STUDIES ON CERCOSPORA LEAF SPOT

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STUDIES ON CERCOSPORA LEAF SPOT

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STUDIES ON CERCOSPORA LEAF SPOT

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Kassie N. Conner, daughter of Terry Whitaker Gunter and Mike Conner, was born on July 24, 1981 in Montgomery, AL. She has one older sister, Misty Conner. She graduated from Wetumpka High School in Wetumpka, AL in 1999. Mrs. Conner spent her first year of college at Auburn University in Montgomery. She entered Auburn University in 2000. She received a Bachelor of Science degree in Horticulture from Auburn University in 2004. She entered the Graduate School of Auburn University in January of 2005, where she began work towards a master’s degree in Plant Pathology. Mrs. Conner married John Allen Cannon on August 5, 2005.

THESIS ABSTRACT

STUDIES ON CERCOSPORA LEAF SPOT

Kassie N. Conner

Masters of Science, December 15, 2006
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Directed by Kira L. Bowen

Fresh *Pseudocercospora cornicola* specimens were compared to an isotype specimen to confirm species identity. The two specimens proved to be the same taxonomic entity. The fungus was then redescribed and illustrated from these specimens while consideration was given to reclassification of the genus. This anamorph has some characteristics in common with both *Pseudocercospora* and *Pseudocercosporella*. However, possession of intermediate characteristics between the two genera warrants its placement in *Pseudocercospora*.

The epidemiology of Cercospora leaf spot was examined with respects to maximum radial growth and sporulation *in vitro* based on nutritional and environmental parameters and maximum lesion development based on environmental parameters. No sporulation occurred in culture during the experiments. Optimal radial growth occurred on water agar and V-8 agar, the optimum temperature for growth was 30 C for

both media, and optimum growth occurred with a 12 hour diurnal light cycle. Optimum lesion development occurred at 35 C and with a 12 hour diurnal light cycle. Humidity did not affect lesion development.

Flowering dogwood cultivar resistance to Cercospora leaf spot was evaluated at two locations for each of two years. At location one, seven cultivars were evaluated in the first year and eight cultivars the second year; at location two there were five cultivars. Three cultivars at location one were determined to be disease free both years: 'Pumpkin Patch', 'Pygmy', and 'Red Pygmy'. 'Cherokee Brave', 'Cherokee Chief', 'Cherokee Princess', and 'Cloud 9' showed high levels of resistance both years at both locations. 'Little Princess' and 'Stellar Pink' showed lower levels of resistance.

Flowering dogwoods were also evaluated for infection occurrence of *P. cornicola* by applying a protectant fungicide during each of six months. Trees in landscapes were divided into sections and each was sprayed in a different month under the assumption that the section(s) with the lowest level of disease at the end of the season would indicate which month(s) infection occurs. Preliminary results suggest that infection occurs from May to July and this period would be the appropriate time to apply protective fungicides to prevent Cercospora leaf spot.

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I. LITERATURE REVIEW

Cercospora leaf spot, caused by *Pseudocercospora cornicola* (Tracy & Earle) Guo & Liu*,* is a fungal leaf spot that affects flowering dogwoods (*Cornus florida*). This late season disease does not appear on its host until mid-summer or early fall. As the disease progresses the leaf spots can coalesce and the leaves become chlorotic, after which they abscise from the tree causing premature defoliation. Tree vigor may be reduced if the premature defoliation is repeated over several years (13).

The genus *Cornus* is the only documented host of *P. cornicola* (10). The flowering dogwood is among one of the most common and popular plants in the United States. The northern limits of the flowering dogwood include extreme southwestern Maine west to New York, extreme southern Ontario, central Michigan, central Illinois, and central Missouri (15). The southern range of flowering dogwood includes north Florida; and to the west includes southeast Kansas, eastern Oklahoma, and east Texas (15). There is also one variety that grows in Mexico in the mountains of Nuevo Leon and Vera Cruz (15).

There are almost 100 cultivars and varieties of the flowering dogwood. A study was conducted at Auburn University in 1996 through 1998 to determine which cultivars perform best in the Southeast based on plant mortality (14). The best performers of white bract selections include: ‘Barton’, ‘Cloud 9’, ‘Fragrant Cloud’, ‘Ozark Spring’, and ‘Welch Bay Beauty’ (14). The worst performers of the white bract selections include: ‘Autumn Gold’ and ‘Wonderberry’ (14). The best performers of the red and pink bract selections were ‘Cherokee Brave’, ‘Cherokee Chief’ and ‘Pink Beauty’ (14). The only good performer of the selections with variegated foliage was ‘First Lady’, while ‘Cherokee Sunset’ and ‘Rainbow’ performed poorly (14).

P. cornicola has a distribution that spans the Gulf States, Japan (5), and China (12). The Gulf States include Alabama, Georgia, Florida, Mississippi, Louisiana, and Texas. These five states account for 14% of the total operations in the United States that grow dogwoods with a total of 191 operations (16). These states account for 15.3% of the total number of dogwoods sold in the United States with a total of 226,000 trees (16). These five states also account for 9.2% of the total revenue brought in for the sale of dogwoods with a total of $2,439,000 (16).

The state of Alabama ranks second, only behind Tennessee, for the greatest quantity of dogwoods sold in the United States (16). Alabama also ranks seventh (behind Tennessee, Oregon, North Carolina, California, New Jersey, and Illinois) for the most revenue brought in from the sale of dogwoods (16).

Dogwoods are so common in the landscape that they are somewhat overused. Their popularity is due to their year-round ornamental value, with four distinct seasonal characteristics: excellent flower display in spring, summer foliage, fall foliage and fruit, and winter form and bark (8). Another attribution to their popularity is that they never become a problem due to spreading, as is the case with other early blooming plants (8).

Dogwoods are susceptible to a number of fungal foliar diseases. Among some of the most prevalent in the southeast are spot anthracnose, Discula anthracnose, powdery mildew, Phytophthora leaf and shoot blight, Septoria leaf spot, and Cercospora leaf spot. The most common foliage disease on flowering dogwood is spot anthracnose, which is caused by *Elsinoe corni* (13). The more destructive anthracnose is Discula anthracnose, which is caused by *Discula destructiva* (13). The most widespread disease of flowering dogwoods in Alabama and surrounding states is powdery mildew, which is caused by *Oidium* sp. (13). The disease that occurs primarily on container grown flowering dogwoods in nurseries along the Florida and Alabama gulf coast is Phytophthora leaf and shoot blight. The two most prevalent species causing this disease are *Phytophthora parasitica* and *P. palmivora* (13). Septoria leaf spot, caused by *Septoria* spp., is a late season defoliator on dogwoods similar to Cercospora leaf spot. Cercospora leaf spot is caused by *P. cornicola.*

P. cornicola is in the kingdom Fungi. It belongs to the phylum Deuteromycota or Fungi Imperfecti. In early literature the deuteromycetes were called imperfect fungi because they were not known to produce sexual spores, unlike those by the species of the Ascomycota and Basidomycota (1). Classifications and descriptions of these fungi were based on production of conidia, mycelia characteristics, or both (1). Typically, conidia are produced on conidiophores, which are specialized hyphae (1). *Pseudocercospora* belongs to the order Hyphomycetales, which indicates that the conidiophores are formed individually and not enclosed in specialized structures (1). It also belongs to the family Dematiaceae, which indicates the dark color of the hyphae and spores (1).

P. cornicola was formerly named *Cercospora cornicola* and was first described on September 29, 1895 in Ocean Springs, Mississippi by S. M. Tracy and F. S. Earle

(18). The fungus was later reclassified as *P. cornicola* in 1989 by Y.-l. Guo & X.-j. Liu, but was not redescribed (12).

Fresenius was the first to describe the genus *Cercospora* in 1863, which he based on the species found on celery (5). Fresenius gave a composite description to characterize his conception of *Cercospora*, but he did not describe species individually (5). He described the conidiophores as biophilous, colored, geniculate, in fascicles, straight or crooked, with or without septa, bearing one or more spores at one time and bearing spores laterally as well as at the tip (5). He described the conidia as obclavate, straight or curved, multiseptate, not cylindrical, and hyaline (5). His description allowed a broad concept of the genus to be adopted. As a result of which hundreds of species were classified within it. Eventually this genus was broken down to several *Cercospora*-like genera, including *Cercospora*, *Pseudocercospora*, *Cercosporella*, and *Pseudocercosporella*.

