# Abiotic Stressors

# in the

# Dogwood Anthracnose Complex

by

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Blacksburg, Virginia

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DEATH AND LIFE

Apparently with no suprise
To any happy flower,
The frost beheads it at its play
In accidental power.
The blonde assassin passes on,
The sun proceeds unmoved
To measure off another day
For an approving God.

Emily Dickinson

From "Collected Poems of Emily Dickinson"
Gramercy Books, New York, 1982.

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**Chapter 2**

**EFFECTS OF ACIDIC FOG ON LEAF SURFACE ANATOMY OF *CORNUS FLORIDA* AND *CORNUS KOUSA* SEEDLINGS**

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**Abstract**

Acidic precipitation reportedly enhances disease severity of dogwood anthracnose (DA) caused by *Discula destructiva*, on *Cornus florida*, the flowering dogwood. Seedlings of *C. florida* and *C. kousa*, the Chinese dogwood which is moderately resistant to dogwood anthracnose, were subjected to acidic fog episodes at pHs 2.5, 3.5, 4.5, and 5.5, using a simulated acidic rain solution. Leaf discs from these and non-treated plants were examined by scanning electron microscopy (SEM). Damage was noted at all pH levels and was primarily confined to the trichomes and stomata. Trichomes appeared dehydrated on both *C. florida* and *C. kousa* leaves, while the "lips" of *C. florida* stomata were increasingly eroded by decreasing pH; *Cornus kousa* stomata were relatively unharmed. At pH 2.5, trichomes of both species seemed to be brittle and fractured, causing deep holes in the lamina. *Discula destructiva* conidia may germinate at trichome bases where damage may cause the leaching of nutrients. Also, the difference in stomatal damage may account, in part, for differences in disease susceptibility.

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Dogwood anthracnose, (DA), caused by *Discula destructiva* Redlin (Redlin, 1991), is possibly the third "great" introduced tree disease in the United States, rivaling only Dutch elm disease and chestnut blight (America). DA is a fungal disease which was first noted about 1978, and was reported by Daughtrey and Hibben (1983) as a lower branch dieback in New York, Connecticut, New Jersey, and Pennsylvania. Byther and Davidson (Byther and Davidson, 1979) described a similar disease in Washington state, Oregon, Idaho, and British Columbia and named it "dogwood anthracnose" in 1983.

Dogwood anthracnose has devastated natural stands of the flowering dogwood, *Cornus florida* L., in the eastern United States. Severe disease is often associated with higher elevations, lower temperatures, and higher humidities, i.e. mountainous understory locations (Hibben and Daughtrey 1988). Outplanted trees in predominantly sunny, warmer, drier areas often show little or no disease. Initial symptoms are leaf spots and twig cankers, characteristically purple rimmed or at times water soaked in appearance, resulting in eventual twig dieback and the girdling of the main stem.

Chellemi and Britton (1992) documented an inverse relationship between evaporative potential and dogwood anthracnose severity in *C. florida*. There was more disease in understory trees where there were the lowest evaporative potential readings, and more disease. This, along with other factors, such as droplet contact angle (Smith, 1992), probably contributes to a tree's suceptibility to the disease. Smith (1992) has noted that the water contact angle was much less on *C. florida* than on *C. kousa*. While *C. florida* is susceptible, *C. kousa*

is moderately resistant to the disease, exhibiting only leaf spots without twig dieback (Redlin, 1991). Since the disease first appeared near two ports of entry, i.e. New York and Seattle, this fungus may have been imported on the resistant species, *C. kousa* (Redlin, 1991).

Anderson et al. (1993) applied simulated acidic rain (pHs 2.5, 3.5, 4.5, and 5.5) from needle points to *C. florida* seedlings. The solution used was similar to ambient rain. There was statistically significant evidence that with increasing acidity there was an increase in dogwood anthracnose symptoms. The approximate pH of rainfall in this area according to Boris Chevone (personal communication, 1993), and Binkly et al. (1989) is 4.7.

In 1992, Thornham et al. examined hydrochloric acid treated leaves by scanning electron microscopy (SEM). Analysis revealed that damage to the trichomes had occurred. Differences in the surface anatomy of sun and shade dogwood leaves were noted (J. B. Crozier and K. T. Thornham, unpublished data); Sun dogwood leaves were waxier. Factors such as evaporative potential or thicker cuticular wax deposits found in sunny locations may deter fungal penetration. A thicker cuticle may also sustain more acidic cuticle erosion.

The objective of this study was to examine by SEM untreated *Cornus florida* and *C. kousa* laminae, and assess damage at four pH exposure levels. Acidic fog may be more damaging than acidic rain due to fog droplet size. In nature, fog episodes can occur as low as pH 2.0 (Bytnerowicz et al., 1986); Bytnerowicz et al. indicated that the very small droplet size involved allowed condensation of the fog on both leaf surfaces allowing more liquid to cover the leaf surface before runoff than rain drops.

## MATERIALS AND METHODS

**Plant materials.** Seeds of *Cornus florida* were stratified for three months at 34 C and were planted in Pro-mix (Premier Brands Inc., Yonkers, NY) in a greenhouse in the winter of 1993. Seedlings were transplanted into Pro-mix at the first true leaf stage. For those plants which were either "sun" or "shade" plants, three seedlings were placed into each pot, with half of the test pots moved under shade cloth, while the other half were placed into full sun in the greenhouse next to the shadecloth test group. Sun pots were periodically moved outdoors to allow for full irradiation of the leaves. The photosynthetically active radiation (PAR) was measured using a LI-COR model LI-185 quantum/radiometer/photometer on the quantum millivolt setting using a quantum sensor. Readings were taken at 11:30am and 12:30pm. With the outdoor sunlight at 100.0% of total PAR, light intensity in the sun section in the greenhouse was an average 86.5% of total PAR and under the shade cloth an average 22.0% of total PAR. Plants were hand irrigated being sure not to get water onto the leaves. Plants were randomly rotated periodically within their sections to ensure even sun exposure. Other seedlings, used for the acidic deposition treatments, were planted individually and were grown in the greenhouse under non-measured but lower light conditions.

**Fog treatments.** Plants were taken from the greenhouse and were placed into mist chambers (approximately 66 cm X 61 cm by 72 cm = 289,872 $cm^3$) in the laboratory under low light conditions (1-4 microeinsteins $m^{-2}$ $s^{-1}$) where they were subjected to a 2-hour fog regime once a day for 4 days using two DeVilbiss Humidi-Kleen model

460 cool mist humidifiers with an approximate mist rate of 330 ml/h. Droplet size was determined by placing a slide with immersion oil spread across its surface and allowing the slide to remain in the fog chamber for several seconds. Droplets were measured immediately using a compound microscope. Droplet size range was 10-60 $\mu$m with a 32 $\mu$m average. Groups of plants were subjected to fog of various pHs (three plants of each species per pH; each treatment was repeated once) simulating acidic fog found in the eastern United States consisting of a mixture of sulfuric and nitric acids. Solutions of $H_2SO_4$ (.5 M) and $HNO_3$ (1 M) were produced using trace metal grade acids and deionized water in acid-washed glassware, and were mixed at a ratio of 5:1 sulfuric to nitric acid. This was used as a stock solution. Fog solutions were made fresh prior to each episode using 3 L deionized water, adding stock solution dropwise until the desired pH was obtained; approximate molarities of H+ for pHs 2.5, 3.5, 4.5, and 5.5 were $5.47 \times 10^{-3}$, $3.00 \times 10^{-4}$, $2.00 \times 10^{-5}$, and $5.00 \times 10^{-6}$ respectively. Components were in a ratio of 12:5:2, H+ to sulfate to nitrate, respectively. For the pH 5.5 solution, (carbonated) deionized water was amended with dilute NaOH to obtain a pH close to 5.5, and then stock acid rain solution was added to achieve the proper pH. Occasionally this was also required of the pH 4.5 solution. Plants were fogged for 2 h daily and allowed to dry by opening the chambers after each episode. Plants were treated approximately every 24 h, were rotated daily to change their position within the chamber, and examined daily for any visible signs of injury due to the treatment. On the fifth day, leaf discs were taken for SEM.

**Scanning electron microscopy.** A 6-mm leaf sample was taken from the distal end of the lamina including the midvein of three leaves (one disc from each plant) per pH per experiment per species, yielding approximately 60 discs including non-fogged controls. We had previously confirmed by SEM that most damage occurs at the distal end of the leaf when treated in the laboratory. For each pH, "young", "middle-aged", and "old" leaves were sampled; age was determined by position on the plant. Discs were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2 (with one drop of Triton X100 in several hundred ml as a surfactant). Samples were subjected to a vacuum for several minutes and then refrigerated for 2-3 days. Discs were rinsed with sodium phosphate buffer three times for 15 min each, and then rinsed two times for 15 min each with distilled water. Discs were frozen in liquid nitrogen, freeze dried in a Virtis Sentry Benchtop 3L freeze dryer, placed on stubs, grounded using colloidal silver, and coated to 15 nm with gold/palladium in an Anatech Hummer X sputter coater. Leaves were scanned with a Philips 505 SEM with an ion getter pump.

**RESULTS**

After fogging episodes, middle-aged to young *Cornus kousa* leaves exhibited extensive beading of the fog, whereas older leaves tended to be evenly wet. *Cornus florida* leaves consistently appeared wet over the entire leaf surface after fogging episodes.

