

Canker of Dogwood Caused by *Lasiodiplodia theobromae*, a Disease Influenced by Drought Stress or Cultivar Selection

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ABSTRACT

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Lasiodiplodia theobromae caused cankers in both nondrought-stressed and preinoculation drought-stressed, wounded, container-grown seedling dogwoods (*Cornus florida*) (1.2–1.5 m tall). Drought-stressed inoculated trees developed larger cankers than nondrought-stressed inoculated trees. Nonstressed, inoculated Cherokee Chief (red flowered) dogwoods developed larger cankers than Welsh's Jr. Miss (pink flowered) and Barton White (white flowered) dogwoods. No cankers developed on any uninoculated dogwoods regardless of drought stress.

In the spring of 1985, numerous field-grown, 3-yr-old pink dogwoods (*Cornus florida* L.) (0.9–1.2 m tall) in a localized area of a nursery in northern Alabama began to decline. The initial symptom was wilting, which often began on one side of the tree and later spread to involve the entire tree. Wilt gradually progressed into a dieback of the affected branches. By late spring and early summer, whole limbs had died with most of the dead and dying foliage remaining attached. All declining trees developed large, inconspicuous trunk cankers where the bark appeared slightly darker than normal. Beneath the affected bark, underlying tissues were brown and discolored. By late summer, many affected trees had completely died, and the brown, dried foliage had dropped.

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. (= *Botryodiplodia theobromae* Pat.), the anamorph of *Botryosphaeria rhodina* (Cooke) Arx (= *Physalospora rhodina* Cooke) (38,40), was consistently isolated from canker margins (27). Because *L. theobromae* is

often considered a disease agent of stressed, weakened plants (10,23,26,29,37) and drought conditions were present at the time of disease development, we considered the possible involvement of drought stress in disease occurrence and development.

L. theobromae (*B. theobromae*) and its teleomorph *B. rhodina* (*P. rhodina*) have been reported to cause disease on a wide variety of plant species (30,37,42). Leaf spots, cankers, root rots, fruit rots, and seed decays have been reported (5,22,29,30,37,40). In addition to disease reports and descriptions, host indices have listed this fungus on a variety of hosts with and without comments on the presence or absence of disease symptoms (1,2,9).

In 1960, dogwood (*C. florida*) was included in a host listing for *P. rhodina* from Louisiana. No information, however, was given as to the presence of disease (2). A 1987 host listing for *B. rhodina* again included the reference to dogwood in Louisiana (9). Although other recent references have been made to the occurrence of *L. theobromae* on *C. florida* (13,31,37), these citations are traceable back to either the 1960 Louisiana host record (2) or to a paper by Voorhees (42) in 1942 which does not, in fact, make any reference to dogwood.

Because *L. theobromae*, as its teleomorph *B. rhodina*, has been previously reported to occur on dogwood (2), ours is not the first record of this fungus on this host. However, the earlier report on dogwood as a host listing does not establish the presence of or describe the particulars of disease occurrence.

In the mid-1970s, Gouin (11,12) and Lamb (18,19) reported a dogwood canker of unknown etiology that had been observed since the late 1960s in Maryland and Virginia. Cankers on young trees (2–3 yr old) in nursery and landscape settings initially appeared as almost insignificant localized roughenings on the bark. These rough spots might further develop into sunken, necrotic cankers that could girdle the stem and ultimately cause dieback and death of the branches. On larger trees, rough areas might become swollen, deeply cracked, and fissured. Later, similar fissured bark cankers might develop on other areas of the affected trunk. Often, internodes exhibited the dry, cracked, sunken cankers whereas nodes showed the swollen galllike areas (20,43). No signs of fungi were reported. Insects, usually dogwood borers, were often found as secondary occurrences within the cankers (20).

After several years of investigation, the etiology of this dogwood canker is still in question. In 1986, Windham and Montgomery (43) reported that it was the most important disease of field-grown nursery dogwoods (3-yr-old and older trees) in Tennessee. In addition to Maryland, Virginia, and Tennessee, dogwood canker has been reported in Mississippi, Georgia, South Carolina, Kentucky, West Virginia, Indiana, Washington, Oregon, North Carolina, and Ohio (M. Windham, *personal communica-*

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tion). Although several fungi including *L. theobromae* (20) and a few nematode species (32,36) have been associated with cankered areas, only *L. theobromae* has proven to be pathogenic, causing sunken, necrotic cankers on container-grown dogwood (*M. Windham, personal communication*).

