Effects of Pretreatment with Simulated Acid Rain on the Severity of Dogwood Anthracnose

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ABSTRACT

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The effects of simulated acid rain on dogwood anthracnose severity were evaluated in a series of greenhouse and field experiments over a 4-year period. In 1990 and 1991, *Cornus florida* seedlings received 10 weekly foliar applications of simulated rain adjusted to pH 2.5, 3.5, 4.5, and 5.5. They were then placed under mature dogwoods naturally infected with *Discula destructiva*. In both years, the percent leaf area infected increased significantly as the pH of the simulated rain solution decreased. In 1992 and 1993, seedlings were wrapped in plastic bags below the root collar to permit separate application of simulated acid rain (pH 2.5) or normal rain (pH 5.5) to the foliage or the growing medium or both. Application of pH 2.5 rain to the growing medium increased disease severity. Foliar applications alone did not increase disease. These results suggest that changes in nutrient availability, rather than foliar damage, are responsible for the increase in anthracnose severity in dogwoods pretreated with simulated acid rain.

Additional keyword: flowering dogwood

Dogwood anthracnose, caused by *Discula destructiva* Redlin, was first reported in 1976 (12). The rapid spread of this disease has led to controversy concerning its origin. The fungus may be exotic (32), but it has not been found elsewhere. However, environmental factors, such as hard winters or air pollution, may also have increased susceptibility of flowering dogwood (*Cornus florida* L.) (21,22).

The relationship between acid rain and forest diseases has been the subject of debate (18,23,34,38). Acid rain has both direct and indirect effects on plants and associated microorganisms (20,24,27,29,37). For many fungi, especially rusts and wood decay organisms, the effects are inhibitory (13,20,27,34,36,38). Bruck and Shafer (11) suggest that acid deposition, by wounding host tissues, can induce generalized resistance responses effective against obligate parasites. However, wounding and stress can also increase senescence, and thus increase susceptibility to facultative parasites (11). In some plants, acid rain increases leaf wettability, foliar water-holding capacity, foliar nutrient uptake, and the leaching of polar solutes (16,25, 31). Acid fog affects water relations (15, 28) in some plants. Effects such as these may have more impact in trees than in annual plants. Acid rain can also produce cumulative effects indirectly, through the soil. It can increase the concentration of aluminum and base cations in the soil solution and provide nitrogen, a limiting nutrient in many southeastern soils (4).

Madden and Campbell (26) note that the effects of air pollutants on pathogen virulence and host resistance have rarely been studied. Most of the literature considers effects on spore germination and penetration or the ultimate effects on disease severity. Information about how acid rain influences disease severity is limited. The effects of acid rain on Scleroderris canker, caused by *Gremmeniella abietina*, illustrate the potential for complexity when a disease and a pollutant interact. *G. abietina* infection is usually latent in Norway spruce. Acid rain enables the pathogen to produce disease symptoms by reducing competition and enhancing spore germination (2). Competition is reduced when acid rain affects epiphytes and endophytes (3). At the same time, acid rain causes ion leakage that enhances spore germination of *G. abietina*. Intriguingly, Scleroderris canker severity on Scots (3) and red pines (6), which are normally more susceptible than spruce, is not affected by acid rain treatments.

In 1993, Anderson et al. (1) reported that simulated acid rain (SAR) increased the susceptibility of dogwoods to anthracnose under laboratory conditions. However, the mechanism is unknown.

D. destructiva is inhibited by acidic conditions in vitro. Conidial germination on agar was zero at pH 2.0, but not significantly affected at pH 3.0 to 5.6 (7). Mycelial growth was also reduced by acidic conditions (9). Thus, SAR treatments do not directly favor growth of the pathogen.