Several pH treatments yielded visible indications of damage after the four episodes. *Cornus kousa* plants subjected to the pH 2.5 fog treatment had brown, circular, necrotic lesions (<0.5 mm - 2 mm) over all portions of middle-aged to younger leaves. Some leaf tips and

margins were necrotic. *Cornus florida* leaves subjected to fog of pH 2.5 exhibited fewer circular necrotic areas, generally 0.5 mm - 1 mm diameter, on young to middle-aged leaves. Some leaf tips became necrotic. *Cornus kousa* plants subjected to pH 3.5 fog exhibited lesions, 0.5 and smaller, and a few necrotic leaf tips and margins. *Cornus florida* plants had no visible necrotic lesions, but a few leaf tips were injured. There were a few necrotic lesions less than 0.5 mm in diameter on *C. kousa* plants subjected to pH 4.5 fog, with no damage being noted on *C. florida* plants at the same pH fog. No visible damage was observed at pH 5.5 for either plant species.

**Trichomes.** By SEM, damage was observed at all fog pH levels. Trichomes from non-fogged *C. florida* and *C. kousa* plants (Figures 1-1 and 1-2 ) were turgid in appearance, exhibiting rounded protrusions. Plants exposed to fog at pH 2.5 and 3.5 exhibited many trichomes that were desiccated by the treatment, or injury due to the treatment caused desiccation of portions of various trichomes (Figures 3 & 4). Both *C. florida* and *C. kousa* seemed to be equally affected by the treatments. At pH 2.5, about 40-50% of the trichomes were damaged; some exhibited dehydration of trichome tips while the central portion remained turgid, others appeared completely dehydrated. At pH 3.5, damage also was similar for both species. Middle-aged leaves showed very little damage; only several trichome tips per disc were damaged. On younger leaves, much more extensive damage was noted. Trichomes were damaged much as they were at pH 2.5, but there were fewer whole trichomes damaged. Fog of pH 4.5 caused much less damage than pH 2.5 or 3.5. Only several trichomes were damaged per disc from middle-aged leaves, and only somewhat more extensive damage occurred

on younger leaves. Damage consisted of dehydration of the extreme tip or tips only, and just one or two trichomes per disc, with more extensive damage on younger leaves. Damage at pH 5.5 was slight and resembled damage at pH 4.5 closely. Leaves fixed in the same manner which had not been exposed to the fogging treatment had no trichome damage. Leaves exposed to pH 2.5 fog had several fractured trichome bases. This phenomenon was only noted at this pH level (Figures 1-5 and 1-6).

**Stomata.** Observation of stomata by SEM revealed morphological differences between *Cornus florida* and *Cornus kousa* plants. In both species, stomata were present only on the abaxial surface. Most *C. kousa* stomata exhibited a large, raised outer ledge and lips, and distinct, slightly raised tissue surrounding the outer ledge. Stomata observed on *C. florida* leaves exhibited a large outer ledge, a somewhat less extensive lip region, and had less rounded tissue surrounding the outer ledge. Closed as well as slightly open stomata were noted on unfogged leaves. Both *C. florida* and *C. kousa* stomata had lips which could close the outer aperture completely. Occasionally the outer aperture was open while the inner aperture was closed. On fogged leaves, observations indicated that many *C. florida* stomata lost portions of their lip region, widening the outer aperture considerably (compare figures 1-7, 1-9, and 1-10). Many *Cornus kousa* stomata seem to be affected only slightly if at all by the acidic fog treatment; compare figures 1-8 and 1-11.

**Sun vs shade.** A small number of sun and shade plants were examined by SEM to determine if visible differences existed. No distinct differences could be noted by SEM, and therefore methods such

as TEM and infection studies will need to be performed to confirm differences between sun and shade plants.

**Discussion**

Over the last 35 years we have seen an overall decrease in the pH of precipitation, and in some cases pH levels between 2.0 and 3.5 have been measured (Ferenbaugh, 1976). Rainwater, containing dissolved carbon dioxide, normally has a pH of 5.5, but with the increasing pollution of the atmosphere with industrial waste and automobile exhaust, sulfuric and nitric acids have become common component of precipitation (Evans et al., 1977, Evans and Curry, 1979, Ferenbaugh, 1976). Many groups have examined the effect acidic precipitation has on leaves, documenting pitting of lamina, physiological pH effects, and structural damage to stomata (Adams et al., 1984, Anderson et al. 1989, Anderson et al. 1993, Bytnerowicz et al., 1986, Evans et al., 1977, Evans and Curry, 1979, Ferenbaugh, 1976, Wood and Bormann, 1975).

Increasing acidity enhances dogwood anthracnose infection (Anderson et al., 1993). Some factor causes plants to become more susceptible after acidic precipitation. By SEM, we have seen distinct damage of the trichomes at pH levels 2.5, 3.5, 4.5, and even some at 5.5. It has been noted as well that as trichomes break or fracture, a large hole forms, and a large trough exists where the "arms" rested on the leaf surface (Figures 5 and 6). I believe this is a prime area for possible leaching of metabolites that could be used as a nutrient source by the pathogen. By SEM it has been noted that *Discula destructiva* conidia get caught in this area like a trap (Crozier and Thornham, 1993, unpublished data). Wood and Bormann (1975) found that

there is a significant amount of leaching of potassium, magnesium, and calcium from pinto bean and maple leaves after application of acid precipitation of pH 2.3, but did not find a significant amount of leaching at pH levels 3.0 and 5.0.

A recent study of stomatal ontogeny and morphology in bean, *Phaseolus vulgaris*, in relation to appressorium formation in *Uromyces appendiculatus* (Terhune et al., 1991) sheds light on the structure of stomata, and their possible importance in allowing infection. In this study it was noted that physical features of leaf surfaces often have important roles in the development of infection structures of powdery mildew, anthracnose, and rust fungi. Topographical features may include depressions located at cell junctions, cuticular wax crystals, trichomes, and stomata (Terhune et al., 1991). SEM revealed topographical changes after acidic precipitation, even at a pH as high as 5.5.

The trichomes of both *C. florida* and *C. kousa* seemed to be equally affected by the acid precipitation, but the stomata were not. Stomata of *C. florida* were affected more and more as the pH dropped, whereas *C. kousa* stomata seemed to be fairly unaffected. It is thought that the *C. kousa* leaves may naturally have a thicker waxy cuticle, which was supported by the beading of fog during precipitation episodes, and Smith's contact angle work (Smith, 1992). Erosion of the cuticular lips, even in a low percentage, could cause a prime entry point for the fungus by widening the stomatal aperture. TEM and infection studies are needed to further clarify these phenomena.

**Fig. 1-1.** Scanning electron micrograph of the adaxial surface of an untreated *Cornus florida* leaf. Note the T-shaped trichome. Bar = 0.1 mm.

