-like deformities, and mortality of all sizes of container-grown plants also occurred Twigs, branches, growing points, and portions of main trunks up to 3 cm in diameter were rapidly killed (Fig. 1C). Branches or limbs of seemingly symptomless trees often died quickly once foliage growth resumed in the spring. On affected branches, leaves initially drooped downward, but did not wilt severely; later, they became silvery, gray-green and eventually died, but did not immediately drop (Fig. 1B). New leaves which developed on diseased portions of trees were small, deformed, and did not fully expand. Affected trees produced flower buds, but the buds did not open. Young, container-grown trees turned brown and died within a few months after infection. Diseased plants often died during the second year after propagation.

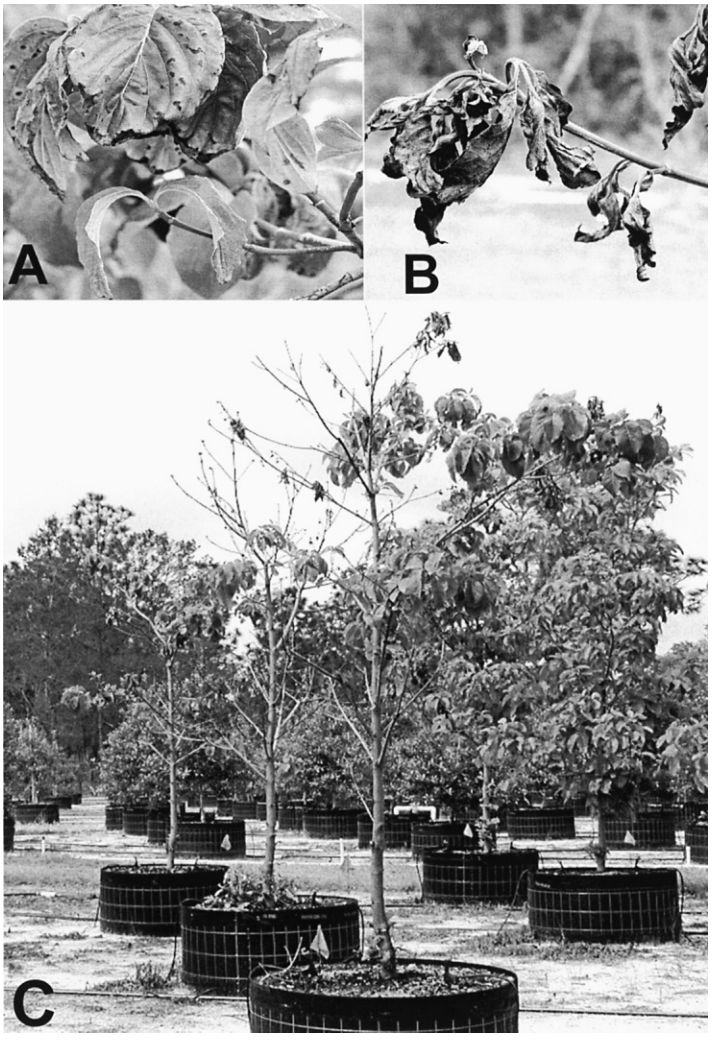

Fig. 1. Common symptoms of limb dieback of flowering dogwood caused by *Colletotrichum acutatum*. **A**. Leaf spots on mature leaves; **B**. Wilting and death of leaves on small, diseased limb; **C**. Extensive limb dieback on large, container-grown flowering dogwood tree.

In warm, humid weather, the pathogen occasionally produced striking masses of orange-colored conidia in patches of acervuli that formed on the bark of dead limbs or just below portions of limb or twig extremities killed by the pathogen. Severely-affected trees often produced numerous adventitious shoots at the base of the tree; the leaves on these shoots were almost always affected by the leaf spot phase of the disease. Cut sections of affected limbs revealed brownish-black or necrotic pith tissue. Wood in affected areas was similarly discolored. It is important to note the similarity of these symptom to those of dogwood anthracnose caused by *Discula destructiva*, because this pathogen has not yet been identified in Florida (Anderson et al., 1994; Redlin, 1991).

Pathogen identification and pathogenicity studies. In the 1992 studies, a *Colletotrichum spp.*, *Eppicoccum nigrum* Link, and *Pestalotiopsis* spp. were consistently isolated from diseased dogwood trees. The *Colletotrichum spp.* was identified on the basis of conidium morphology and colony growth as *Colletotrichum acutatum* J. H. Simmonds, Walker et al. (1991). At 50 d after agar plug inoculations, only plants inoculated with *C. acutatum* produced limb and twig dieback symptoms similar to those observed on the original affected trees. Trees inoculated with *Eppicoccum nigrum*, and *Pestalotiopsis* spp. remained symptomless. Numerous other fungi were also isolated from cankers and from diseased and deformed trunks, but no pathogens were isolated consistently enough to be considered as a causal agent of the disease.

In the 1999 experiments, a *Colletotrichum* species was consistently isolated from acervuli produced in abundance on diseased stems of 1-yr-old rooted cuttings and small container-grown plants. The same fungus was also consistently isolated from acervuli produced on diseased leaves, twigs, branches, trunks of larger plants and small trees, and from discolored wood and pith tissues taken from affected portions of diseased dogwood. On plants and small trees inoculated in June with conidia of the suspected pathogen, leaf spots developed within 30 d after inoculation. After 3 months, numerous acervuli were produced on inoculated leaves and on adjacent small twigs from which the same fungus was consistently reisolated. Following the spring growth flush that occurred 8 months later (in March), all inoculated trees died. Acervuli of the fungus were produced on some, but not all of the dead plants.