Haines et al. (19) reported no visible damage on dogwoods treated with pH 2.0 SAR. However, scanning electron microscopy studies have since shown that SAR treatments eroded epicuticular wax and altered trichome morphology of dogwood leaves (10,40). Willey and Hackney demonstrated increased leaching of calcium and magnesium ions from dogwood leaves treated with SAR droplets (41). Plant leachates often contain sugars and amino acids, although these have not been investigated in dogwood. Leakage of foliar nutrients may either stimulate or inhibit pathogenic fungi (5). An excessive loss of nutrients through leaching could also stress the host. Stress from other sources (i.e., drought) increases susceptibility to anthracnose (17).

The 1990 and 1991 studies reported here were designed to determine whether pretreatments with SAR that had increased anthracnose susceptibility in the laboratory (1) would also increase susceptibility in the field. Studies during 1992 and 1993 were undertaken to determine whether aboveground factors (e.g., cuticular erosion) or belowground factors (e.g., altered nutrient availability) were responsible for the observed increase in susceptibility.

MATERIALS AND METHODS

Field verification (1990 and 1991). One-year-old flowering dogwood seedlings were obtained in January of each year from the Georgia Forestry Commission nursery near Montezuma. In February, they were planted individually in 2.5-liter pots in a mixture of equal parts by volume of peat moss, perlite, and topsoil. Seedlings were placed in an air-conditioned greenhouse maintained at 23 to 27°C at the Center for Forest Environmental Studies in Dry Branch, GA. The seedlings were fertilized 2 weeks after planting with an excess volume of a dilute commercial fertilizer (15:30:15 at 100 µg of N per ml).

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Before pretreatment assignment, 200 seedlings were selected for uniformity in size and vigor. Fifty seedlings were randomly assigned to each of four pretreatments and subdivided into five replications (10 seedlings/treatment/replication). The pH of SAR solutions was adjusted to 2.5, 3.5, 4.5, or 5.5 with a 0.65 M mixture of sulfuric and nitric acids in approximately the same ratio found in ambient rain (70 mequiv SO_4^{-2}:30 mequiv. NO_3^-) (35).

Rain pretreatments were 1 cm applied once weekly for 10 weeks by means of a rain simulator (14). Droplets formed on the tips of the simulator needles and fell onto seedlings that had been placed on rotating tables to ensure uniform application. To maintain adequate levels of soil moisture, 300 ml of SAR of the appropriate pH was applied directly to the growing medium in each pot approximately once a week. All seedlings were lightly fertilized once during the pretreatment application period (14 kg of N per ha).

After SAR pretreatment, all pots with dogwood seedlings were transported to Coweeta Hydrologic Laboratory in southwestern North Carolina and placed under mature dogwoods that were naturally infected with *D. destructiva*. To reduce the effects of spatial variation in inoculum density, the 40 pots in each replication were arranged in 10 groups of four, with each group containing one seedling from each pretreatment (40 seedlings per replication; 5 replications).

In June and September, disease severity was assessed by visually estimating the percent leaf area infected for each seedling. The evaluator, who was unaware of SAR pretreatments, trained by using a ForestHealth computer training module (30) and tested at 80% reliability on repeated assessments of dogwoods in these studies. The means for the 10 seedlings receiving each pretreatment in each replication were used as data points ($n = 5$) in a regression analysis (SAS Institute, Cary, NC). An analysis of variance was also performed, and the means were separated using Duncan's multiple range test.

Aboveground versus belowground effects (1992 and 1993). Seedlings were obtained and planted as previously described. Due to seedling size, 5-liter pots were used. Two concurrent experiments were run each year. For each experiment, sixty seedlings (10/treatment/replication; 6 replications) were assigned to each of the following four pretreatments: (i) received pH 2.5 SAR on the growing medium surface and pH 5.5 SAR on the foliage; (ii) with pH 2.5 SAR applied to both medium surface and foliage; (iii) received pH 2.5 SAR on the foliage and pH 5.5 SAR on the medium surface; and (iv) with pH 5.5 SAR applied to both medium surface and foliage. Before each rain event, a plastic bag was tied around the root collar of each seedling, covering the growing medium and pot. Foliar pretreatments were applied using the rain simulator as previously described. The plastic bags were removed between rain events. Treatments to the growing medium were applied by pouring 300 ml of SAR of the appropriate pH directly on the soil surface. Variable amounts of SAR of the appropriate pH were applied as needed to maintain roughly equivalent levels of soil moisture for an average of about 5 liters per pot over the duration of the pretreatments.