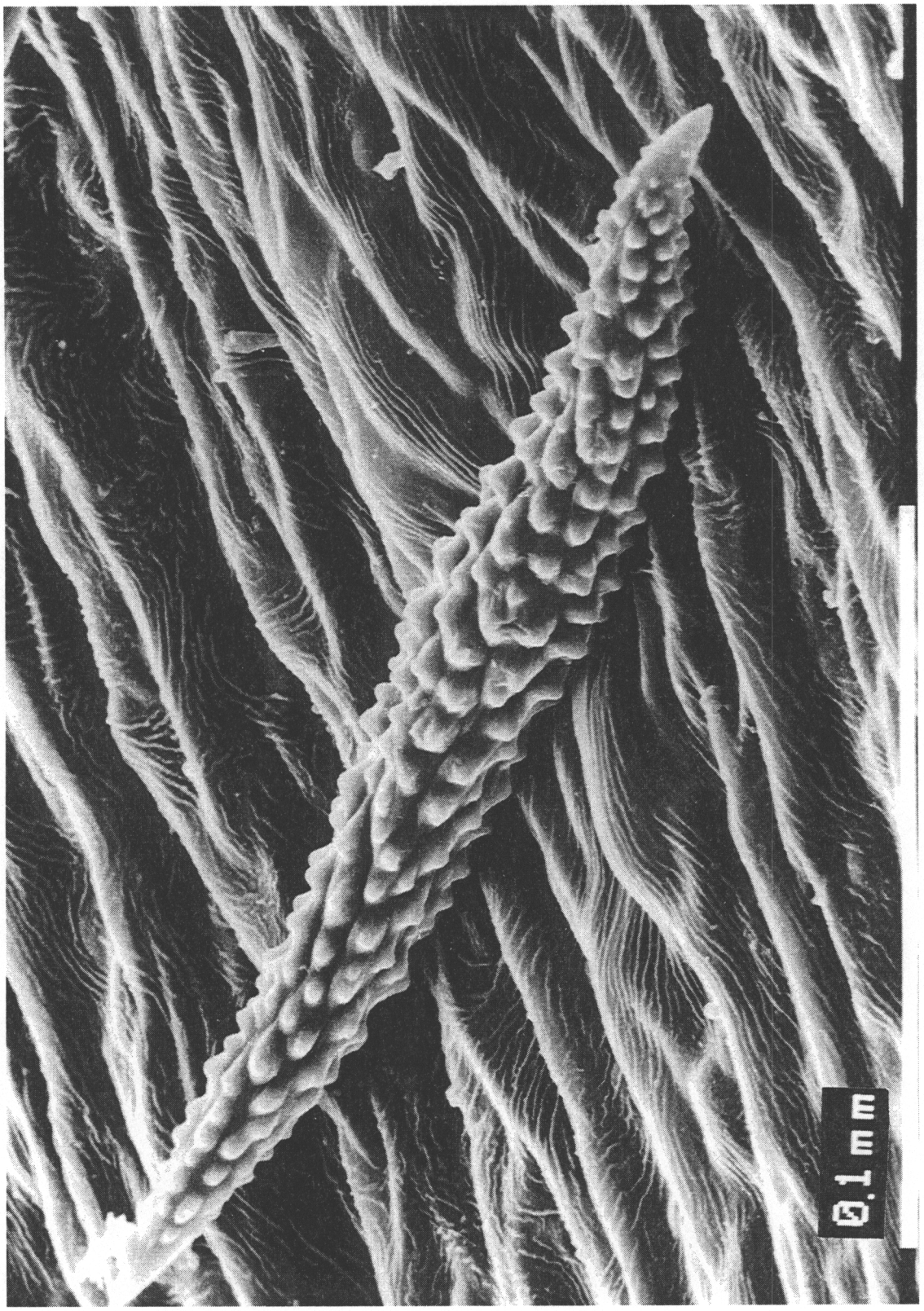

0,1 mm

**Fig. 1-2.** Scanning electron micrograph of the abaxial surface of an untreated *Cornus florida* leaf. Note a trichome tip and several closed stomata. Stomata exhibit distinct outer ledges and lips.
Bar = .1 mm.