Stress has often been implicated as a predisposing factor for canker diseases in a wide variety of hard and soft wood trees. This was well documented by Schoeneweiss in 1975 and 1981 (33,34). Numerous papers have continued to implicate stress, usually drought stress, as a predisposing factor to many types of fungal canker diseases (3,4,7,8,14,15,24,26). *L. theobromae* has been reported on a wide variety of plant species in the tropics, subtropics, and temperate zones (30,31,37). Many of the reports of temperate zone diseases involve cankers of woody trees and shrubs.

Disease reports and descriptions vary with respect to the virulence of the particular isolate on the particular host species studied. Although some *L. theobromae* isolates will cause disease on vigorous plants (5,21,23,25,26,30,41), many more citations describe this fungus as a weak disease agent that will cause disease only on weakened, stressed trees (23,25,26,28,30) or as a mild to moderately aggressive disease agent that is much more of a problem when stress conditions exist (23,26,28,30,31,37). Lewis and van Arsdel (26) showed that different isolates of *L. theobromae* on sycamore varied greatly with respect to virulence. One isolate caused damaging cankers on vigorous trees whereas another isolate would generally cause disease only if the sycamores had been previously subjected to drought stress.

The objectives of this study were to confirm *L. theobromae* as a primary causal agent of a canker disease of dogwood, to determine the importance of drought stress as a predisposing factor in disease susceptibility and development, and to evaluate the susceptibility of dogwood cultivars to *L. theobromae* in the presence and absence of drought stress. A preliminary report has been published (27).

MATERIALS AND METHODS

Isolation and culture. Pieces of bark and xylem tissue were cut from the margins of surface-sterilized (immersed in 0.5% NaOCl for 1 min, then rinsed with sterile distilled water) cankers with a sterile scalpel and placed into plates of acidified Difco potato-dextrose agar (PDAa). To induce sporulation, culture plates with mycelial growth were placed into unsealed plastic bags in a room kept at 19–25 C under fluorescent illumination 8–10 hr daily.

Drought stress effects on pathogenicity. Two tests were conducted on

containerized white seedling dogwoods to determine the pathogenicity of *L. theobromae* as a wound pathogen on nonstressed dogwoods and dogwoods subjected to drought stress just before inoculation.

In the initial test, dormant white seedlings (1.2–1.5 m tall) were potted in 2.6-L containers of a pine bark mix (6:1 pine bark/sand, v/v) amended with 3.5 kg of dolomitic limestone, 1.2 kg of gypsum, 1.2 kg of superphosphate, 0.9 kg of Micromax, and 8.3 kg of Osmocote 18-2.5-10 (NPK) per cubic meter. Trees were placed in a double-layer polyethylene greenhouse in February 1986 and hand-watered twice daily. All trees were completely leafed-out by early March. In late March, water was withheld from half of the trees until 60% of the trees showed incipient wilt, a wilt which persisted from afternoon until morning the following day.

Five drought-stressed and five nonstressed trees were inoculated with *L. theobromae* by placing a mycelial agar plug from a 4- to 5-day-old culture into a truncated half-elliptical slit in the bark (1 mm thick \times 8 mm long \times 5 mm wide) cut with a sterile scalpel. Similar wounds were made on five drought-stressed and five nonstressed control trees. All slits were wrapped loosely with Parafilm to help maintain a humid environment. Trees were then arranged in a randomized block design and watered twice daily. Parafilm wraps were removed 2 wk after inoculation. Trees were fertilized 1 mo after inoculation with 18-6-12 slow-release fertilizer at 1.6 g/L. Cankers were measured 2 mo after inoculation.

This study was repeated in the early fall of 1986 with eight trees per treatment; procedures were identical to those described for the first test.