The genus *Pseudocercospora* was established in 1910 by Spegazzini. In 1971 Ellis, author of *Dematiaceous Hyphomycetes*, described the genus precisely indicating that the colonies are effuse, tufted, hairy, mid to dark brown or olivaceous brown (9). He described the mycelium as immersed, the stroma as present, and the setae and hyphopodia as absent (9). The conidiophores were described as individual threads that are unbranched, flexuous, often narrow, cylindrical and closely adpressed near the base, splaying out and somewhat swollen towards the apex, pale to mid-brown or olivaceous brown, and smooth (9). Ellis described the conidia as solitary, dry, acrogenous on young conidiophores, later acropleurogenous, simple, mostly obclavate, often rostrate, conico-truncate at the base, pale to mid-brown, smooth or rugulose, and with numerous transverse and occasionally one or two longitudinal or oblique septa (9).

In 1896, S. M. Tracy and F. S. Earle first described the symptoms that *C. cornicola* causes as irregular, brown, deadened spots without a definite border that are 5-10 mm in diameter (18). The hyphae was described as densely clustered from a modular base, very short, continuous, somewhat flexuous, olivaceous, and 11-15 by 3-4 μ in size (18). The conidia were described as slender, thread-like, somewhat curved, mostly continuous, hyaline or light olivaceous and 60-70 by 2-3 μ in size (18).

In 1953, Charles Chupp, author of *A Monograph of the Fungus Genus Cercospora*, added onto Tracy and Earle's description of *C. cornicola*. He described the leaf spots as irregular brown areas without definite borders that are 5-10 mm in extent (5). He described the fruiting as epiphyllous, and the stroma as small, dark, globular, and 20-40 μ in diameter (5). The fascicles were described as dense to very dense, and the conidiophores as very pale olivaceous brown, delicate, wavy, uniform in width and color, without septa, not or rarely mildly geniculate, not branched, have a rounded tip, without visible spore scars, and 2-3.5 x 10-25 μ (5). Chupp described the conidia as narrowly obclavate, subhyaline to very pale olivaceous, mildly curved, obconic base, sub-acute tip, without distinct septa, and 2-3 x 20-70 μ (5).

There has been no special attempt to include literature dealing with the sexual stage of *Cercospora*-like species (5). Presence of sexual stages is recorded only incidentally (5). All known species are *Mycosphaerella* (5).

The most recent work with Cercospora leaf spot was done by Hagan. He described the severity of the disease as mild and reported that the disease may appear on flowering dogwood any time from mid-summer to early fall after several days of showers (13). The effect of the disease has little impact on tree health, but tree vigor may be reduced if repeated defoliation occurs over several years (13). Leaf spot damaged trees have little fall color because they lose their leaves in 2-3 weeks after symptom development (13). Hagan described the symptoms of Cercospora leaf spot as angular to irregularly shaped spots with tan-brown areas (1/8 to ¼ inch in diameter) and diffuse borders (13).

P. cornicola is believed to over-winter on diseased leaves that could be on the ground or hanging in the tree (13). Spores likely infect leaves in the lower portions of the tree canopy first during wet weather (13). Following a heavy dew or rain shower, numerous dark tufts, called fascicles, can be seen on the upper surface of the leaf spot with a hand lens (13). The disease will intensify from July until November during favorable weather conditions (13).

In 1932 the recommended control for Cercospora leaf spot consisted of a general covering spray of Bordeaux at two or three week intervals beginning when the leaves are about full grown (11). Hagan noted that specific control measures of Cercospora leaf spot have not been developed fully (13). A serious reduction in plant vigor resulting from early leaf drop should be prevented by good tree management practices (13). Fungicides can provide good protection from the disease and it is recommended that application begins shortly after an extended period of rain starting in July or August and continuing at a 10 to 14 day interval afterwards (13). Protectant applications are only suggested for trees damaged nearly every year by the disease (13). Recommended fungicides for Cercospora leaf spot include: azoxystrobin, chlorothalonil, myclobutanil, and thiophanate-methyl (13).

The description of the fungus *P. cornicola,* up to now, is still open to interpretation. Few references to Cercospora leaf spot are found in the literature. The lack of interest in this disease may be due to its occurrence late in the growing season, or its degree of damage, since it causes little harm to its host.

Defoliation due to *P. cornicola* infection decreases the plant's aesthetics and longevity, and therefore warrants additional research. First the organism's morphology needs to be adequately described. Favorable conditions for the organism need to be precisely determined *in vitro* and *in vivo* to gain insight on its epidemiology. Finally, since there is not adequate research on control of the fungus, cultivar resistance should be examined as part of a complete control strategy.

II. REVISED DESCRIPTION AND RECLASSIFICATION OF *PSEUDOCERCOSPORA CORNICOLA*

ABSTRACT

Pseudocercospora cornicola (Tracy & Earle) Guo & Liu [basionym *Cercospora cornicola* Tracy & Earle], the causal organism of dogwood Cercospora leaf spot on flowering dogwood, is redescribed and illustrated from its type material and from fresh collections made in Alabama, where it is of common occurrence. Consideration is given to reclassification in the genus *Pseudocercosporella*. Comments are made on previous descriptions and circumscription of the genera *Cercospora*, *Pseudocercospora*, and *Pseudocercosporella*.

INTRODUCTION

The hyphomycetous anamorph *Cercospora cornicola* was first named and described in 1896, based on a collection made the previous year on languishing leaves of *Cornus florida* at Ocean Springs, Mississippi by S. M. Tracy and F. S. Earle (18). The fungus was described as follows: "Epiphyllous, on irregular brown deadened spots without a definite border, 5-10 mm. Hyphae densely clustered from a nodular base, very short, continuous, somewhat flexuous, olivaceous, 11-15 by 3-4 μ; conidia slender, thread-like, somewhat curved, mostly continuous, hyaline or light olivaceous, 60-70 by

2-3 μ" (18). Type specimens were deposited in the herbaria of Cornell University (CUP), the U. S. Department of Agriculture (BPI), Rutgers College (RUT), Columbia University, and Harvard University (FH) (18).

In 1953 Chupp, in '*A Monograph of the Fungus Genus Cercospora*', amended Tracy & Earle's description of *C. cornicola*, describing it thusly: "Leaf spots irregular brown areas without definite borders, 5-10 mm in extent; fruiting epiphyllous; stroma small, dark, globular, 20-40 μ in diameter; fascicles dense to very dense; conidiophores very pale olivaceous brown, delicate, wavy, uniform in width and color, septa not visible, not or rarely mildly geniculate, not branched, rounded tip, spore scars not visible, 2-3.5 x 10-25 μ; conidia narrowly obclavate, subhyaline to very pale olivaceous, mildly curved, obconic base, sub-acute tip, septa indistinct, 2-3 x 20-70 μ". Hosts were listed as *Cornus florida* L., *C. officinalis* Sieb. & Zucc., *C. controversa* Hems., and *Cornus* spp. (5).

The description of *Cercospora*, as given by Fresenius, allowed a broad concept of the genus to be adopted, as a result of which hundreds of species were classified within it. However, it has subsequently been broken down into smaller, more narrowly-defined, segregate genera within a complex of Cercospora-like fungi. *Cercospora cornicola* lacks the prominent, thickened conidiophore and conidial scars typical of *Cercospora* and is, therefore, not considered appropriately classified. In 1989, Y.-l. Guo & X.-j. Liu reclassified it as *Pseudocercospora cornicola*, but it was not given a comprehensive, updated description.

The genus *Pseudocercospora* was established in 1910 by Spegazzini. In 1971 Ellis, in '*Dematiaceous Hyphomycetes*', described the genus in some detail: "Colonies effuse, tufted, hairy, mid to dark brown or olivaceous brown. Mycelium immersed.