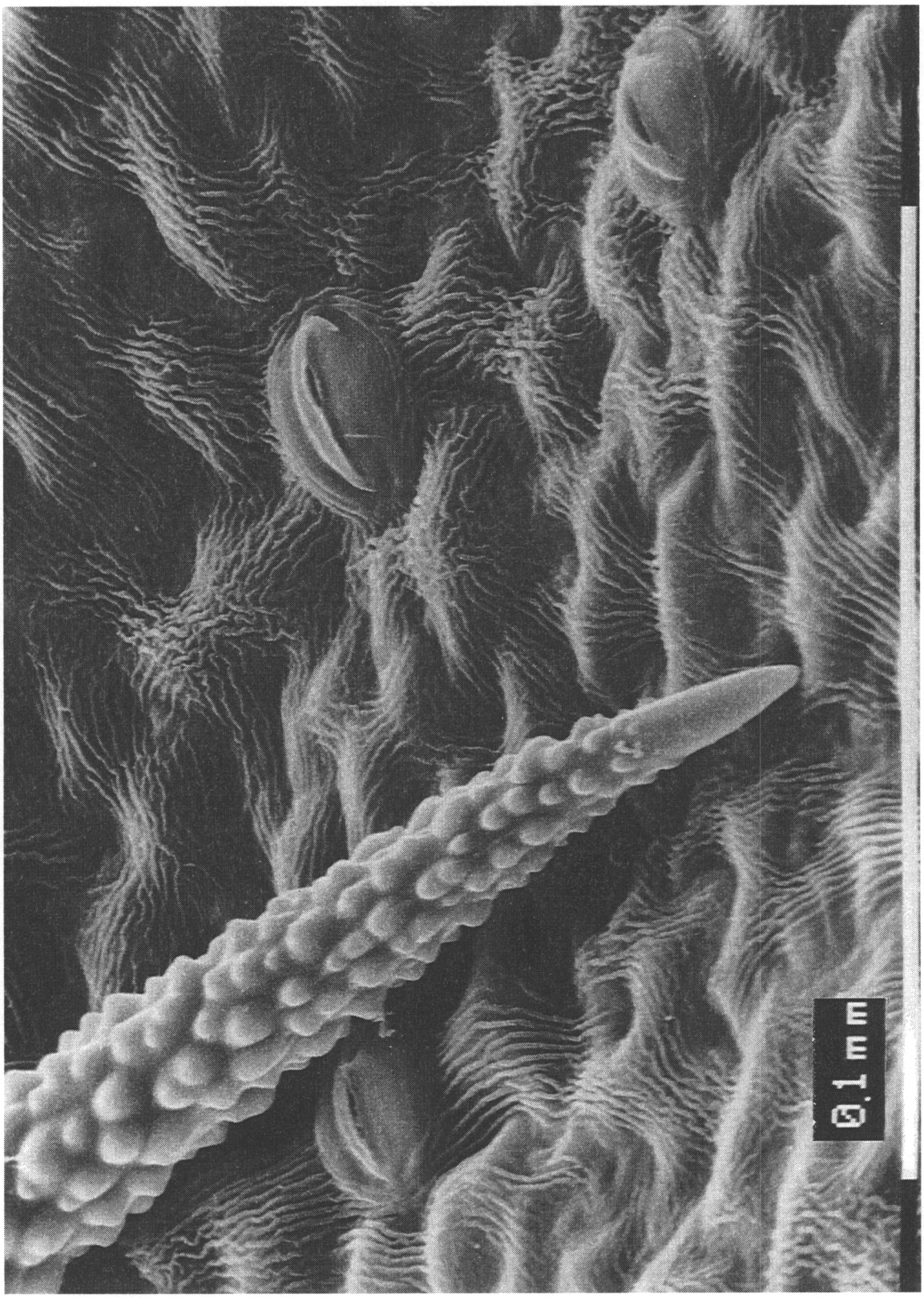

0.1 mm

**Fig. 1-3.** Scanning electron micrograph of a damaged *Cornus kousa* trichome, pH 2.5. Bar = 0.1 mm.

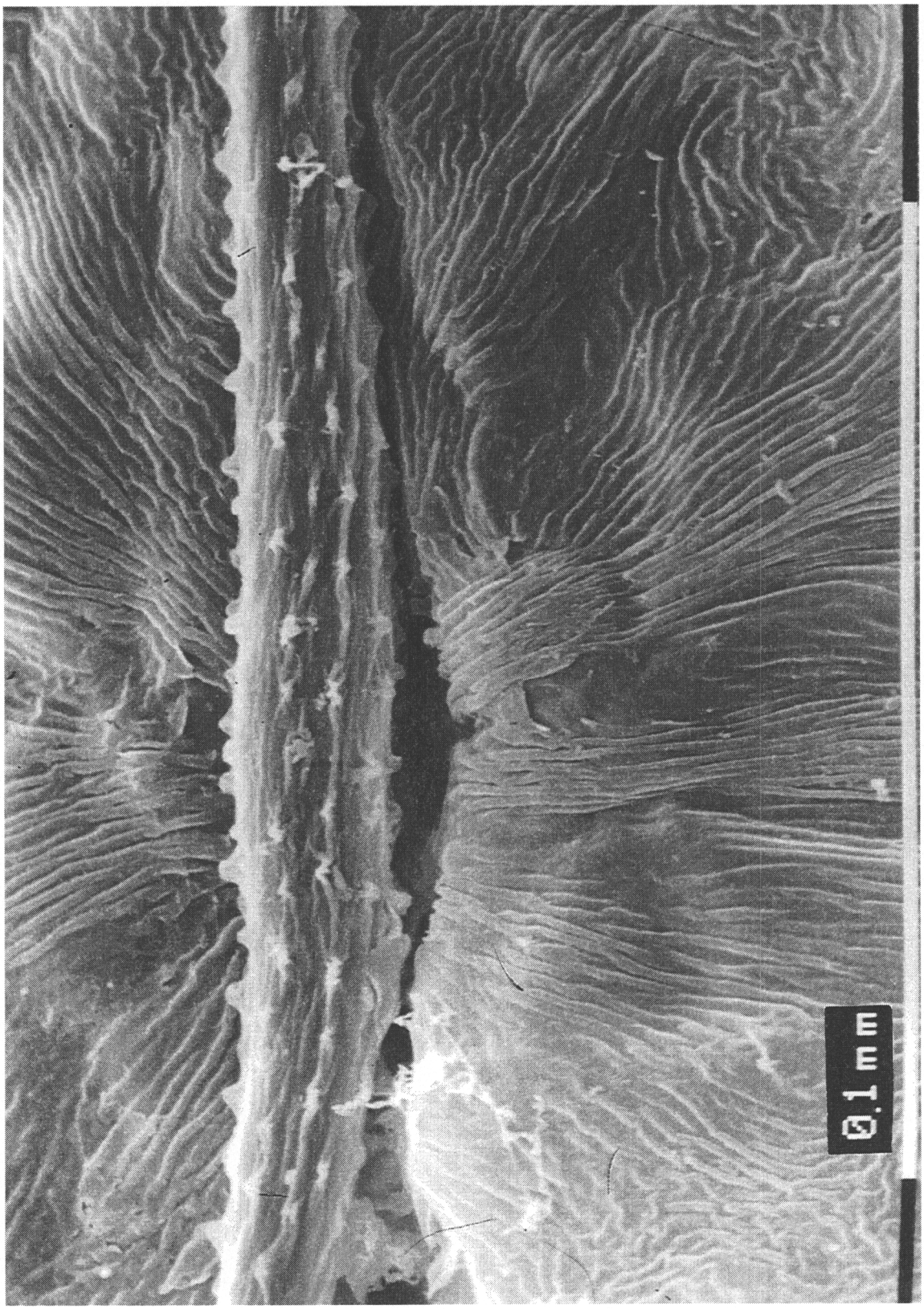

EE 10.1

**Fig. 1-4.** Scanning electron micrograph of a damaged *Cornus kousa* trichome, pH 3.5. Bar = 0.1 mm.

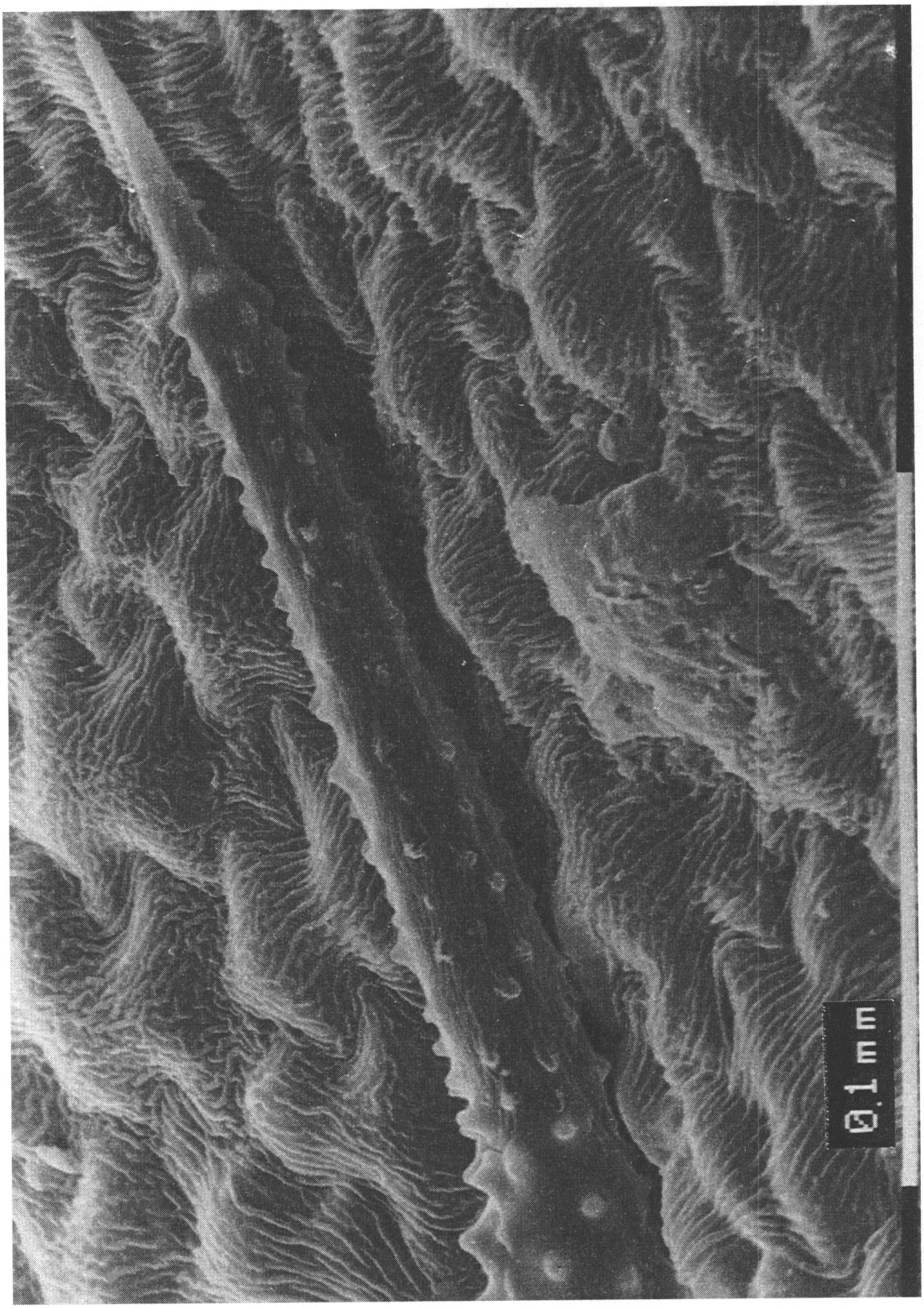

0.1 mm

**Fig. 1-5.** Scanning electron micrograph of a fractured trichome and subsequent hole, *Cornus florida*, pH 2.5. Bar = 0.1 mm.

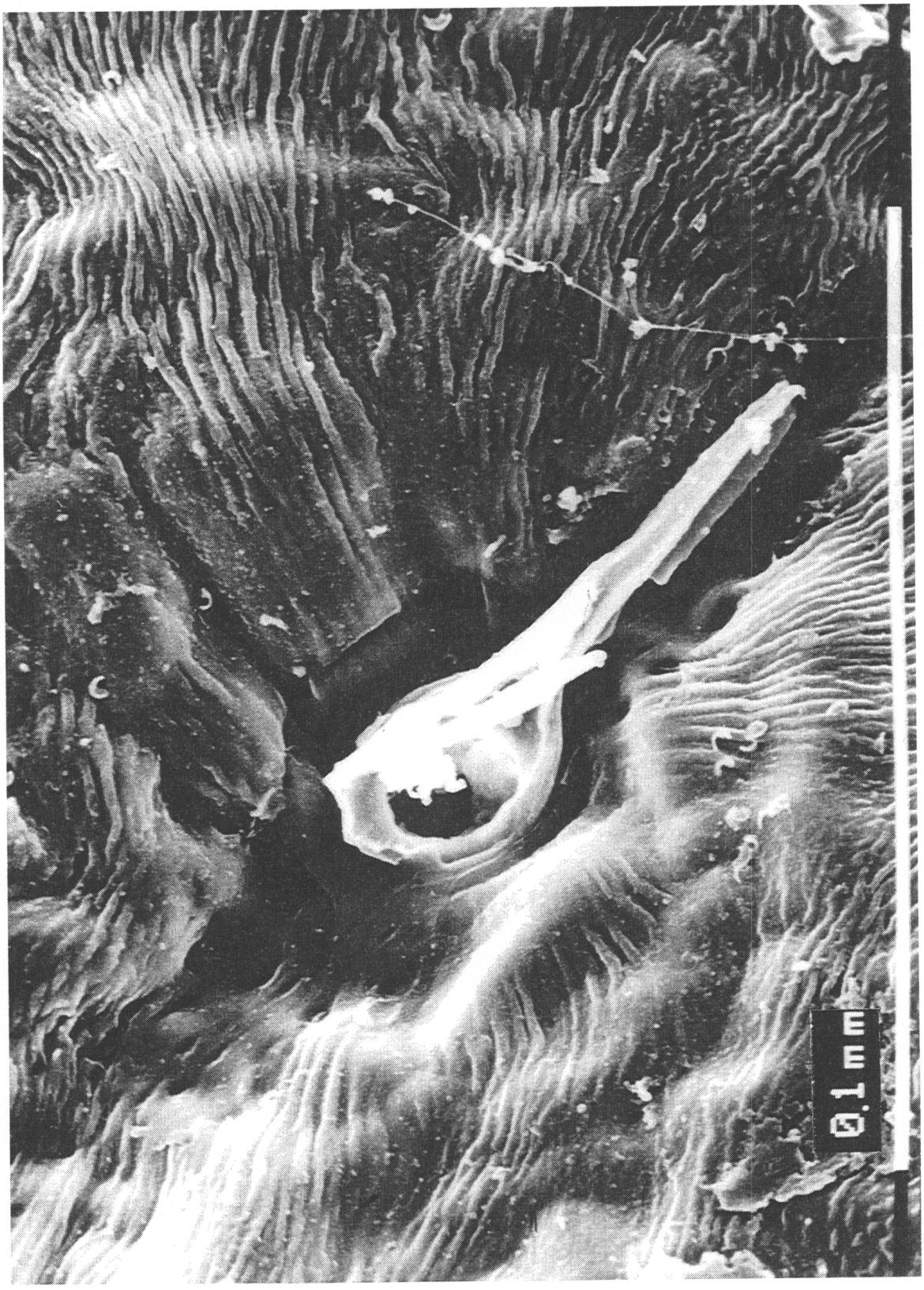
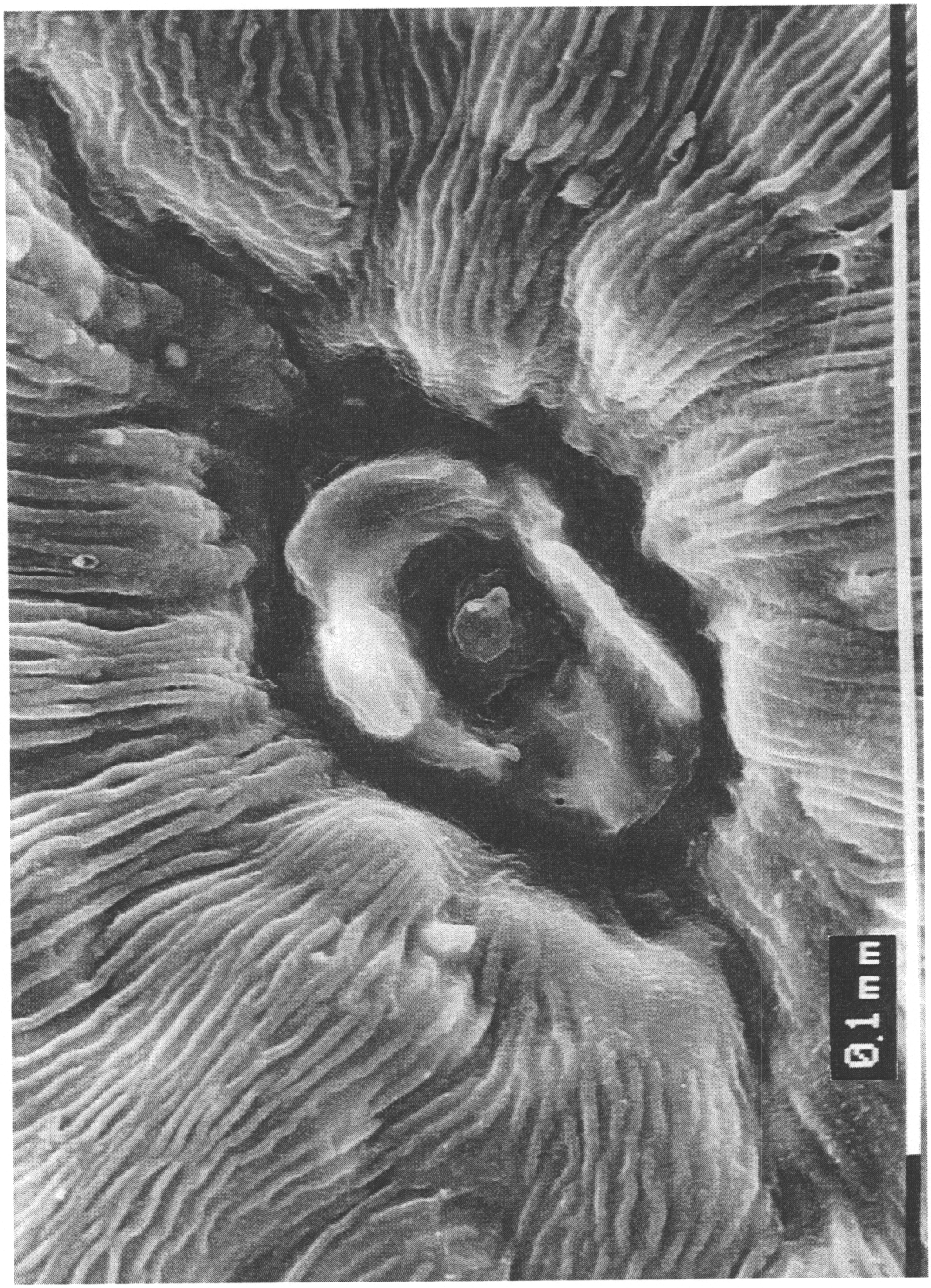