Timing of drought stress conditions. This test was conducted in the early fall of 1986 using procedures previously described. Four treatments of four single-plant replicates each were included in the test. Treatments included drought stress before inoculation, no drought stress before inoculation, drought stress 2 mo after inoculation, and drought stress before inoculation and 2 mo after inoculation. Identical procedures were followed for companion controls for each treatment except for inoculation with *L. theobromae*. Cankers were measured 3 mo after inoculation.

Cultivar susceptibility. Barton White, Welsh's Jr. Miss, and Cherokee Chief dogwoods (0.9–1.2 m. tall) were maintained in 1.9-L containers of an amended pine bark mix. Irrigation was applied twice daily with overhead sprinklers. Recommended nursery maintenance practices were followed. Tests ran from early June to early September 1987. For each cultivar, eight drought-stressed (incipient wilt on 60% of the trees) and

eight nonstressed trees were inoculated with *L. theobromae* as described earlier. All treatments were matched with uninoculated controls. Canker development was determined 6 and 12 wk after inoculation.

Significance of treatment effects (i.e., drought stress, drought stress timing, and cultivar selection) were tested by analysis of variance (ANOVA) and treatment means were compared with Fisher's protected least significant difference (LSD) test (39) using canker area measurements (canker length in centimeters multiplied by canker width in centimeters).

RESULTS

Isolation and culture. Fungal cultures placed in unsealed plastic bags under fluorescent light produced pycnidia and mature conidia after 10–14 days of incubation. Mature conidia were two celled, 20–30 \times 10–15 μ m, and were a light olive brown in color. Based on characteristics of the pycnidia and conidia, the fungus was identified as *L. theobromae* (27).

Drought stress effects on pathogenicity. In the spring test, drought-stressed trees developed dark brown sunken cankers that measured 5 cm² in area. Pycnidia with conidia were scattered over the surface of the cankers. Cankers did not develop on nondrought-stressed, inoculated trees. Slit wounds on the nonstressed inoculated trees healed, but *L. theobromae* pycnidia and conidia were present at the inoculation site. Wounds on the uninoculated controls healed without any evidence of canker development. Results from the fall test were similar except that small elliptical cankers (1.2 cm² area) developed on the nonstressed inoculated trees. Cankers on drought-stressed trees were larger (2.0 cm² area) ($P = 0.05$). Wounds on the uninoculated control trees healed without any evidence of canker formation.

Timing of drought stress conditions. Cankers on nonstressed trees (1.3 cm² area) were significantly smaller ($P = 0.05$) than cankers on trees that received a preinoculation drought stress (2.5 cm² area) or a pre- and postinoculation drought stress (2.5 cm² area) (Table 1). Cankers on trees subjected only to a postinoculation stress were intermediate (1.9 cm² area) and not significantly different from the nonstressed trees or the preinoculation stressed trees (Table 1). Cankers were not observed on the uninoculated controls.

Cultivar susceptibility. All three dogwood cultivars subjected to a preinoculation drought stress developed large, elliptical, brown, sunken, cracked cankers with canker areas ranging from 6.0 to 25.6 cm² (Table 2). Cankers on nonstressed trees of all three cultivars were smaller ($P = 0.05$) than those on preinoculation stressed trees, and canker areas measured from 1.6 to 15.2 cm².

Canker sizes among the three drought-stressed cultivars were not significantly different. However, with the nonstressed trees, cankers on Cherokee Chief were larger ($P = 0.05$) than those on Welch's Jr. Miss and Barton White (Table 2). Cankers did not develop on any uninoculated control trees.

DISCUSSION

The pathogenicity of *L. theobromae* on drought-stressed and nonstressed seedling white, Barton White, Welsh's Jr. Miss, and Cherokee Chief dogwoods has been clearly demonstrated. Drought stress applied before fungal inoculation influenced canker development because significantly larger cankers consistently developed on all trees stressed before inoculation, but drought was not necessary for disease development. Thus far, our documentation of this disease in nature indicates that stress was involved. As mentioned before, there are numerous reports of fungi causing more damage when stress conditions exist just before, during, or just after inoculation. Drought stress generally results in a higher incidence of canker disease and an increase in canker size (3,4,7,8,14,16,17,26).