Stroma present. Setae and hyphopodia absent. Conidiophores macronematous, synnematous or mononematous caespitose; individual threads unbranched, flexuous, often narrow, cylindrical and closely adpressed near the base, splaying out and somewhat swollen towards the apex, pale to mid brown or olivaceous brown, smooth. Conidiogenous cells integrated, terminal, often monoblastic and percurrent whilst the conidiophores are young, later polyblastic, sympodial and denticulate, with short, broad conical denticles and no scars. Conidia solitary, dry, acrogenous on young conidiophores, later acropleurogenous, simple, mostly obclavate, often rostrate, conico-truncate at the base, pale to mid brown, smooth or rugulose, with numerous transverse and occasionally one or two longitudinal or oblique septa."

A similar genus to *Pseudocercospora* is *Pseudocercosporella*, which was established in 1973 by Dreighton. In '*A Monograph of Cercosporella, Ramularia and Allied Genera*', Braun describes the genus *Pseudocercosporella*: "Phytopathogenic, mostly causing leaf spots, vegetative mycelium usually internal, sometimes with internal primary and external secondary mycelium, hyphae colorless or pale, septate, branched, smooth; stroma lacking to well-developed, substomatal to intraepidermal, large stroma often rupturing the stomata or epidermal cells, sometimes erumpent, hyaline to faintly pigmented. Conidiophores semi-micronematous to macronematous, mononematous, solitary to fasciculate, emerging through stomata or erumpent through the cuticle, arising from inner hyphae or stromata, sometimes formed as lateral branches of superficial hyphae, large stromata with numerous, dense, short conidiophores often forming flat, crustose to subglobose sporodochial conidiomata, conidiophores simple, rarely branched, straight and subcylindrical to geniculate-sinuous, hyaline, occasionally faintly greenish, continuous (reduced to a single conidiogenous cell) or septate; Conidiogenous cells separate (conidiophore reduced to a single Conidiogenous cell) or integrated, terminal, monoblastic, determinate to polyblastic, sympodial, intermediate, conidial scars inconspicuous, unthickened, colorless. Conidia formed singly, rarely in simple or branched chains, subcylindric, filiform, somewhat obclavate, euseptate, usually multiseptate, hyaline, thin-walled, apex obtuse to subacute, base more or less truncate, hilum unthickened, not darkened, conidial secession schizolytic."

Pseudocercospora and *Pseudocercosporella* are closely related genera and are connected by intermediate species (3). The main difference between the two is that *Pseudocercosporella* consists of fungi with colorless conidiophores and conidia, and well-developed, hyaline or subhyaline, rarely pigmented, stromata (3). Meanwhile, *Pseudocercospora* species have pigmented conidiophores and conidia (3).

Pseudocercospora cornicola occurs commonly on living leaves of flowering dogwood (*Cornus florida*) in the southeastern United States. Records exist of its occurrence in Japan (5) and China (12). A recent fresh collection made in Alabama, and examination of the type material of *C. cornicola*, have allowed the opportunity to study the fungus and consider its reclassification. A new combination is considered herein and the species is redescribed and illustrated.

TAXONOMIC PART

Pseudocercospora cornicola (Tracy & Earle) Guo & Liu, Mycosystema 2:232, 1989.

≡ *Cercospora cornicola* Tracy & Earle, Torrey Botanical Club 23:205, 1896.

Leaf spots necrotic lesions, vein-limited, angular, irregularly shaped, up to 10 mm in diameter, and often confluent. Mycelium internal; composed of branched, septate, pale brown hyphae; and 2-3 μ in diameter. Caespituli epiphyllous, consisting of punctiform fascicles, olivaceous brown, discrete, usually abundant, and gregarious to somewhat scattered. Stroma well-developed; erumpent; partly superficial, partly immersed; pale to mid-brown; composed of densely packed, predominately isodiametric, subglobose to somewhat angular cells; pseudoparenchymatous; and up to 70 μ in diameter. Conidiophores numerous in dense fascicles, pale olivaceous brown, smooth walled, cylindrical, straight, or slightly curved, becoming geniculate distally with age, usually one septum, 2-3 μ in width, up to 4 μ at the base, and up to 25 μ in length. Conidia narrowly obclavate, hyaline to very pale olivaceous-brown, straight to slightly curved, faintly septate, usually 1-2 septa, sometimes 3 septa, obtuse at apex, truncate at base, 2-3 μ in width, and 20-70 μ in length.

On living leaves of *C. florida* L. (Cornaceae); Cosmopolitan.

Collections examined: on *C. florida*, Ocean Springs, Mississippi, September 29, 1895, Cornell University, CUP-039517, isotype; on *C. cornicola*, Auburn, Lee County, Alabama, August 31, 2005, K. N. Conner, AUA.

DISCUSSION

Although currently classified in the genus *Pseudocercospora*, *P. cornicola* has some characteristics in common with taxa placed in *Pseudocercosporella*; particularly the presence of conidiophores bearing inconspicuous, unthickened, colorless conidial scars and filiform, thin-walled conidia whose base is unthickened (3). On account of this, *Pseudocercosporella* might be a more appropriate generic home for this species. However, the stromata and conidiophores are somewhat pigmented and therefore its placement in *Pseudocercospora* is probably warranted. This taxon is, essentially, an entity which has features that are intermediate between the two genera.

The revised description differs notably from previous accounts in that the stroma is well-developed and up to 70 µ in diameter, the conidiophores become geniculate distally with age and usually contain one septum, and the conidia are faintly septate, usually containing 1-2 and sometimes 3 septa. These differences may be due to having access to numerous, developed specimens. With this new description there should be no confusion to the identity of *P. cornicola* on flowering dogwoods.

