Anthracnose Infection of Dogwood Seedlings Exposed to Natural Inoculum in Western North Carolina

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ABSTRACT

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Groups of 25 healthy dogwood seedlings were exposed for 2 wk to naturally occurring inoculum under mature, diseased trees at 2-wk intervals for three growing seasons. After exposure, seedlings were placed in an incubation room and supplied with trickle irrigation and fluorescent lighting for 2 wk. Following incubation, percent leaf area infected (LAI) was estimated visually. In 1989, LAI remained less than 5% until June. However, consistent rainfall throughout the summer created conditions conducive to infection of seedling groups exposed from 6 June through September, and LAI ranged from 11 to 47% during that period. In 1990 and 1991, LAI was less than 5% until early May, and heavy infection began in mid-May. Midsummer droughts reduced LAI to less than 5%. LAI increased with renewed rainfall but dropped below 10% for the remainder of the season beginning in mid-July 1990 and in September 1991. Numerous secondary infection cycles occurred in each of the 3 yr. Stepwise regression analysis showed that 34% of the variance in LAI was explained by 2-wk rainfall total and 17% was explained by the LAI of the previous seedling group. This supports the hypothesis that secondary infection cycles in southwestern North Carolina depend on consistently recurring rainfall and inoculum buildup.

Additional keywords: *Cornus florida*, dieback, *Discula destructiva* Red.

Dogwood anthracnose was first observed in Washington State in 1976 and was described by Byther and Davidson (3) in 1979. On the west coast it attacked the Pacific dogwood (*Cornus nuttallii* Audubon) most severely, although ornamental plantings of flowering dogwood (*C. florida* L.) and even some of the usually resistant Japanese dogwood (*C. kousa* Hanse) were also affected. Salogga (11) identified the causal organism as a *Discula* sp. in 1982 and reported anthracnose in Washington, Oregon, Idaho, and Vancouver, British Columbia.

Pirone (8) first documented the occurrence of the disease on the east coast in New York in 1978. Anthracnose spread south as far as northern Alabama within 10 yr (1). Hibben and Daughtrey (6) fulfilled Koch's postulates in 1988, and in 1991 Redlin (9) established that the same fungus was responsible for both eastern and western epidemics and described it as a new species, *Discula destructiva* Red.

The first symptoms are leaf spots, usually in the lower two-thirds of the tree. These can be small (1 cm or less), angular, discrete spots with red to purple margins. However, "blight phase" lesions are prevalent when environmental conditions are particularly favorable for disease development. These are large, rapidly expanding, water-soaked, dark brown to black blotches without a reddish border. Lesions generally begin at the margin of the leaf and expand up the veins into the petiole. In North Carolina, leaves infected early in the season usually abscise. Leaves infected late in the season remain on the tree over the winter. Further invasion from these leaves often results in twig dieback (6).

Inoculum apparently overwinters on dead twigs and leaves that fail to abscise. Conidia are produced in acervuli, in a slime matrix, and are dispersed by splashing rain. No perfect stage of the fungus has been found. Hibben and Daughtrey (6) demonstrated the occurrence of secondary cycles by repeated observation of symptoms on marked leaves and new leaves from June through October in New York. They also found that twig dieback was frequent and more severe on understory trees than on trees growing in a semiopen location. Chellemi and Britton (4) reported that more rapid disease development in the understory and in the interior canopy of exposed trees was related to the low evaporative potential characteristic of these microclimates. The study reported here is the first attempt to associate disease development with macroclimate events, e.g., rainfall.

The current control strategy is to maintain tree vigor and general tree health and to apply protectant fungicides. The recommended schedule of applications varies with regional climate. In the northeastern United States, protectant applications are recommended every 10–14 days during the period of leaf expansion (7). In the Pacific Northwest, one application soon after budbreak and a second application 1 mo later provide adequate control (R. Byther, *personal communication*). In the southeastern Appalachians, full-season applications every 14 days are required to provide adequate control (10,16), and fungicide use in the southeast has therefore been discouraged as both costly and tedious.