0.1 mm

**Fig. 1-6.** Scanning electron micrograph of a fractured trichome and subsequent hole, *Cornus kousa*, pH 2.5. Bar = 0.1 mm.


0.1 mm

**Figs. 1-7.** Scanning electron micrograph of untreated *Cornus florida* leaf. Note stoma with distinct outer ledge, lips, and outer aperture. Bar = 10 $\mu$m.

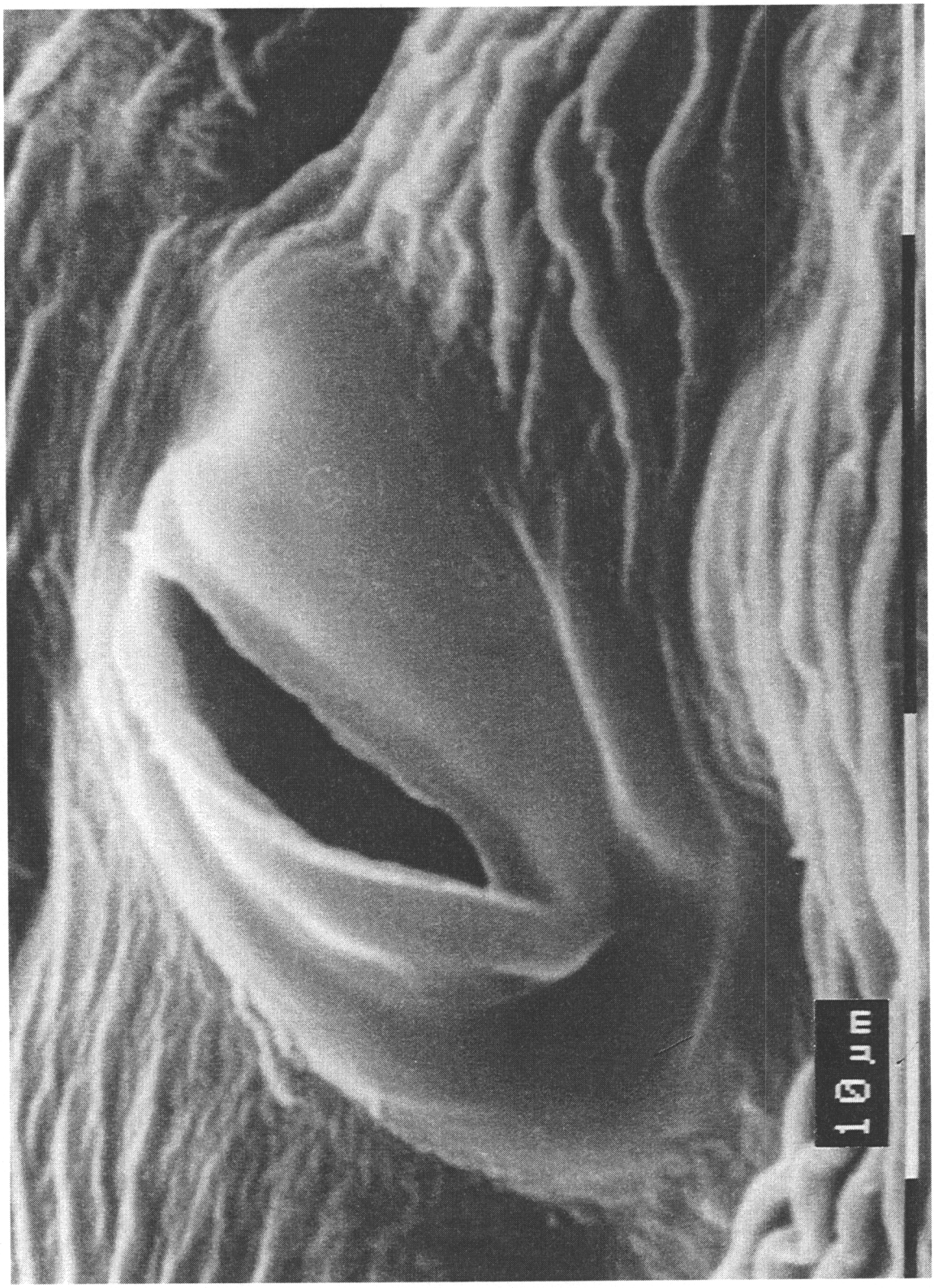

10 µm

**Fig. 1-8.** Scanning electron micrograph of untreated *Cornus kousa* leaf. Note stoma with distinct outer ledge, lips, and outer aperture. Bar = 10 μm.