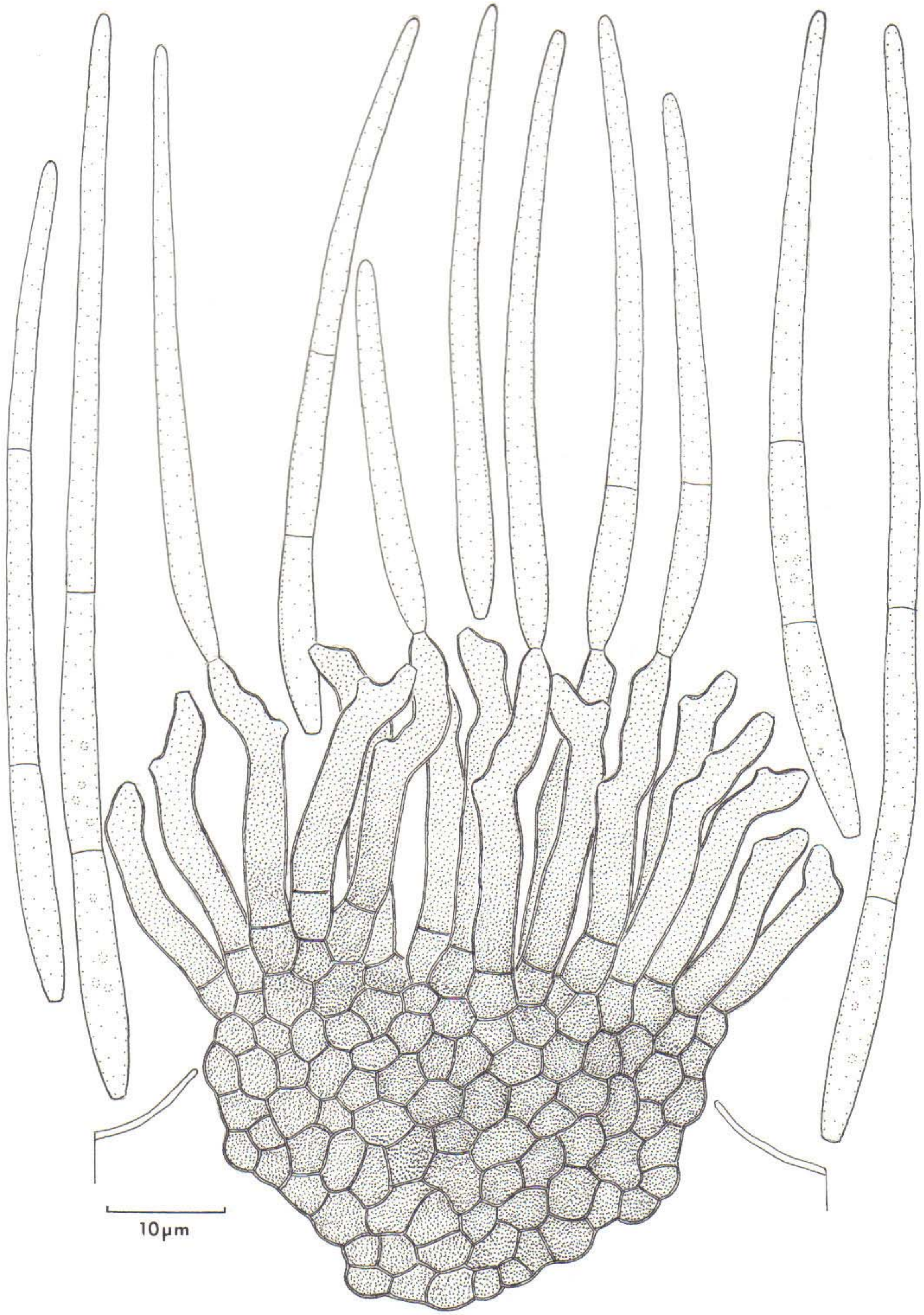

Figure 1. *Pseudocercospora cornicola* caespituli by Dr. G. Morgan-Jones

III. FACTORS INFLUENCING GROWTH AND SPORULATION OF *PSEUDOCERCOSPORA CORNICOLA* AND CERCOSPORA LEAF SPOT DEVELOPMENT IN FLOWERING DOGWOOD

ABSTRACT

Factors influencing growth and sporulation of *Pseudocercospora cornicola* and Cercospora leaf spot development in flowering dogwood were examined with respects to maximum radial growth and sporulation *in vitro* based on nutritional and environmental requirements, and maximum lesion development based on environmental requirements. No sporulation occurred in culture during these experiments. Maximum radial growth occurred on water agar and V-8 agar, at 30 C, and under a 12 hour diurnal light cycle. Maximum lesion development occurred at 35 C, under a 12 hour diurnal light cycle, and at any humidity level within a range of 76 % to 100 % relative humidity.

INTRODUCTION

The flowering dogwood (*Cornus florida*) is a common ornamental landscape tree with exceptional seasonal characteristics. Its four valuable characteristics include: excellent flower in spring, lush summer foliage, fall foliage color and fruit, and winter form and bark (8). Cercospora leaf spot, caused by *Pseudocercospora cornicola*, is a late season leaf spotting fungal disease that affects flowering dogwoods. As the disease progresses through the fall, the leaf spots coalesce and leaves become chlorotic. After chlorosis, the leaves abscise from the tree causing premature defoliation before fall color sets in, decreasing aesthetics.

Cercospora leaf spot may appear on flowering dogwoods from mid-summer to early fall. In Auburn, Alabama in 2005 the disease appeared in late July, but in 2006 appearance was delayed until mid-August. The symptoms consist of necrotic lesions that are vein-limited, angular, irregularly shaped, often confluent, and up to 10 mm in diameter.

Species within the Cercospora complex have specific nutritional and environmental requirements for growth and sporulation (6). There have been successful attempts to induce sporulation in some species in this complex using various media, temperatures, and light regimes (2,4,6), although none have been reported for *P. cornicola*. Decoction media from host tissues are used to induce sporulation in some species, while many sporulate well on carrot leaf-decoction agar and others on V-8 juice agar (7).

Limited knowledge about growth requirements of *P. cornicola* and the effects of environment on disease development has hindered progress in managing Cercospora leaf spot. The purpose of this experiment was to determine optimum cultural conditions for fungal growth and sporulation, and to determine optimum environmental factors for disease development.

METHODS AND MATERIALS

Isolates of *P. cornicola* were obtained from lesions on flowering dogwood in Lee County, Alabama in 2005. A moistened, sterilized needle was used during isolation to dislodge the conidia from conidiophores. Isolates were maintained on acidic potato dextrose agar (APDA) plates and stored at room temperature as stock plates. Three experiments were performed *in vitro* to determine the optimal conditions for growth and sporulation of *P. cornicola* (media, temperature, and light); and three were performed *in vivo* to determine optimal environmental conditions for lesion development (temperature, light, and humidity).

All data collected in experiments were analyzed through analysis of variance using the general linear model procedure (GLM) of the Statistical Analysis System (SAS) and means were separated using Fisher's protected least significant difference test ($P \leq 0.05$).

Effects of Media on Radial Growth and Sporulation

Five media were tested for their effect on radial growth and sporulation of *P. cornicola*: APDA, V-8 juice agar, carrot-leaf decoction agar (CLDA), dogwood leaf agar (DLA), and water agar. Blocks of mycelium from stock plates were ground with 10 ml sterile water and poured onto a clean APDA plate to create a uniform fungal lawn. The resulting lawn was used to inoculate the test media by transferring 0.8 cm plugs to each test plate. The experiment was set up in a completely randomized design consisting of five treatments (the five different media) and six replications of each treatment. Each plate was measured for radial growth and sporulation weekly for four weeks after which the experiment was repeated.

CLDA was prepared by steaming 300 g of pureed carrot leaves in 500 ml distilled water for one hour; the decoction was strained through a double layer of cheesecloth and diluted to 500 ml; the filtrate was mixed with 500 ml distilled water and 16 g agar and steamed for another hour (7). DLA was prepared in a similar manner to CLDA by using 300 g flowering dogwood leaves (50% of which were steamed and 50% left raw). The extract was squeezed out of the leaves using a grinder and 500 ml distilled water; the extract was filtered through a double layer of cheesecloth and diluted to 500 ml; the dilution was microwaved to boiling and added to 500 ml distilled water and 16 g agar.

Effects of Temperature on Radial Growth and Sporulation

Optimal temperature for radial growth and sporulation of *P. cornicola* was assessed in a 12 hour diurnal light cycle at temperatures ranging from 15 to 40 C at five degree intervals. The temperatures were maintained in incubators. Mycelial plugs, 0.8 cm in diameter, were taken from a fungal lawn as described earlier, and placed on freshly prepared culture plates. The experiment was set up in a randomized complete block with a 6x2 factorial arrangement. There were six temperatures (15, 20, 25, 30, 35, and 40 C), two media (water and V-8), and four replications. Colony diameter and sporulation was measured weekly for four weeks. The experiment was repeated, switching the temperatures among the incubators.

Effects of Light on Radial Growth and Sporulation

Optimal light regimes for radial growth and sporulation of *P. cornicola* was assessed at 30 C on water agar and V-8 juice agar using incandescent lights at four different cycles: continuous dark, 12 hours light / 12 hours dark, 16 hours light / 8 hours dark, and continuous light. The lights were set up in incubators and a timer was set to control the light cycles. Mycelial plugs from a fungal lawn were used to inoculate plates. The experiment was set up in a randomized complete block with a factorial arrangement using four light cycles, two media, and five replications. Fungal growth and sporulation was measured as earlier described, and the test was repeated switching the light regimes between the incubators.

Environmental Effects on Lesion Development

Flowering dogwood twigs with attached leaves naturally inoculated with Cercospora leaf spot were collected to determine the effects of temperature, light, and humidity on lesion development. A 2x2 mm lesion on each leaf was marked for monitoring and the twigs were placed in moistened floral foam which was placed in a plastic storage container to prevent desiccation. Effects of temperature on lesion development were tested in incubators on a 12 hour diurnal cycle with temperatures ranging from 20 to 40 C at five degree intervals. Effects of light on lesion development were tested at 35 C using four light cycles: continuous dark, 12 hours light / 12 hours dark, 16 hours light / 8 hours dark, and continuous light. The light cycles were maintained in incubators using a timer.

The effect of relative humidity on lesion development was tested in a similar manner as previous lesion development experiments except the floral foam was wrapped in plastic wrap to retain moisture while ambient relative humidity was manipulated. Small holes, large enough for the base of the twig to penetrate the floral foam, were made in the plastic wrap. Saturated salt solutions were made based on Winston and Bates' method for establishing relative humidity (RH) for biological research (19). The experiment was conducted at room temperature (20 C) with a 12 hour diurnal light cycle.