The objective of this study was to determine when infection occurs in the southeastern Appalachians so that fungicide applications might be timed more effectively and economically.

MATERIALS AND METHODS

Plant materials. The study was conducted at the Coweeta Hydrologic Laboratory, an experimental forest of the USDA Forest Service in southwestern North Carolina. Rainfall averages 180 cm per year in this area (14). In 1990, 87% of the dogwoods in the Coweeta Basin were infected and an average 20% of the total leaf area was affected (5).

Five mature dogwoods infected with dogwood anthracnose were selected as inoculum sources. These were understory trees in a 36-yr-old yellow poplar (*Liriodendron tulipifera* L.) stand. Five 21-cm-diameter holes 15 cm deep and 20 cm apart were dug under one infected branch of each dogwood. The holes were located along a radial line from the exterior canopy zone toward the trunk. These holes were used throughout the study to standardize the locations where potted seedlings were exposed to inoculum.

Two-year-old seedlings produced by the Georgia Forestry Commission near Montezuma, Georgia, were transplanted into 21-cm pots and maintained in a lathhouse under 55% shade in Athens, Georgia. Since these locations were south of the reported range of the disease, seedlings were presumed to be anthracnose-free. Scattered leaf spots, mostly *Septoria*-induced, were sometimes present on the foliage. The first two groups of seedlings exposed each year were placed in a greenhouse for 1 mo prior to exposure in order to hasten leaf development and increase the probability

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of inoculum interception.

Infection studies. During 1989–1991, five healthy seedlings were placed under each inoculum tree every 2 wk from dogwood bloom in mid-April through September. After 2 wk of exposure to natural inoculum and weather conditions, the seedlings were removed to an enclosed incubation room and provided with trickle irrigation and fluorescent light (8 $\mu E \cdot m^{-1} \cdot s^{-1}$. Temperature remained at 18 ± 5 C. Plant foliage was kept dry for 2 wk to permit latent infections to develop. The percent leaf area infected (LAI) on each seedling was estimated visually. Any nonanthracnose lesions were disregarded. Isolations from atypical lesions were occasionally necessary to confirm pathogen identity, but most assessments were based on characteristic symptom expression.

Weather data. A tipping bucket gauge (Belfort Reading Rain Gauge, Belfort Instrument Co., Baltimore, MD) located in a clearing 150 m downhill from the study site was used to measure rainfall.

Data analysis. Stepwise multiple regression analysis (12) was used to regress LAI on 2-wk rainfall total, the number of rain events, previous 2-wk rainfall, and LAI from the previous seedling group. This last variable was included as an estimate of inoculum availability. The alpha level used to control the inclusion and exclusion of independent variables was 0.15. Adjusted R^2 values were calculated and are reported for each analysis.

RESULTS

The stepwise regression analysis on LAI indicated that 51% of the variation in seedling group infection was explained by 2-wk rainfall total (34%) and by LAI of the previous seedling group (17%). Parameter estimates were 1.5 and 0.4, respectively. The interaction of these variables was not significant, and the adjusted R^2 for the model was 0.47. Deleting data accumulated prior to 1 June, when temperature and inoculum might have been limiting, improved the model adjusted R^2 to 0.62. Separate models for each summer (after 1 June) were calculated using these same two independent variables and yielded an adjusted R^2 of 0.26 in 1989, 0.82 in 1990, and 0.88 in 1991.

1989 Results. Less than 5% of the leaf area of seedlings exposed in April or May became infected even though more than 20 cm of rain fell from 14 April to 23 May (Fig. 1). The first severe infections occurred in the group of plants that received 20.4 cm of rain from 6 June to 20 June. Subsequent plant groups developed high LAI despite much lower rainfall. The last two plant groups, which received 12.4 and 19.5 cm rain, respectively, were very severely infected.