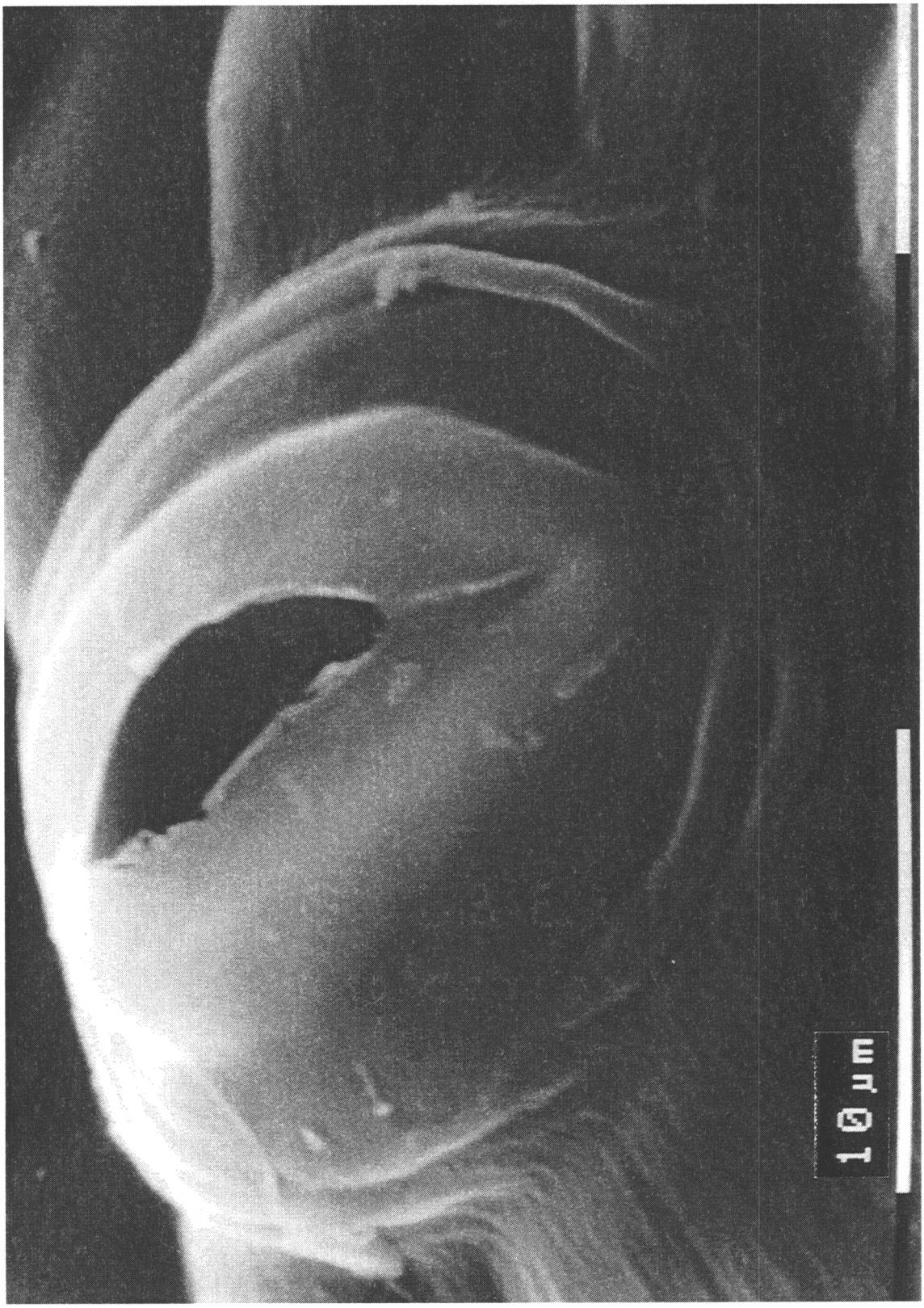

10 µm

**Fig. 1-9.** Scanning electron micrograph of treated *Cornus florida* leaf, pH 5.5. Note stoma with distinct outer ledge, lips, and outer aperture. Bar = 10 $\mu$m.

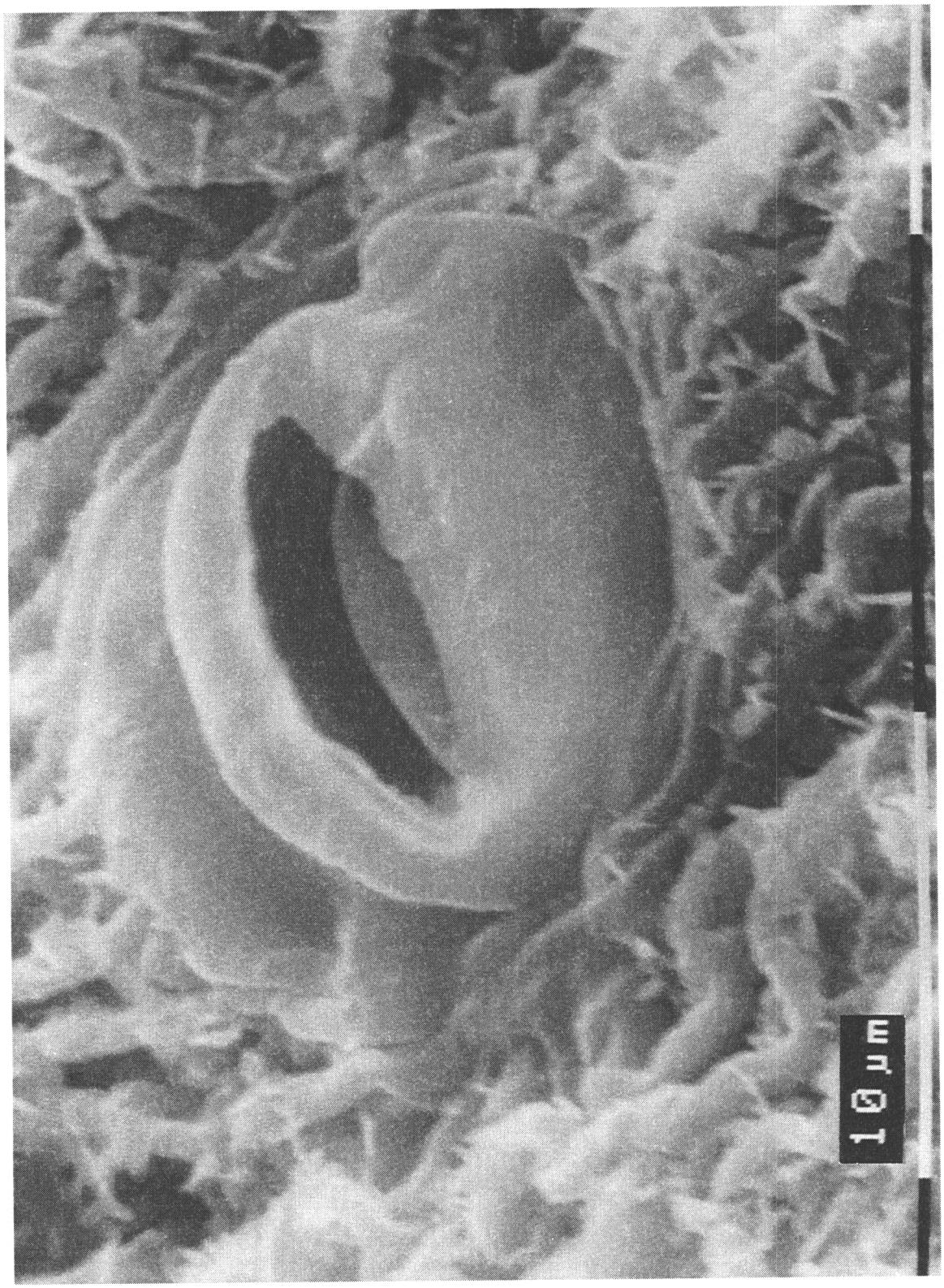

10 µm

**Fig. 1-10.** Scanning electron micrograph of treated *Cornus florida* leaf, pH 2.5. Note stoma with distinct outer aperture, damaged lips, and inner aperture. Bar = 10 μm.