At 20 C, a saturated NaCl solution produces 76% RH, KCl produces 85%, Na_2CO_3 produces 92%, and KH_2PO_4 produces 96.5% RH (19). Distilled water was used to produce 100% RH. One liter of distilled water was brought to a boil for each solution and 325g, 435g, 315g, and 418g of NaCl, KCl, Na_2CO_3, and KH_2PO_4, respectively, were added to the boiling water. The excess salt was allowed to settle and the saturated solution was decanted off the top. 300 ml of solution was placed in a beaker inside the plastic box with the twigs and leaves. The boxes were sealed with plastic wrap to keep humidity levels constant and a psychrometer was used to ascertain humidity daily.

All lesion development experiments were set up in a completely randomized design using 12 replications. All marked lesions were measured every 48 hours for seven days by taking a width, length, and calculating an area measurement. Each experiment was repeated switching the environmental conditions between incubators to prove reproducibility.

RESULTS

Effects of Media on Radial Growth and Sporulation

P. cornicola had more discriminating nutritional requirements for sporulation than for radial growth. Mycelial growth occurred on all media, but sporulation did not occur on any of the tested media. A dense mat of mycelium formed from the mycelial disk after one week, although growth occurred slowly over the four weeks during which they were measured.

The physical characteristics of the mycelial growth differed on the different media. The growth on the water agar was extremely sparse, while the growth on all other media was thick. The thickest growth was observed on DLA, while radial lines dividing cultures into sections were observed on CLDA and APDA.

Data from repeated experiments was combined and the radial growth means ranged from 4.1 to 2.9 cm (Table 1). Growth on water agar had the greatest mean of 4.1 cm and differed from that on other media. V-8 agar, CLDA, and DLA had similar growth with V-8 agar having the largest mean of the three, 3.8 cm. APDA differed from the other media with a radial growth mean of 2.9.

Effects of Temperature on Radial Growth and Sporulation

P. cornicola grew in culture over a wide range of temperatures (Table 2). When the results from both experiments were combined, mycelial growth occurred from 15 to 35 C, but did not occur at 40 C. The interaction between temperature and media was not significant, thus each temperature on both media was examined separately. Optimal growth on water agar and V-8 agar occurred at 30 C with a mean of 5.2 cm, and these two treatments were similar to one another while different from other treatments. V-8 agar at 25 and 20 C had similar means of 5.1 and 5.0, respectively, and supported slightly less than optimal growth. Fungal growth on water agar at 35 and 40 C and V-8 agar at 40 C had similar means and supported the least amount of growth, if any. All other treatments differed from one another with means ranging from 4.6 to 1.0. Sporulation did not occur at any temperature on either media.

Effects of Light on Radial growth and Sporulation

P. cornicola grew under all 4 light regimes (Table 3). The 12 hour diurnal light cycle and the continuous darkness proved to be similar and optimal for radial growth, when the data from both experiments were combined, with a mean of 3.7 cm. The 16 hour light cycle produced less growth with a mean of 3.5 and continuous light produced the least amount of growth with a mean of 2.4. Sporulation in culture was not observed under any light regime.

Environmental Effects on Lesion Development

Lesion development of Cercospora leaf spot was best at 35, 25, and 30 C, with mean areas of 16.8, 14.7, and 13.8 mm, respectively, after all data was combined (Table 4). At 20 C, lesion development was minimal with a mean of 7.7, and at 40 C leaves became completely desiccated before lesions could develop. 12 hours light / 12 hours dark and 16 hour light / 8 hours dark proved to allow similar lesion development with means of 12.4 and 10.6 mm, respectively (Table 5). Continuous dark and continuous light allowed similar but reduced lesion development with means of 7.2 and 5.5, respectively. There was no significant difference in lesion development between the five different levels of humidity.

DISCUSSION

P. cornicola grew over a wide range of conditions *in vitro* but did not sporulate in any of the conditions tested. All factors tested influenced radial growth including culture media, temperature, and light.

Radial growth was greatest on water agar while V-8 agar produced the highest growth rate among the three remaining media, although differences were not significant. On water agar, growth was exceptionally sparse, and this might be explained by stress on the fungus with no nutritional source. Water agar and V-8 agar were therefore selected to conduct the remaining *in vitro* experiments.

P. cornicola grew at a wide range of temperatures, from 15 to 35 C. Optimum temperature for radial growth occurred at 30 C which corresponds with normal average maximum temperatures for the month of May and September versus normal average maximum temperatures of 33 to 34 C in June, July, and August (17). This may indicate that infection occurs early in the season and the fungus colonizes dogwood leaves in May with a long latency period before symptom development or that colonization occurs late in the season around September.

A 12 hour light cycle and continuous darkness significantly increased radial growth in culture at 30 C. The 12 hour light cycle corresponds to the natural light cycle in spring and fall, which also coincides with those months when normal average maximum temperatures are 30 C. That continuous darkness was observed to increase radial growth leads to speculation that long periods of light in mid-summer may hinder fungal growth and symptom development.

There was no significant difference between lesion development at 25, 30, or 35 C, although temperatures of 35 C did allow larger lesions. The 30 C effect on lesion development corresponds to the 30 C effect on radial growth in culture. Temperatures of 35 C occur in July and August, when symptom development begins (17). At 40 C, physiological stress on the leaves occurred without allowing for lesion development.

Lesion development was significantly higher when exposed to a 12 hour and 16 hour light cycle versus continuous light or dark. The 12 hour light cycle that was observed to increase lesion development corresponds with the 12 hour light cycle increasing radial growth in culture. The 16 hour light cycle corresponds to natural light cycles in July and August when normal average ambient maximum temperatures are 35 C. Lesion development was decreased by continuous darkness but radial growth in culture was increased by continuous darkness, which might be explained by host stress without light discouraging symptom development.

Sporulation did not occur on any of the media, at any temperature, or with any light cycle tested. *P. cornicola* has more fastidious nutritional and environmental requirements for sporulation than for mycelial growth. The relationship between host physiology and nutritional requirements of the pathogen may play a large role in sporulation and should be investigated further.

In 1932, the recommended control for Cercospora leaf spot consisted of a general covering spray of Bordeaux at two or three week intervals beginning when the leaves are about fully grown (11). Hagan noted that specific control measures of Cercospora leaf spot have not been developed fully and a serious reduction in plant vigor resulting from early leaf drop should be prevented by good tree management practices (13). He also noted that fungicides can provide good protection from the disease and recommended that applications begin shortly after an extended period of rain starting in July or August and continuing at 10- to 14- day intervals (13). Protectant applications were only suggested for trees damaged nearly every year by the disease (13). Recommended fungicides for Cercospora leaf spot include azoxystrobin, chlorothalonil, myclobutanil, and thiophanate-methyl (13).

Protectant fungicides only protect the leaf from what is present on the surface at the time of application and what may land on the surface as long as fungicidal residues remain. The mycelium of *P. cornicola* is internal. If a protectant fungicide is sprayed after spore germination and penetration, the sprays will be ineffective. The time period of inoculation of *P. cornicola* on dogwoods is not known. If inoculation occurs in spring and there is a long latency period, as theorized, results from the environmental requirements for growth of *P. cornicola in vitro* and lesion development *in vivo* suggest that protectant fungicidal sprays should begin in May and continue through September, reapplying according to the fungicide label. More experiments need to be performed in order to determine when inoculum of the fungus arrives on the host plant.

Table 1. Radial growth of *Pseudocercospora cornicola* on different culture media after four weeks.

Media	Radial Growth (cm)
Water agar	4.10 a
V-8 agar	3.78 b
Carrot leaf decoction	3.73 b
Dogwood leaf agar	3.70 b
Acidic potato dextrose agar	2.91 c
LSD ($P \leq 0.05$)	0.1931

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$).

Table 2. Radial growth of *Pseudocercospora cornicola* cultured on two media at varying temperatures.

Temperature (C)	Media	Radial Growth (cm)
15	Water agar	2.06 f [y]
15	V-8 agar	3.26 e
20	Water agar	4.55 c
20	V-8 agar	5.01 b
25	Water agar	4.40 d
25	V-8 agar	5.06 b
30	Water agar	5.24 a
30	V-8 agar	5.16 a
35	Water agar	0.84 h
35	V-8 agar	0.99 g
40	Water agar	0.80 h [z]
40	V-8 agar	0.80 h
LSD ($P \leq 0.05$)		0.0878

[y] Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$)

[z] Original plug transfers were 0.8 cm in diameter

Table 3. Radial growth of *Pseudocercospora cornicola* when exposed to varying light regimes.