1990 Results. The first group of seedlings exposed in 1990 died from drought stress, and data for these seedlings were

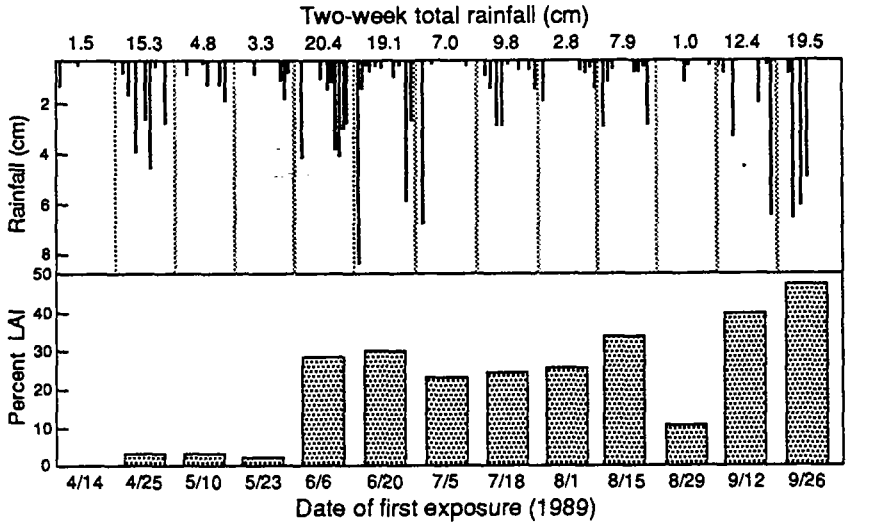

Fig. 1. Mean percent leaf area infected (LAI) on five seedling groups exposed to natural inoculum on each date in 1989. LSD = 24%. Upper bars indicate daily rainfall beneath the 2-wk total rainfall for each exposure group.

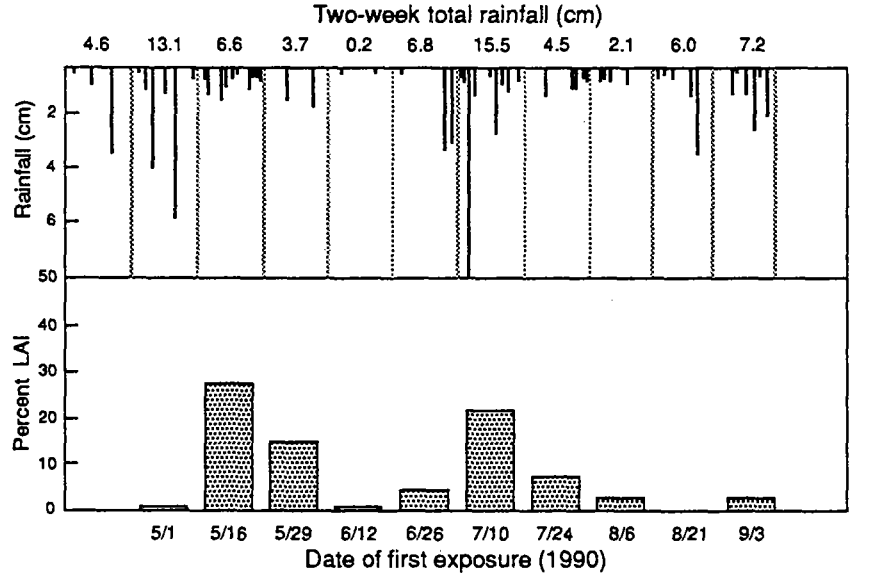

Fig. 2. Mean percent leaf area infected (LAI) on five seedling groups exposed to natural inoculum on each date in 1990. LSD = 17%. Upper bars indicate daily rainfall beneath the 2-wk total rainfall for each exposure group.