In the first pathogenicity test, nonstressed inoculated dogwoods did not develop any cankers. Callus tissue healed over the wounds such that it was difficult to locate the inoculation sites. But, in all other pathogenicity tests, both non-

stressed and stressed inoculated dogwoods developed cankers. Similar results were reported with *Cytospora kunzei* Sacc. on Colorado blue spruce (17,35). Schoeneweiss (35) reported that *C. kunzei* caused cankers in Colorado blue spruce that had been subjected to a preinoculation drought stress, but not in nonstressed trees. Contrarily, Kamiri and Laemmlen (17) had previously reported that *C. kunzei* caused cankers in both drought-stressed and nonstressed Colorado blue spruce. The cankers in the drought-stressed trees were much larger and caused 100% tree mortality whereas those that developed in the nonstressed trees were small and did not appear to affect the overall health of the infected trees. Variation in isolate virulence and host susceptibility modifications by stress (26), as well as genetic variations in host resistance (6), may combine and account for some conflicting reports.

Although cankers did not develop in inoculated nonstressed dogwoods in the first pathogenicity test, scattered surface-protruding pycnidia containing viable spores of *L. theobromae* were produced over the wound inoculation site. Apparently, *L. theobromae* can survive in dogwood bark and presumably could cause disease with the onset of favorable conditions. Schoeneweiss (35) reported that *C. kunzei* colonized the wood of nonstressed Colorado blue spruce without causing canker development. A similar observation was made by Coggeshall working with *L. theobromae* infections of sycamore (6).

White seedling dogwoods are not as susceptible to the canker disease as the cultivars Barton White, Welsh's Jr. Miss, and Cherokee Chief. Based on canker dimensions, these three cultivars were equally susceptible to *L. theobromae* when subjected to preinoculation drought stress. On nonstressed trees, the largest cankers developed on Cherokee Chief followed by Welsh's Jr. Miss and Barton White.

This is the first description of *L. theobromae* canker disease on dogwood (27). The previous report of this fungus on dogwood was a host listing which did not indicate disease type or involvement.

Our results agree with Punithalingam (29,30) and Sinclair et al (37) who reported that *L. theobromae* was an opportunistic pathogen which often caused disease on weakened plants but could, in some situations, cause disease of apparently healthy, nonstressed plants. We regard our isolate of *L. theobromae* as a stress-influenced pathogen that may be partially restricted by appropriate cultivar selection.

We can only speculate on the importance of *L. theobromae* on dogwood in the future. The original trees diagnosed with this disease in 1985 were small and drought stressed. We did not see the fungus on any other dogwoods during 1986–1987. However, we did isolate *L. theobromae* from a sunken, cracked branch canker on an approximately 15-yr-old dogwood submitted for diagnosis during the summer of 1988. This raises the possibility that *L. theobromae* might be the cause of the dogwood canker reported throughout the Southeast (11,12,18–20,32,36,43).

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Table 1. Canker development on white-flowered seedling dogwoods subjected to drought stress before and/or after inoculation with *Lasiodiplodia theobromae*^a

Drought stress	Canker area ^b (cm ²)
Preinoculation	2.5
Postinoculation	1.9
Preinoculation and postinoculation	2.5
None	1.3
LSD ^c ($P = 0.05$)	0.9

^aUninoculated dogwoods did not develop cankers.

^bMeasurements are mean values for four trees.

^cMean separation within columns according to Fisher's least significant difference test.

Table 2. Susceptibility of drought-stressed and nonstressed dogwood cultivars to *Lasiodiplodia theobromae*^a

Cultivar	Canker area ^b	
	Stressed	Nonstressed ^c
Barton White	16.8	3.7
Cherokee Chief (red)	16.0	7.5
Welsh's Jr. Miss (pink)	14.7	4.9
LSD ^d ($P = 0.05$)	4.7	2.6

^aUninoculated dogwoods did not develop cankers.

^bCanker measurements in square centimeters are mean values for eight trees.

^cMean canker areas for nonstressed trees across all varieties were significantly different from stressed trees according to ANOVA ($P = 0.05$).

^dMean separation within columns according to Fisher's least significant difference test.

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