After 10 weeks of rain pretreatments, the seedlings were transported to Coweeta, exposed to natural inoculum and rainfall (average pH = 4.5) under mature dogwood trees infected with *D. destructiva*, and evaluated as before. An analysis of variance was used to test main effects of soil and foliar applications. In 1993, significant interaction between soil and foliar applications necessitated comparison of simple effect means by Duncan's multiple range test.

RESULTS AND DISCUSSION

Field verification (1990 and 1991). Seedlings treated in 1990 developed marginal burning, thought to be the result of intense sunlight in the greenhouse. Even though the amount of burning was not related to rain treatment, data were taken separately on old (SAR-treated) and newly produced (nontreated) leaves. For both sets of leaves, anthracnose severity was significantly greater in seedlings receiving pH 2.5 SAR than in any other pretreatment. However, only 2.7% of leaf area was infected in 1990. Natural epidemic development halted in early summer, presumably because no rain occurred for 6 weeks after plants were exposed to natural inoculum. Therefore, the same seedlings were kept in the field through the following winter and exposed to inoculation during the spring and the summer of 1991. Several infection periods occurred in the spring of 1991 (8). By mid-June 1991, seedlings treated in 1990 with pH 2.5 SAR had 84% leaf area infected (Fig. 1), significantly more than the seedlings treated with pH 5.5 (34%). Regression analysis of the data indicated that the pH of the SAR accounted for 43% of the variance in disease severity.

When the study was repeated in 1991, seedlings were shaded in the greenhouse, which greatly reduced marginal burning. After exposure to inoculum, seedlings treated with pH 2.5, SAR had 18% leaf area infected, compared with 7% on those treated with pH 5.5 SAR. The pH of the SAR accounted for 57% of the variance in percent leaf area infected in 1991 (Fig. 1).

Thus, pretreatments with SAR significantly increased anthracnose severity on both treated and nontreated leaves of greenhouse-grown dogwood seedlings exposed to inoculum under field conditions. The fact that nontreated, newly produced leaves were similarly affected suggested that further studies should examine the role of aboveground versus belowground effects.