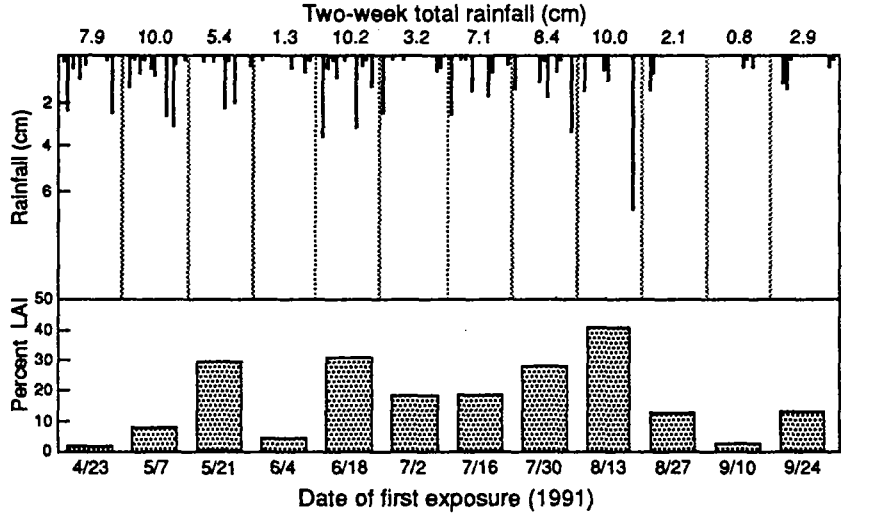

Fig. 3. Mean percent leaf area infected (LAI) on five seedling groups exposed to natural inoculum on each date in 1991. LSD = 17%. Upper bars indicate daily rainfall beneath the 2-wk total rainfall for each exposure group.

omitted from the study. Seedlings exposed on 1 May did not become infected, although they received 13.1 cm of rain (Fig. 2). The first severe infections occurred in the group exposed 15 May. Only 0.2 cm of rain fell from 12 to 26 June, and LAI dropped to 1%. Heavy rains did not occur until near the end of the next exposure period, and LAI for that period was only 5%. Frequent rainfall events resumed in July, and the next exposure group, which received 15.5 cm of rain, sustained 24% LAI. LAI fell below 10% for the remainder of the season, although rainfall averaged 4.9 cm per exposure period. A final group of seedlings exposed 18 September also died from drought stress, and data for these seedlings were excluded from the study.

1991 Results. LAI was very low early in the season (Fig. 3). Severe infection occurred in the third exposure group, with 30% LAI after only 5.4 cm of rain. Early June was dry, and LAI was low. Severe infection resumed with increased rainfall in late June and August. Infections continued to occur through September.

In all 3 yr, very little infection occurred until after 6 May (Fig. 4). Secondary infection cycles occurred throughout the growing season. The correlation between LAI and amount of rainfall is consistent with the hypothesis that infection depends on rainfall, among other conditions.

DISCUSSION

These studies clearly establish that infection can occur at any time throughout the summer, given sufficient rainfall. They also show that in the mountains of southwest North Carolina, LAI is lower before 15 May than later in the summer.

Salogga (11) reported that the first symptoms on Pacific dogwood in the northwestern United States appeared in late May or early June. First symptoms generally appeared about 1 mo after bloom on native flowering dogwood trees in southwestern North Carolina during the 3 yr of this study, although small lesions were found earlier in 1990. These lesions were observed on the tips of expanding primary leaves approximately 2 wk after bloom, resulted only in leaf tip necrosis, and did not expand into the blight phase. These lesions may be the result of infections that take place in the bud or during bud expansion (F. F. Hendrix, *personal communication*).

Data obtained in this 3-yr study suggest that early-season infections are much less common in the mountains of North Carolina than those occurring during late spring and early summer. Heavy infections were not observed in the first month following bloom, which usually occurs in mid-April at the study elevation (777 m). Early-season infection of trees in natural stands was even less severe than infection of seedlings exposed in our study. Since young leaves are quite susceptible, presumably less infection occurred on native trees because in early spring they had less leaf area than the forced seedlings; this would reduce inoculum interception. Rainfall was usually plentiful in early-season exposure periods, but both inoculum density and temperatures may have been limiting. Conidia are probably produced de novo in spring by overwintering mycelia or acervuli. If conidia produced in the autumn survive over winter in infected leaves and twigs, spore viability may be low. Furthermore, temperatures in May averaged only 15–18 C during wet periods. The optimum temperature for in vitro spore germination is 20–24 C (2). Cool early spring temperatures might reduce conidial production or infection efficiency of already scarce or low-vigor inoculum. After 1 June, average temperatures during wet periods were higher (18–21 C). The effect of temperature on infection by *D. destructiva* has not been studied under suitably controlled conditions.