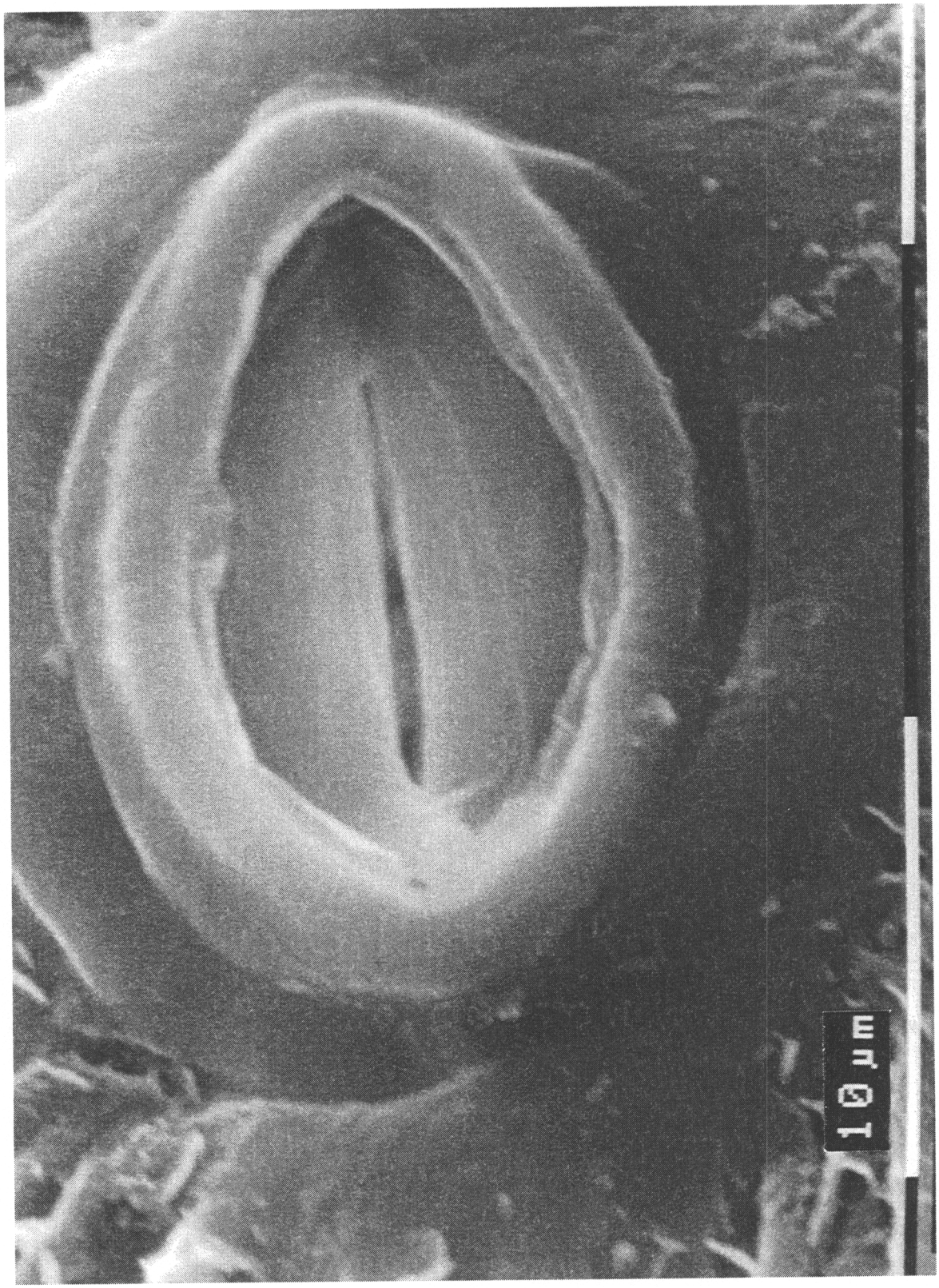
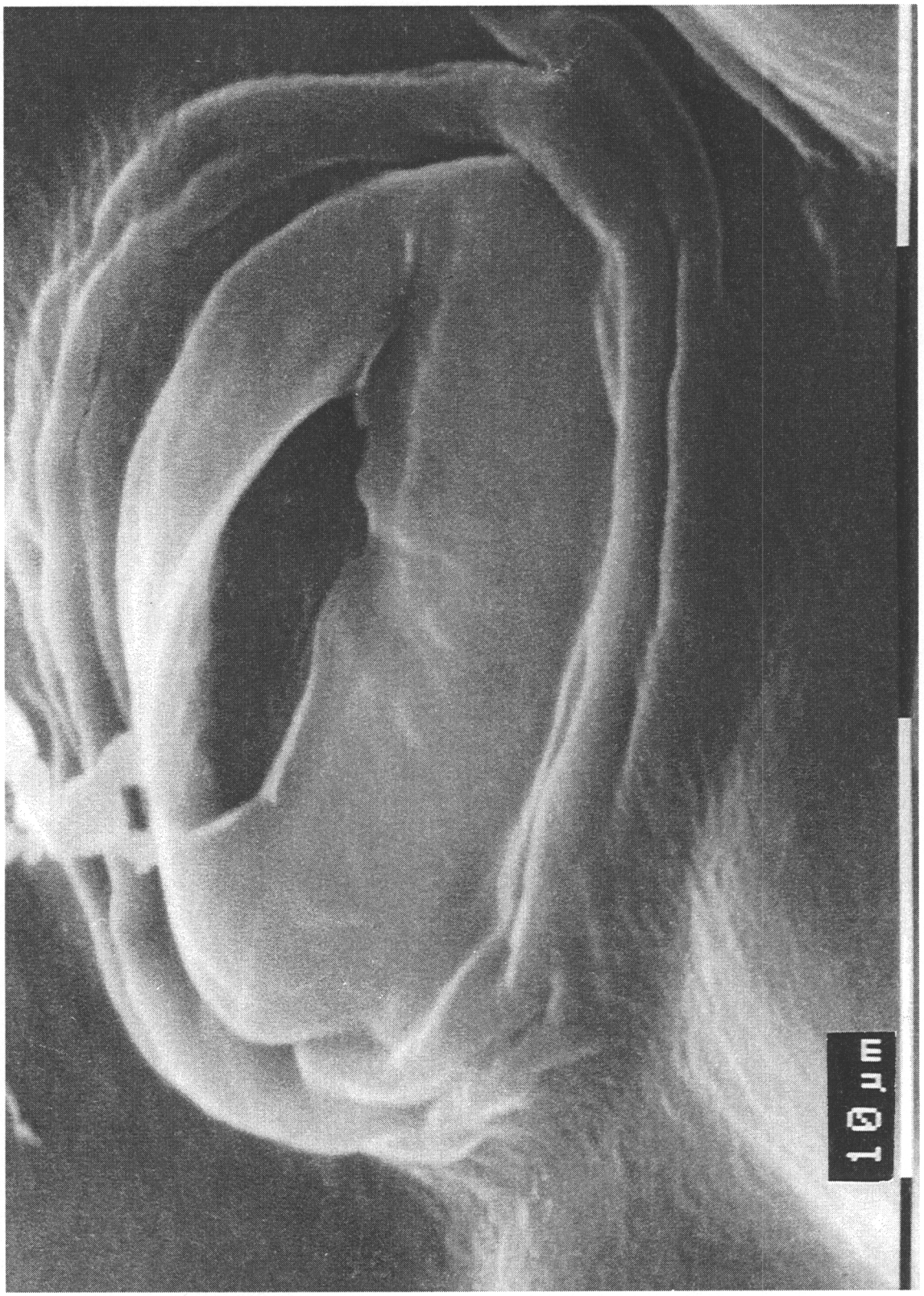

10 µm

**Fig. 1-11.** Scanning electron micrograph of treated *Cornus kousa* leaf, pH 2.5. Note stoma with distinct outer ledge, unharmed lips, and outer aperture. Bar = 10 μm.


10 µm

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## EFFECTS OF TEMPERATURE ON THE GROWTH AND SURVIVAL OF *DISCULA DESTRUCTIVA*


### Abstract

Cardinal growth temperatures and response to thermal stress regimes were determined for isolates of *Discula destructiva*, causal agent of dogwood anthracnose. The optimum temperature was between 20 and 22 C, with 4 of 6 isolates growing best at 20 C. All isolates grew within 7 d at 1 C and 28 C, but no growth was noted after 7 d at 30 C, although regrowth occurred after transfer to a lower temperature. All isolates were killed after 7 d at 35 C. The fungus was alive in 88% of 4-mm mycelium agar discs after 5 min at 45 C in water, while it was alive in 51% after 10 min, and in 0% after 15 min. The fungus was alive in 89% of 4-mm discs from autoclaved dogwood leaves on amended PDA, on which *D. destructiva* was allowed to grow, after 10 min at 45 C. The thermal death point of conidia in free water was 46-47 C, and the thermal death times for 45 and 55 C were 20 min and 30 s, respectively, for conidia from oatmeal agar plates. Conidia from autoclaved dogwood leaves on amended PDA were killed within 5 min at 45 C. This information may lead to an understanding of possible climatic barriers, and the thermal treatment of plant material.

Dogwood anthracnose (DA) is possibly the third "great" imported tree disease in the United States, rivaling only Dutch elm disease and chestnut blight (America). DA is a fungal disease which was first noted in approximately 1978, and was reported in 1983 by Daughtrey and Hibben as a lower branch dieback in New York, Connecticut, New Jersey, and Pennsylvania. Byther and Davidson (1979) described a similar disease in Washington state, Oregon, Idaho, and British Columbia and named it "dogwood anthracnose" in 1983. The disease appears as necrotic, spreading, purple-rimmed or watersoaked lesions, often on leaves on lower branches, forming dark cankers under the bark. Trees are killed when a canker girdles the tree in the main stem.

Flowering dogwood, *Cornus florida*, native to eastern North America, and the Nuttall dogwood, *C. nuttallii*, native to the Pacific Northwest, are most susceptible, although the ornamental dogwood native to China, *C. kousa*, and other species of dogwoods are susceptible to a lesser extent (Brown et al., 1992). Disease progression seems to take place in higher elevations in cool, moist times of the year (Anonymous, 1990).

Hibben and McArdle (1992) established three study plots in a forest site in the Mohonk Preserve in New Palts, New York, where dogwoods had severely declined in the late 1970's. In 1991 only 10.8% of the dogwoods were alive, and leaf symptoms were present within the plots, indicating that the fungus is still active within this area. The disease has quickly spread down the Appalachian mountain range in the eastern United States, extending from Maine to Alabama (Knighten and Anderson, 1991). In the south-eastern states from Virginia to Alabama, 164 counties have reported the disease (Knighten and

Anderson, 1991). In 1988 0.5 million acres were affected; in 1991 it increased to 9.6 million acres (Knighten and Anderson, 1991). By 1993 the disease had exceeded the boundaries of the dogwood test plots set up by the USDA forest service in the south-eastern United States.

The DA pathogen is *Discula destructiva* Redlin (Redlin, 1991), a deuteromycetous fungus producing subcuticular acervular conidiomata in leaf and twig tissues. Acervuli may exude hyaline ellipsoid conidia in a mass or matrix, rarely as a spore horn (Redlin, 1991).The teleomorphic stage of this fungus has never been found, but would most likely be either in the genus *Apiognomonia* or *Gnomoniella* (Redlin, 1991). Recently it has been shown that there may be two pathogens involved, *D. destructiva* and another *Discula* sp. labeled type I and type II, respectively, based on enzymatic and genetic work (Trigiano et al., 1993).

Much of the basic biology and epidemiology of the fungus is still unknown, including how it has moved so rapidly down the east coast of the United States. The objective of this study was to determine growth temperatures for 6 *Discula destructiva* isolates, and to determine heat stress parameters for 8 isolates. In 1991, Stipes and Ratliff reported that in their 8 isolates, the optimum temperature was 20-25 C, with little growth at 5 C, and none at 28 or 32 C.

**MATERIALS AND METHODS**

**Fungal isolates.** Isolates used were obtained from S. C. Redlin, R. W. Roncadori, or were isolated by J. B. Crozier (Table 2-1), and originated from several geographic regions and a variety of *Cornus* plant materials. Isolation from living twig tissue is relatively easy, and pure cultures are commonly obtained. Twigs are

coated with 95% EtOH and ignited (Crozier, unpublished, 1993). The flame is then quickly extinguished and chips of the dark inner tissue are taken aseptically with a razor and forceps. Chips are then placed onto potato-dextrose agar (PDA), or any suitable medium. Isolation from leaf tissue is more difficult, and less reliable, but can be accomplished by placing leaves into 10% Clorox with a drop of liquid detergent for 20 min with pieces plated on PDA containing 200 mg of each chloramphenicol and streptomycin. The fungus can be cultured in the laboratory on a variety of media, including potato-dextrose, yeast extract, and oatmeal agars, the latter of which induces copious sporulation. Conidia are also produced on sterile dogwood leaf discs on PDA.