Light Regime	Radial Growth (cm)
0 hours light	3.71 a
12 hours light / 12 hours dark	3.73 a
16 hours light / 8 hours dark	3.45 b
24 hours light	2.37 c
LSD ($P \leq 0.05$)	0.1705

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$).

Table 4. Cercospora leaf spot lesion size on leaves after seven days at varying temperatures.

Temperature (C)	Area of Lesion (mm)
20	7.68 b
25	14.70 a
30	13.75 a
35	16.79 a
40	-
LSD ($P \leq 0.05$)	3.5153

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$).

Table 5. Cercospora leaf spot lesion size on leaves after seven days when exposed to varying light regimes.

Light Regime	Area of Lesion (mm)
0 hours light	7.21 b
12 hours light / 12 hours dark	12.41 a
16 hours light / 8 hours dark	10.63 a
24 hours light	5.50 b
LSD ($P \leq 0.05$)	2.9408

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$).

IV. FLOWERING DOGWOOD CULTIVAR RESISTANCE TO CERCOSPORA LEAF SPOT

ABSTRACT

In October of 2005 and 2006, a minimum of five cultivars of flowering dogwoods were evaluated for resistance to Cercospora leaf spot at each of two locations. All cultivars were evaluated for incidence and severity of the disease on a 0-100% scale. In 2005, ‘Pygmy’, ‘Red Pygmy’, and ‘Pumpkin Patch’ were disease free. ‘Cherokee Brave’, ‘Cherokee Chief’, ‘Cherokee Princess’, and ‘Cloud 9’ had low levels of disease, while ‘Little Princess’ and ‘Stellar Pink’ had the highest levels of disease. In 2006, weather conditions differed from 2005, causing lower levels of Cercospora leaf spot. ‘Pygmy’, ‘Red Pygmy’, ‘Pumpkin Patch’, ‘Cloud 9’, and ‘Cherokee Chief’ remained disease free. ‘Cherokee Brave’, ‘Little Princess’, and ‘Stellar Pink’ were susceptible with ‘Stellar Pink’ having the highest level of disease. Identifying disease resistant flowering dogwood cultivars is critical to controlling Cercospora leaf spot.

INTRODUCTION

Cercospora leaf spot, caused by the fungus *Pseudocercospora cornicola,* affects flowering dogwoods (*Cornus florida*). This disease does not appear on its host until mid-summer or early fall and causes premature defoliation, which results in a decrease in host aesthetics due to little or no fall color. Cercospora leaf spot does not kill its host, but may reduce tree vigor if defoliation is repeated over several years (13). *P. cornicola* has a documented distribution that spans the Gulf States, Japan (5), and China (12), although the disease is spreading north within the United States. The genus *Cornus* is the only documented host of *P. cornicola* (10). The flowering dogwood is among one of the most common and popular plants in the United States. The northern limits of the flowering dogwood include extreme southwestern Maine, west to New York, extreme southern Ontario, central Michigan, central Illinois, and central Missouri (15). The southern range of flowering dogwood includes north Florida; and to the west includes southeast Kansas, eastern Oklahoma, and east Texas (15). There is also one variety that grows in Mexico in the mountains of Nuevo Leon and Vera Cruz (15).

There are almost 100 cultivars and varieties of the flowering dogwood. A study was conducted at Auburn University in 1996 through 1998 to determine which cultivars perform best in the Southeast based on plant mortality (14). The best performers of white bract selections include: ‘Barton’, ‘Cloud 9’, ‘Fragrant Cloud’, ‘Ozark Spring’, and ‘Welch Bay Beauty’ (14). The worst performers of the white bract selections include: ‘Autumn Gold’ and ‘Wonderberry’ (14). The best performers of the red and pink bract selections were ‘Cherokee Brave’, ‘Cherokee Chief’ and ‘Pink Beauty’ (14). The only good performer of the selections with variegated foliage was ‘First Lady’, while ‘Cherokee Sunset’ and ‘Rainbow’ performed poorly (14).

The Gulf States account for 14% of the total nursery operations in the United States that grow dogwoods with a total of 191 operations (16). These states account for 15.3% of the total number of dogwoods sold in the United States with a total of 226,000 trees (16). These five states also account for 9.2% of the total revenue brought in for the sale of dogwoods with a total of $2,439,000 (16).

The state of Alabama ranks second, only behind Tennessee, for the greatest quantity of dogwoods sold in the United States (16). Alabama also ranks seventh (behind Tennessee, Oregon, North Carolina, California, New Jersey, and Illinois) for the most revenue from the sale of dogwoods (16).

Dogwoods are common in the landscape and somewhat overused. Their popularity is due to their year-round ornamental value, with four distinct seasonal characteristics: excellent flower display in spring, summer foliage, fall foliage and fruit, and winter form and bark (8). Another attribute for their popularity is that they never become a problem due to spreading, as is the case with other early blooming plants (8).

Cercospora leaf spot is not lethal to flowering dogwoods, but a reduction in tree vigor may occur due to repeated infection. Symptoms of the disease include necrotic lesions that are vein-limited, angular, irregularly shaped, up to 10 mm in diameter, and often confluent. When leaf spots coalesce, early leaf abscission occurs, possibly reducing food storage and tree vigor.

Little information is available on the control of Cercospora leaf spot. In 1932 the recommended control consisted of a general covering spray of Bordeaux at two or three week intervals beginning when the leaves are about full size (11). Hagan noted that specific control measures of Cercospora leaf spot have not been developed fully, but a serious reduction in plant vigor resulting from early leaf drop should be prevented by good tree management practices (13). He also noted that fungicides can provide good protection from the disease and it is recommended that applications begin shortly after an extended period of rain starting in July or August and continuing at 10 to 14 day intervals (13). Protectant applications are only suggested for trees damaged nearly every year by the disease and recommended fungicides for Cercospora leaf spot include azoxystrobin, chlorothalonil, myclobutanil, and thiophanate-methyl (13).

A complete disease control program should begin with cultivar choice based on disease resistance. Disease resistance may prevent the need for protectant fungicides allowing for lower inputs in disease control. It has been noted during research on simulated landscape plantings of dogwoods that some cultivars are more resistant than others. Some defoliate a few weeks after symptom development, while others defoliate later in the season. The objective of this experiment was to determine which cultivars are resistant to Cercospora leaf spot as the beginning of a control strategy for disease management.

METHODS AND MATERIALS

A minimum of five cultivars of flowering dogwoods or flowering dogwood hybrids were evaluated for resistance to Cercospora leaf spot at each of two commercial nurseries in the fall of 2005 and 2006. Both locations were located in Franklin County, Tennessee. Five year old field grown trees were evaluated at location one, and seven year old stock trees were evaluated at location two. Trees at neither location were treated with a fungicide spray program.

Cultivars were chosen based on common use in Gulf State landscapes or their fall color. There were nine cultivars evaluated all together. At location one, ‘Cherokee Brave’, ‘Cherokee Chief’, ‘Little Princess’, ‘Pumpkin Patch’, ‘Pygmy’, ‘Red Pygmy’, and ‘Stellar Pink’ (a Rutgers hybrid) were evaluated the first year, with the addition of ‘Cloud 9’ in the second year. At location two, ‘Cherokee Brave’, ‘Cherokee Chief’, ‘Cherokee Princess’, ‘Cloud 9’, and ‘Stellar Pink’ were evaluated.

All cultivars were evaluated for incidence and severity of Cercospora leaf spot. The test was set up as a randomized complete block with samples. In each nursery, trees of each cultivar were divided into four blocks and five trees were randomly evaluated from each block. If incidence was 100% for a cultivar, each of 20 trees were rated for severity on a 0-100% scale. If incidence was less than 100% for a cultivar, each of 30 trees were rated for severity on a 0-100% scale. Samples were collected from all cultivars, brought back to the lab, and examined microscopically to confirm the presence of *P. cornicola*. All data collected in evaluations were subjected to analysis of variance using the general linear model procedure (GLM) of the Statistical Analysis System (SAS) and means were separated using Fisher’s protected least significant difference test at $P \leq 0.05$.