The present study confirms the occurrence of secondary infection cycles reported by Hibben and Daughtrey (6), who made repeated observations on flagged branches between June and October in New York. Smith (13) recently reported that anthracnose was apparently monocyclic in Connecticut in 1990 and 1991, with most infections occurring within 4 wk of leaf emergence. Fewer secondary cycles would be expected in drier years or in areas that are warmer and receive less rain than Coweeta in July and August (15). The regression results reported here support the hypothesis that infection, especially for secondary cycles occurring after 1 June, is related to rainfall. Repeated defoliation caused by numerous cycles of infection is the major cause of tree mortality from dogwood anthracnose and probably accounts for the severity of the disease in the mountains of North Carolina.

It is apparent that widely different climatic conditions result in a regional disparity in the initiation of epidemics and require very different fungicide spray schedules for control. Early-season sprays suffice to prevent extensive leaf infections in the northeast and Pacific Northwest. However, in the mountains of the southeast where the disease is very severe and where numerous secondary cycles occur, early-season fungicides would probably provide less control than would sprays applied after 15 May. Secondary cycles throughout the summer necessitate protectant coverage as long as rainfall is likely. However, the recent labeling of propiconazole for anthracnose control may permit future development of a forecasting model to reduce the number of applications required on the basis of leaf wetness duration and temperature.

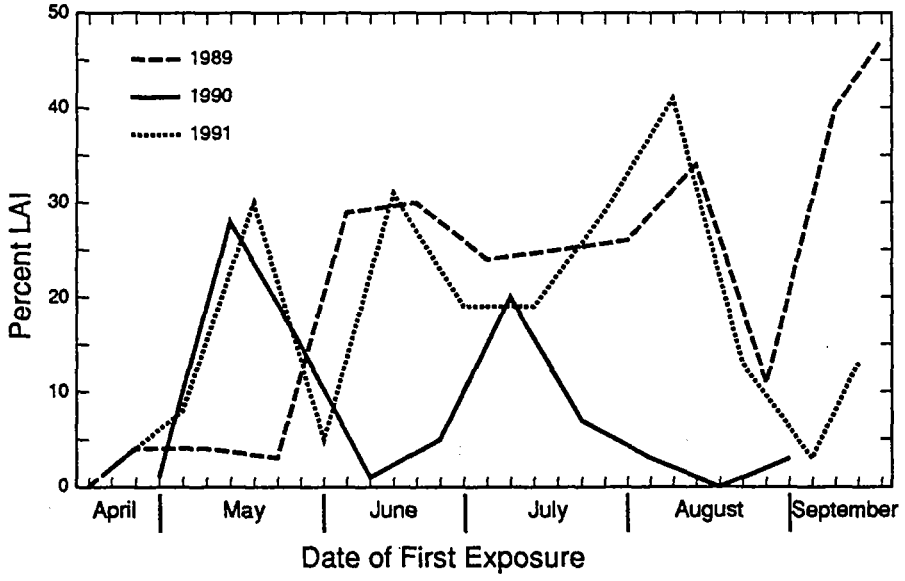

Fig. 4. Mean percent leaf area infected (LAI) on seedling groups exposed to natural inoculation during 1989–1991, superimposed for comparative purposes.

ACKNOWLEDGMENTS

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Leptotrochila medicaginis: Moisture Requirements for Ascospore Discharge, Germination, and Plant Infection

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ABSTRACT

Semeniuk, G. 1993. *Leptotrochila medicaginis*: Moisture requirements for ascospore discharge, germination, and plant infection. Plant Dis. 77:37-42.