On PDA lawn plates inoculated with large numbers of conidia, abundant conidiomata with masses of bright salmon to red-orange conidia were produced. Conidiomata that developed in culture on PDA, or acervuli which formed readily on diseased plant material, did not produce setae. On PDA, mycelial growth was tufted and pale-gray. The underside of colonies was buff to cream-colored or pale-gray to tan, but never dark. With age, a pale-pink or orange-pink pigment usually formed within the agar media surrounding the colony. When produced on PDA, most mature conidia were elliptical and elongated with abruptly-tapering ends. An average of 18% had both ends slightly rounded; none had only one end abruptly tapered or rounded. Conidia measured 15.3 × 4.78 μm; the length/width ratio was 3.20. These results are consistent with published descriptions of *C. acutatum* (Adaskaveg and Hartin, 1997; Adaskaveg and Förster, 2000; Sutton, 1992; Walker et al., 1991). The teleomorph was not found on diseased plant material or in cultures on PDA.

Analysis of DNA by PCR using the CaInt2 - ITS4 and CgInt - ITS4 primer pairs (Adaskaveg and R. J. Hartin, 1997; Brown et al., 1996) indicated the fungus was *C. acutatum*. Comparison of PCR products with those produced by other known *C. acutatum* pathotypes isolated from leatherleaf fern, lime, postbloom fruit drop-affected Navel orange fruit, and strawberry fruit, and with isolates of *C. gloeosporioides* from citrus, Camellia, Nandina, holly, and strawberry further supported the identity of *C. acutatum*. An amplified PCR product (approximately 490-bp) was obtained from all dogwood isolates using primers CaInt2 and ITS4. This result was also consistent with the size of PCR product expected from *C. acutatum* DNA. With the CgInt—ITS4 primer pair specific for *C. gloeosporioides*, no amplification products were produced with DNA from the fungus isolated from dogwood or from other known *C. acutatum* isolates. For all the known isolates of *C. gloeosporioides*, the expected amplification products were produced (Adaskaveg and. Hartin, 1997; Adaskaveg and Förster, 2000; Brown et al., 1996).

Discussion

Based upon the morphology of conidia, growth characteristics in culture on PDA, and PCR results, it was concluded that the dogwood pathogen was *Colletotrichum acutatum*. The dogwood isolate was not pathogenic on leatherleaf fern, key lime, or strawberry (data not presented) so it was further concluded that the pathogen was likely a distinct pathotype of *C. acutatum*.

Although symptoms and damage produced by this disease were strikingly similar to those described for dogwood anthracnose incited by *Discula destructiva* (Anderson et al., 1994; Redlin, 1991), *D. destructiva* was not found on any of the samples tested in this study, whereas, *C. acutatum* was consistently isolated from diseased tissues collected at several locations. It is important to be able to distinguish between dogwood anthracnose caused by *D. destructiva* and the dogwood disease caused by *C. acutatum*, because *D. destructiva* is a major threat to native *Cornus florida* trees in the eastern United States. Its spread and distribution are being extensively monitored (Anderson et al., 1994; Knighten and Anderson, 1993), and movement of infected plants has been prohibited in some areas. Environmental conditions conducive to *D. destructiva* can also occur in North Florida (Chellemi et al., 1992; Chellemi and Britton, 1992). However, *D. destructiva* has not yet been found in Florida (Anderson et al., 1994; Knighten and Anderson, 1993). It is also remarkable that the dogwood disease caused by *C. acutatum* should appear in Florida about the same time that several other disease caused by this fungus were recognized and described in the USA and elsewhere (Adaskaveg and Hartin, 1997; Adaskaveg and Förster, 2000). For example, Britton and Redlin (1995 b) reported that *C. acutatum* could also cause a disease similar to the dogwood disease described here on black gum (*Nyssa sylvatica*) in which leaf spot, stem lesions and branch and twig dieback are also produced. Smith (1993) reported that fruit of flowering dogwood trees growing in Connecticut woods and landscapes were frequently infected with *C. acutatum* which caused irregular, black lesions on the calyx end of fruits. Britton and Redlin (1995 a) investigating the germination of dogwood seeds, found a seed-borne phase of *C. acutatum* which could infect young seedlings and cause damping-off. Presumably, *C. acutatum* can be seed-borne. However, in Florida, the majority of the damage caused by *C. acutatum* thus far has occurred on the widely-grown cultivar 'Weaver' which is propagated from

cuttings. Recently, the disease has also been reported to occur on nursery-grown dogwood propagated from seeds that were collected in the landscape and from wild *C. florida* trees in northern Florida. We have not yet surveyed *Cornus spp.* growing in the wild for this disease.

This report describes an apparently new and destructive disease of flowering dogwood. It is important to recognize this disease, because it has caused significant losses and continues to do so. Moreover, the symptoms can easily be confused with those of dogwood anthracnose and the two diseases produce similar damage to trees. Although infection by *C. acutatum* can produce a wide variety of symptoms and damage on flowering dogwood, we propose that the common name of dogwood limb dieback be used to refer to this disease.

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