RESULTS

A difference was observed in resistance among the dogwood lines to Cercospora leaf spot. In 2005, at location one, the incidence of Cercospora leaf spot on ‘Cherokee Brave’, ‘Cherokee Chief’, ‘Little Princess’, and ‘Stellar Pink’ was 100% (Table 6). The cultivars ‘Pygmy’, ‘Red Pygmy’, and ‘Pumpkin Patch’ were disease free. Severity of Cercospora leaf spot at location one on ‘Cherokee Chief’ and ‘Cherokee Brave’ were similar with low mean levels of 6.2% and 3.8%, respectively. ‘Stellar Pink’ and ‘Little

Princess' differed from other cultivars evaluated with high mean levels of severity, 25.2% and 12.2%, respectively.

Within the same year at location two, the incidence of Cercospora leaf spot on 'Stellar Pink', 'Cherokee Princess', and 'Cloud 9' was 100%, while 'Cherokee Brave' and 'Cherokee Chief' had lower levels of 76% and 72%, respectively (Table 6). Severity of Cercospora leaf spot was highest on 'Stellar Pink' with a mean of 21.2%. 'Cloud 9', 'Cherokee Princess', 'Cherokee Brave', and 'Cherokee Chief' differed slightly, all within the mean range of 5.0 to 1.2%.

In 2006, at location one, 'Pumpkin Patch', 'Red Pygmy', 'Pygmy', 'Cherokee Chief', and 'Cloud 9' were all disease free, while 'Cherokee Brave', 'Little Princess', and 'Stellar Pink' had a disease incidence of 100%. The three cultivars that had disease differed significantly for disease severity. 'Cherokee Brave' had the lowest levels of severity and 'Stellar Pink' had the highest levels of severity, with means ranging from 4.0% to 13.0%, respectively. At location two in 2006, all cultivars evaluated were disease free except 'Stellar Pink' which had 100% incidence and a mean severity of 2.6%.

DISCUSSION

In 2006, disease levels were lower than in 2005 likely due to below average rainfall levels creating dry conditions that probably limited Cercospora leaf spot infection and development. Cultivars that showed a high level of resistance in 2005 showed complete resistance in 2006, while cultivars that showed a low level of resistance in 2005 showed a higher level in 2006.

In both years, 'Pygmy', 'Red Pygmy', and 'Pumpkin Patch' remained disease free. These cultivars would be considered maintenance free with respects to disease management of Cercospora leaf spot, but they are not commonly planted in the landscape.

'Cherokee Chief', 'Cherokee Brave', 'Cherokee Princess', and 'Cloud 9' had low levels of severity in 2005 and were disease free or had low levels of severity in 2006. These cultivars show a high level of resistance and would require little, if any, disease management of Cercospora leaf spot to avoid premature defoliation. They are also common cultivars in the landscape. 'Little Princess' would require an increased level of disease management or fall color would be minimal, while 'Stellar Pink' would require a higher level of disease management or the tree will prematurely defoliate before fall color begins.

The severity of Cercospora leaf spot is also dependent on other factors. Flowering dogwoods planted in shaded areas do not develop this disease as early as those planted in full sun, but trees planted in shade do not develop fall colors as well as those growing in full sun (personal observation). Resistance is also dependent on sanitation because *P. cornicola* over-winters on diseased leaves on the ground or in the tree (13). The overall health of the tree may also affect the level of resistance by maintaining a low level of stress in the tree.

Table 6. Incidence and severity of Cercospora leaf spot in 2005 on various dogwood cultivars.

	Location 1		Location 2	
Dogwood Line	Incidence	Severity	Incidence	Severity
Flowering Dogwood (*Cornus florida)*				
Cherokee Brave	100 a	3.85 c	76 b	2.13 cd
Cherokee Chief	100 a	6.15 c	72 c	1.17 d
Cherokee Princess	-	-	100 a	3.85 bc
Cloud 9	-	-	100 a	4.95 b
Little Princess	100 a	12.15 b	-	-
Pumpkin Patch	0 b	0 d	-	-
Pygmy	0 b	0 d	-	-
Red Pygmy	0 b	0 d	-	-
Rutgers hybrid dogwood (*C. kousa x florida*)				
Stellar Pink	100 a	25.25 a	100 a	21 25 a
LSD (*P*=0.05)	0	3.3384	0	2.5109

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$)

Table 7. Incidence and severity of Cercospora leaf spot in 2006 on various dogwood cultivars.

	Location 1		Location 2	
Dogwood Line	Incidence	Severity	Incidence	Severity
Flowering Dogwood (*Cornus florida)*				
Cherokee Brave	100 a	4.00 c	0 b	0 b
Cherokee Chief	0 b	0 d	0 b	0 b
Cherokee Princess	-	-	0 b	0 b
Cloud 9	0 b	0 d	0 b	0 b
Little Princess	100 a	7.85 b	-	-
Pumpkin Patch	0 b	0 d	-	-
Pygmy	0 b	0 d	-	-
Red Pygmy	0 b	0 d	-	-
Rutgers hybrid dogwood (*C. kousa x florida*)				
Stellar Pink	100 a	13.05 a	100 a	2.65 a
LSD (P=0.05)	0	1.1732	0	0.4538

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$)

V. INFECTION OCCURENCE OF *PSEUDOCERCOSPORA CORNICOLA* ON FLOWERING DOGWOOD

ABSTRACT

Established flowering dogwoods in a landscape setting were evaluated for infection period of *Pseudocercospora cornicola*. Experiments were performed by dividing trees into sections and spraying one section monthly from spring to early fall with a protectant fungicide. Each section was evaluated weekly, after symptom development, for severity on a 0 to 100% scale to determine infection periods. Lowest severity of disease was assumed to result from protection during infection periods. May, June, and July had the lower disease severity levels and infection is presumed to occur during this period with a latent period lasting until symptom observation in August. Knowledge of infection period is important in scheduling protectant fungicide sprays to manage Cercospora leaf spot.

INTRODUCTION

Cercospora leaf spot, caused by *Pseudocercospora cornicola,* is a fungal leaf spot that affects flowering dogwoods (*Cornus florida*). This late season disease does not appear on its host until mid-summer or early fall. As the disease progresses the leaf spots can coalesce and the leaves become chlorotic, after which they abscise from the tree causing premature defoliation. The severity of Cercospora leaf spot is usually mild and the disease may appear on flowering dogwood any time from mid-summer to early fall after several days of showers (13). The effect of the disease has little apparent impact on tree health, but tree vigor may be reduced if repeated defoliation occurs over several years (13). Leaf spot damaged trees have little or no fall color because they lose their leaves in 2-3 weeks after symptom development (13). Leaf spot symptoms are described as necrotic lesions, vein-limited, angular, irregularly shaped, up to 10 mm in diameter, and often confluent.

P. cornicola is believed to over-winter on diseased leaves that could be on the ground or hanging in the tree (13). Spores likely infect leaves in the lower portions of the tree canopy first during wet weather (13). Following a heavy dew or rain shower, numerous dark tufts, called fascicles, can be seen on the upper surface of the leaf spot with a hand lens (13). The disease will intensify from July until November during favorable weather conditions (13).

In 1932, the recommended control for Cercospora leaf spot consisted of a general covering spray of Bordeaux at two or three week intervals beginning when the leaves are full grown (11). Hagan noted that specific control measures of Cercospora leaf spot have not been developed fully, but a serious reduction in plant vigor resulting from early leaf drop should be prevented by good tree management practices (13). Fungicides can provide good protection from the disease and Hagan recommended that applications begin shortly after an extended period of rain starting in July or August and continuing at 10- to 14- day intervals (13). Protectant applications are only suggested for trees damaged nearly every year by the disease, and recommended fungicides for Cercospora leaf spot include azoxystrobin, chlorothalonil, myclobutanil, and thiophanate-methyl (13).

Based on optimal environmental conditions for growth of *P. cornicola in vitro* occurring in spring with symptom development in late-summer, it is theorized that Cercospora leaf spot has a long latency period. The purpose of this experiment was to determine the infection period of *P. cornicola* on dogwood in order to gain a greater understanding of the biology of this fungus.