In humidity-controlled atmospheres above water or different concentrations of aqueous NaCl solutions at 20–22 C, mature apothecia on dry, infected alfalfa leaves (*Medicago sativa*) rehydrated and discharged ascospores of *Leptotrochila medicaginis* more abundantly near 99.5 and 100% RH and less abundantly at progressively lower RH values; RH limits for discharge were 98.0% at 14.4 and 15.5 C and 97.5% at 20–22 and 26-28 C. On glass in a moist chamber (~100% RH) and on fresh water agar (WA) at 20–25 C, discharged ascospores swelled and formed short germ tubes within 4 hr, which lengthened to spore width (approximately 5 μm) within 6 hr. Ascospores on fresh potato-dextrose agar completed these two processes within 4 hr. They also completed these two processes on WA fortified with glucose, glycerol, and sucrose as humectants within 4 hr at water potentials ranging from −40.6 to −30.7 bar and within 12–24 hr at water potentials ranging from −70.8 to −63.6 bar, and on WA fortified with NaCl and KCl as humectants within 24 hr at water potentials of −49.2 and −63.6 bar, respectively. Ascospores on alfalfa stem cuttings held in a moist chamber at 20–25 C swelled within 4 hr, and their germ tubes infected leaves within 8 hr. At a constant 17 C, the minimum RH for leaf infection within 24 hr was 95%.

Additional keywords: yellow leafblotch

From 1952 to 1983, yellow leafblotch of alfalfa (*Medicago sativa* L.), induced by *Leptotrochila medicaginis* (Fuckel) H. Schüepp, occurred in varying incidences year after year in the western part of South Dakota. In the eastern part of the state where rainfall is higher, it almost ceased to exist in the early 1960s, having been abundant there during the mid-1950s. The reason for this difference was not understood, but it may have been due to differences in the rate of inoculum regeneration and dissipation as related to moisture availability. Salunska (17) performed field studies of apothecial development, ascospore production, and disease progress by the yellow leafblotch pathogen and deduced that alternating dry and wet periods favored pathogen persistence and disease occurrence, and that ambient moisture conditions above 70% relative humidity (RH) were necessary for ascospore discharge and plant infection. To assess more accurately the latter relationship for *L. medicaginis*, a determination was made of moisture needs for ascospore discharge, ascospore germination, and plant infection. A preliminary summary of the results has been reported (21).

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MATERIALS AND METHODS

Supply of mature apothecia and ascospores. Alfalfa leaves with yellow leafblotch symptoms were collected from fields in western South Dakota in early summer 1981 and exposed, as previously described (19,20,25), to local (Brookings) outdoor moisture conditions until apothecia with mature ascospores developed. Ascospores developed by early fall, whereupon the leaves were pressed flat and air-dried between moisture-absorbing paper, stored in a closed polyethylene box at 4–8 C, and used over the ensuing winter months.

Moisture required for ascospore discharge. Three or four dry leaflets with well-developed apothecia were placed on each of two 25 × 75 mm glass microscope slides contained in transparent plastic boxes (80 × 63 × 30 mm). These slides were supported horizontally and side-by-side in each box by end-wall ledges 10 mm above 60 ml of aqueous NaCl solutions (12,16) or deionized water that produced relative humidities ranging from 95 to 100% at 0.5% intervals. Other slides were supported 3 mm above the leaf-bearing slides in order to catch discharged ascospores. The boxes were covered and sealed tightly with plastic tape.

Four trials were conducted, each with a triplicate array of leaf-containing boxes with a range of RH values. In two trials, boxes were kept in a large, insulated room at a constant temperature of 14.4 ± 0.5 and 15.5 ± 0.5 C, respectively; apothecia were scored for their ascospore discharges at 2- and 4-day intervals. In the other two trials, boxes were held in temperature-controlled cabinets at 21.0 ± 1.0 and 27.0 ± 1.0 C, respectively; ascospore discharge scores were determined after 2 days.

Moisture required for ascospore germination. Dry leaflets bearing mature apothecia were rehydrated for 1–2 days at 2–5 C on a wet paper towel within a closed plastic box. Each leaflet, with apothecia facing upward, was then transferred onto a piece of wet paper towel within the well (18 mm wide × 5 mm deep) of a Syracuse dish and held at 20–25 C. Each well was covered with an ethanol-sterilized microscope coverslip