Aboveground versus belowground effects (1992 and 1993). Application of pH

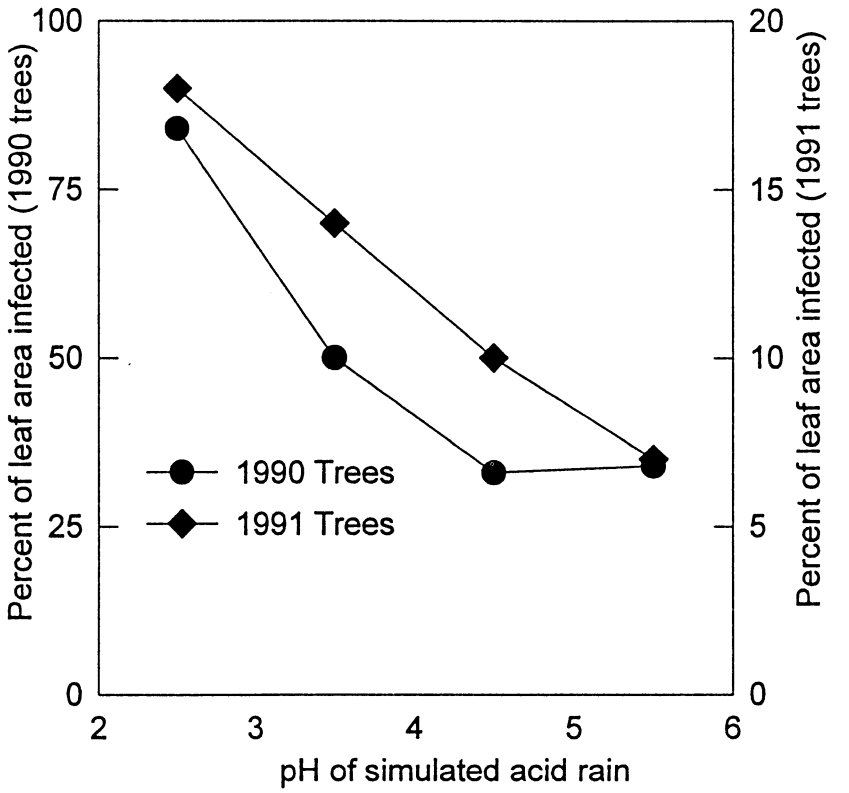

Fig. 1. Percent leaf area infected in 1991 on trees pretreated with simulated rain at pH 2.5, 3.5, 4.5, and 5.5 in 1990 and 1991.

2.5 SAR to the growing medium increased disease severity in three of four experiments (Table 1). Applications of pH 2.5 SAR to the foliage alone did not increase disease in either year. However, in one experiment in 1993, applications to both the foliage and the growing medium significantly increased disease over applications to the growing medium alone (Table 1).

Some abscission of uninfected leaves was noted in August 1993. Earlier than expected for autumn leaf fall, this abscission was more common in experiment 2, which was exposed to natural inoculum in a location receiving less light than experiment 1. The amount of leaf loss was small (2 versus 4%), but significantly less (P = 0.05) in pretreatments of pH 2.5 SAR on the growing medium than in pretreatments of normal rain (pH 5.5) Trees receiving pH 2.5 SAR on the growing medium had higher foliar nitrogen (K. Britton et al., unpublished).

These field studies corroborate laboratory studies that used artificial inoculation conditions (1). Some of the pH treatments used in these studies were far more acidic than average ambient rainfall (39), but short, acute exposures may occur during initial "washing out" of the atmosphere during rain episodes (27). The levels of acidity found in ambient rainfall were not sufficient to significantly increase infection in these studies. But anthracnose field studies must overcome natural variability in inoculum density. The consistent trend of increasing infection with decreasing pH of the rain solution coupled with the significant increases in infection with ambient levels of acidity reported by Anderson et al. (1) suggest that ambient rainfall of pH 4.5, while probably not responsible for the disease outbreak, may increase susceptibility to this disease in the field.

Most research on the interactions between acid rain and dogwood anthracnose has focused on foliar mechanisms (10,40). The results of this study indicate that the belowground effects of acid rain are more important. The simulated acid rain solution supplies nitrogen, and also can change the availability of other nutrients (4,35). Perhaps the addition of nitrogen to the growing medium induces succulence, facilitating penetration of the fungal hyphae into the leaf tissue. Perhaps changes in nutrient composition or changes in carbohydrate levels make the leaves a better substrate for growth of the fungus. Too little is known about the effects of acid rain on dogwood anthracnose to determine how important it is outside an experimental situation, or how to counteract its effects.

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Table 1. Percent leaf area infected with dogwood anthracnose caused by *Discula desctructiva* following pretreatments with either pH 2.5 or 5.5 simulated acid rain (SAR) on the soil and/or foliage

	1992		1993	
SAR pretreatment pH	**Experiment 1**	**Experiment 2**	**Experiment 1**	**Experiment 2**
Soil 2.5/foliage 5.5	5.7[x]	2.0[y]	1.01[x]	4.1 b[z]
Soil & foliage 2.5	5.8	3.1	0.5	7.4 a
Soil 5.5/foliage 2.5	3.5	2.5	0.1	0.9 c
Soil & foliage 5.5	2.9	2.2	0.1	0.9 c

[x] Soil application treatments exhibited significant increase in disease (analysis of variance main effect at $P < 0.01$). Foliage application main effect and soil × foliage application interactions were not significant.

[y] No significant differences in this experiment.

[z] Significant soil × foliage application interactions occurred. Simple effects tested by analysis of variance. Means followed by the same letter do not differ significantly at $P \leq 0.05$ according to Dunan's multiple range test.

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