METHODS AND MATERIALS

Mature flowering dogwood trees were arbitrarily selected in landscape locations in Auburn, Alabama based on attainability of permission for use in this study. Each tree was divided into six sections of branches and marked. Branches were chosen in the lower portion of the tree canopy because *P. cornicola* infects the lower portion first. Buffer zones were left between the marked limbs to minimize fungicide drift. Chlorothalonil, which is considered a protectant fungicide, was applied to the appropriate section twice at two week intervals. Sprays began in April and different sections were sprayed in different months through September. Theoretically, the treatment(s) with the lowest levels of severity at the end of the season would indicate which month(s) infection occurs.

The experiment was set up in a completely randomized design with six treatments and five replications. The six treatments consisted of the six different months during which fungicides were applied. Upon disease observation, sections were rated for severity on a 0 to 100% scale weekly. Final leaf spot severity data was analyzed through analysis of variance using the general linear model procedure (GLM) of the Statistical Analysis System (SAS) and means were separated using Fisher's protected least significant difference test at $P \leq 0.10$. Significance was set at 10% due to the variation of tree selection, location, and shading among the trees.

RESULTS AND DISCUSSION

Symptom development was initially observed in mid-August and ratings were taken through mid-October. Differences were observed between the different months of protectant fungicide applications ($P=0.087$) (Table 8). There was a significant difference between the replications which might be explained by different tree selections and their resistance levels to Cercospora leaf spot or location effects.

April, August, and September were similar with a mean severity range of 7.6% to 19.8%, while May, June, and July were similar with means ranging from 5.8% to 2.7%. These preliminary data suggest that the infection period of *P. cornicola* occurs from May through July, and this is when protectant fungicides should be sprayed to manage Cercospora leaf spot and prevent premature leaf drop. The experiment will be repeated next year.

Figure 8. Disease severities on parts of dogwood trees sprayed during spring and summer months.

Treatment Month	Severity
April	7.60 ab
May	5.80 b
June	3.25 b
July	2.67 b
August	11.33 ab
September	19.75 a
LSD ($P \leq 0.10$)	12.42

Means within columns followed by different letters are significantly different according to Fisher's protected least significant difference test ($P \leq 0.10$)

V. SUMMARY

Pseudocercospora cornicola was redescribed, illustrated, and consideration was given to a reclassification of the fungus into another genus. The revised description differs notably from previous accounts in that the stroma is well-developed and up to 70 μ in diameter, the conidiophores become geniculate distally with age and usually contain one septum, and the conidia are faintly septate, usually containing 1-2 and sometimes 3 septa. These differences may be due to having access to numerous, developed specimens. With this new description there should be no confusion as to the identity of *P. cornicola* on flowering dogwoods. Based on the revised description, it was decided that the current genera is the correct placement of the fungus.

Factors influencing growth and sporulation of *P. cornicola* and Cercospora leaf spot development in flowering dogwood were evaluated including optimum nutritional and environmental requirements for sporulation and radial growth *in vitro*, and optimal environmental requirements for lesion development. Maximum radial growth occurred on water agar and V-8 agar, at 30 C, and under a 12 hour diurnal light cycle. The optimum conditions *in vitro* coincide with the environmental conditions during the months of May and September, and it is believed that these are the months of active fungal growth. Sporulation did not occur in any situation *in vitro*. This is possibly due to a specific interaction between the fungus and the host physiology. Maximum lesion development occurred at 35 C, under a 12 hour diurnal light cycle, and at any humidity level within a range of 76% to 100% relative humidity. The optimum environmental conditions *in vivo* correspond with naturally occurring environmental conditions in July and August.

Flowering dogwood cultivar resistance to Cercospora leaf spot was evaluated for two consecutive years at each of two locations. In both years, ‘Pygmy’, ‘Red Pygmy’, and ‘Pumpkin Patch’ remained disease free. These cultivars would be considered maintenance free with respects to disease management of Cercospora leaf spot, but they are not common in the landscape. ‘Cherokee Chief’, ‘Cherokee Brave’, ‘Cherokee Princess’, and ‘Cloud 9’ had low levels of severity in 2005 and were disease free or had low levels of severity in 2006. These cultivars show a high level of resistance and would require little, if any, disease management of Cercospora leaf spot to avoid premature defoliation. ‘Little Princess’ would require an increased level of disease management or fall color would be minimal, while ‘Stellar Pink’ would require a higher level of disease management or the tree will prematurely defoliate before fall color begins.

Established flowering dogwoods in a landscape setting were evaluated for infection period of *P. cornicola* in order to gain a greater understanding of the biology of this fungus which is important in scheduling protectant fungicide sprays to manage Cercospora leaf spot. Preliminary data suggests that the infection period of *P. cornicola* occurs in May, June, and July, and this is when protectant fungicides should be sprayed to prevent premature leaf drop.

The objective of the current study was to gain insight on Cercospora leaf spot by redescribing and reclassifying *P. cornicola*, examining factors influencing growth and sporulation of *P. cornicola* and Cercospora leaf spot development in flowering dogwoods, determining flowering dogwood cultivar resistance to Cercospora leaf spot, and determining infection occurrence of *P. cornicola* on flowering dogwoods. The knowledge obtained in this study will build a foundation for the control of Cercospora leaf spot.

LITERATURE CITED

1. Baird, R. E. 2004. Deuteromycota: The Imperfect Fungi. Pages 133-139 in *Plant Pathology, Concepts and Laboratory Exercises.* Robert N. Trigiano, Mark T. Windham & Alan S. Windham (eds.). Boca Raton, Florida: CRC Press L.L.C.
2. Beckman, P. M., and Payne, G. A. 1983. Cultural techniques and conditions influencing growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. Phytopathology 73: 286-289.
3. Braun, U. 1995. *A Monograph of Cercosporella, Ramularia and Allied Genera (Phytopathogenic Hyphomycetes) Vol. 1*. Eching bei Munchen: IHW-Verlag. p. 22-23 and 129-130.
4. Chen, M. D., Lyda, S. D., and Halliwell R. S. 1979. Environmental factors influencing growth and sporulation of *Cercospora kikuchii*. Mycologia 71: 1150-1157.
5. Chupp, C. 1953. *A Monograph of the Fungus Genus Cercospora.* Ithaca, New York: Cornell University Press. p. 175.
6. Cooperman, C. J., and Jenkins, S. F. 1986. Conditions influencing growth and sporulation of *Cercospora asparagi* and Cercospora blight development in asparagus. Phytopathology 76: 617-622.

7. Dhingra, O. D., and Sinclair, J. B. 1995. *Basic Plant Pathology Methods Second Edition.* Boca Raton, Florida: CRC Press Lewis Publishers. p. 363 and 347.

8. Dirr, M. A. 1998. *Manual of Woody Landscape Plants (Their Identification, Ornamental Characteristics, Culture, Propagation and Uses) Fifth Edition.* Champaign, Illinois: Stipes Publishing L.L.C. 256 pp.

9. Ellis, M. B. 1971. *Dematiaceous Hyphomycetes.* Kew: Commonwealth Mycological Institute. p. 208-209.

10. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. *Fungi on Plants and Plant Products in the United States.* St. Paul, MN: APS Press. p. 608.

11. Felt, E. P., and Rankin, W. H. 1932. Insects *and Diseases of Ornamental Trees and Shrubs.* New York: The Macmillan Company. Pg 202.

12. Guo, Y. L., and Liu, X. J. 1989. *Pseudocercospora cornicola*. Mycosystema 2: 232.

13. Hagan, A., and Mullen, J. 2004. *Dogwood Diseases in Alabama.* ANR-551. Alabama Cooperative Extension System, Auburn University.

14. Hardin, B. R., Eakes, D. J., Gilliam, G. J., and Williams, J. D. 1999. Mortality and Growth of Dogwood Selections in the Southeastern United States. Auburn University. On-line resource.

15. Little, E. L., Jr. 1979. *Checklist of United States Trees (native and naturalized).* U.S. Department of Agriculture, Agriculture Handbook 541. Washington D. C. p. 375.

16. NASS. 1998. Census of Horticultural Specialties. Nursery plants sold by state. USDA. On-line resource.

17. National Weather Service Forecast Office. 2006. Climate Report. Montgomery airport climate summary. NOAA. On-line resource.

18. Tracy, S. M., and Earle, F. S. 1896. New Species of Fungi from Mississippi. *Torrey Botanical Club Vol. 23*. p. 205-206.

19. Winston, P. W., and Bates, D. H. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237.