**Growth curves.** Plates (100 X 15 mm) of glucose-yeast extract agar (GYEA, 15 g agar, 5 g glucose, 1 g yeast extract, 10 ml per plate) were used to grow isolates DA 11, DA 14, DA 17, LTB, DTD, LTE. Four-mm plugs were obtained from the leading edge of a culture and placed singly in the center of agar plates (20 ml/plate). Plates were placed into a Fisher Scientific Isotemp incubator approximately 24 cm below a 45-cm 15-watt daylight fluorescent light. Radial growth was measured after 7 d (three measurements averaged per plate); 3 replicate plates of each isolate per temperature were used in 3 trials.

***In vitro* thermal regimes.** For thermal stress testing, 4-mm discs from the leading growth edge (GYEA plates, 10 ml/plate) of isolates DA 11, DA 14, DA 17, LTB, DTD, and LTE on GYEA were placed singly into 1 ml sterile distilled in 18 X 150 mm test tubes; water was preheated to 45 C in a water bath. After stress treatment, tubes

were placed into a cool water bath of approximately 15-18 C. Control tubes were kept in the cool water bath for the duration of the companion heat trial. Mycelial discs were then transferred to GYEA plates, incubated at 23 C, and monitored for the presence of mycelial growth indicating that mycelium had not been killed. In a separate experiment, 4-mm discs were taken from cultures on dogwood leaves on amended PDA, (1 L decoction of 250 g potatoes and 30 dogwood leaves plus 20 g dextrose, 2 g yeast extract, 1 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g agar). Leaf discs were treated as previously described.

Spores of isolate DA 14 used in thermal stress tests taken from oatmeal agar, and spores from 2 amended PDA plates (isolates RRP and A2), were dispersed in sterile distilled water; spore concentrations were not determined, but since spores did not germinate at high densities (estimated at 5 X $10^6$ spores/ml), non-cloudy suspensions estimated at 5 X $10^5$ spores/ml were used. Generally a high percentage of spores from control tubes did germinate. One ml of spore suspension was placed into one ml of heated (45 or 55 C) sterile distilled water, as above. After treatment, tubes were cooled as described previously and the spores were pipetted onto a slide with a thin film of GYEA. Control spores were maintained in companion test tubes in a cool water bath as described previously, and were pipetted onto slides similarly. Slides were incubated in sterile glass petri dishes on glass rods above wet filter paper at ca. 22 C for approximately four days then examined by brightfield microscopy for germination.

## RESULTS

**Growth curves.** Young cultures of *Discula destructiva* on GYEA are white in color. After 7 d at 20, 22, and 24 C, however, 4 of the 6 isolates turned green, especially around the plug. DA 14 did not turn green, but consistently exhibited tan streaks radiating from the plug. LTE looked very much like DA 14, sometimes appearing to take on the tan character, but at times also exhibiting slight greening. All of the isolates had their own characteristic growth pattern. Most isolates had undulating leading edges, but growth of DA 14 often was completely circular; growth of LTE was also almost circular, but was not quite as smooth. DA 11 often exhibited a "feathering" of the overall culture.

An immeasurably small amount of growth was noted at 1 C for all isolates. After the cultures were placed at approximately 23 C, they all grew normally indicating that none of the isolates were killed by the low temperature. Growth was measurable between 10 and 24 C, and 4 of 6 isolates grew best at 20 C (Table 2-2), the other two at 22 C; the amount of growth dropped off somewhat by 24 C. The optimum temperature for these 6 *Discula destructiva* isolates therefore is between 20 to 22 C. At 28 C there was an immeasurably small amount of growth, and the plugs were very dark in color. The isolates regrew at approximately 23 C, however, indicating that the plugs were merely inhibited by this temperature. There was no growth at 30 C, and the plugs had varying degrees of light and dark streaks within them. All plugs regrew after being placed at 23 C indicating that this temperature merely inhibited the isolates, and did not kill them. Plugs held at 35 C were clear after 7 d, showed no growth, and did not

regrow after being placed at 23 C indicating the isolates had been killed at this temperature. Growth was similar for all isolates except DA 17 which grew consistently slower.

Analysis of the data as a split-split plot design with three incubators as whole plots and three replications of each isolate during each test as subplots, the growth averages over the 5 temperatures where measurable growth was obtained were significantly different ($P = 0.0001$). There was a significant difference among the 6 isolates over all of the 5 temperatures where growth was measurable ($p = 0.0001$), and the interaction of isolate X temperature was also significant ($p = 0.0001$). Using Tukey's Studentized Range (HSD) Test at the 0.05 level, the mean growth at temperatures 20 and 22 C was not significantly different, mean growth at temperatures 24 and 15 C was not significantly different, and the mean growth at 10 C was significantly different from both of the above temperature groupings.

***In vitro* mycelial thermal regimes.** At 45 C, mycelium in GYEA discs exhibited an average of only 12% mortality among the 6 isolates after 5 min exposure (Table 2-3). After 10 min exposure there was 49% mortality, and after 15 and 20 min there was 100% mortality. Leaf discs taken from amended PDA plates showed an average of only 11% mortality after 10 min exposure to 45 C (Table 2-3).

***In vitro* conidial thermal regimes.** The thermal death point (TDP) for DA 14 spores taken from oatmeal agar plates was 46-47 C with a 10 min exposure. The thermal death times for this isolate at 45 and 55 C were 20 min and 30 s respectively. If all spores were killed, i.e. no heated spores germinated when control spores did germinate, the temperature and time was recorded as the TDP or TDT. Isolates

RRP (type I) and A2 (type II) grown on amended PDA both had living control spores (the type I isolate had ca. 100% germination of control conidia, while the type II had only ca. 40% germination), but neither isolate had living (germinating) spores after being subjected to 45 C after 5 min exposure.

Conidia from amended PDA either germinated or remained unchanged, whereas oatmeal agar conidia either germinated, became a thin walled "sphere", or remained unchanged. Living *D. destructiva* conidia changed from their usual elliptical shape to a swollen irregular or spherical shape. Dead conidia, based on control (germination of spores kept in cool water bath) versus heat-killed (no germination of spores subjected to hot water bath) conidia, did not change shape or size. Since "living" is a criterion for the TDT and TDP, this posed an interesting problem. Even though rarely were "spheres" seen germinating, they were counted as "alive" and were incorporated into the observation of the TDT and TDP data (Figures 2-1 and 2-2). Developmentally complete spores of most fungi swell when placed into a suitable environment; this is a precursor to germination (Gottlieb, 1978). Dead spores swell very little if at all, and not only must spores be alive to swell and germinate, they must be ready to undertake vigorous metabolism (Gottlieb, 1978).

**DISCUSSION**

The 6 isolates used for the growth curve work all fit the description for *Discula destructiva*. The maximum and minimum temperatures were 30 and 1 C respectively, with isolates being inhibited at 30 C, and killed at 35 C. The optimum is approximately 20-22 C with 4 of 6 isolates growing best at 20 C. DA 17 from

Massachusetts consistently grew more slowly than did the other isolates.

It is evident that *D. destructiva* can grow at 1 C, slightly above freezing. Growth of mycelium after one week was observed, but not enough to measure. It is commonly thought by many DA researchers (personal comunication) that over the course of the winter, a time of temperature fluctuation, fungus in twig tissue could grow relatively easily with little host resistance, and may in this time form the destructive cankers which may lead to the death of the tree.

Parham and Windham (1991) measured the growth rate of foliar lesions in shaded versus outplanted trees. Even though leaf temperature of outplanted trees was greater than that of shade trees, lesion size progressed at a similar rate in both sites. Sun leaves tended to exhibit purpling of the lesion edge, and less acervular formation, indicating a possible inhibition due to a factor such as heat intensity.

In 1992 Parham and Windham continued this study and reported that leaf temperatures of sun leaves were 4-5 C (ca. 10 F) warmer than shade leaves. Maximum temperatures were 32 and 27 C (89.6 and 80.6 F) for sun and shade leaves respectively during the duration of the experiment. Lesions were quite restricted in the full sun leaves. Lesions expanded much faster in shade leaves and on the north facing inner canopy shade leaves of full sun plants. Again the dominant lesion type for sun leaves was the purple rimmed type producing fewer acervuli.

My work shows that *D. destructiva* grew slightly at 28 C (82.4 F), but was inhibited at 30 C (86 F) and killed at 35 C (95 F). It is

thought, therefore, that summertime temperatures, especially in outplanted trees, may inhibit the fungus slightly and could reduce inoculum. Since night temperatures may fall into a prime range for fungal growth (below 24 C or 75.2 F), the fungus may be able to partially recover, and therefore, even though summertime leaf temperatures may reach inhibitory or possibly lethal temperatures, they may not be lengthy enough to eradicate disease.

A similar situation exists with *Heterobasidion annosum* (*Fomes annosus*) which causes annosum root rot of conifers. This fungus colonizes freshly cut conifer stumps, and from them enters living trees through root grafts. Less annosum root rot develops in summer cut stands than in winter cut stands (Gooding et al., 1966). Summer inoculated stumps failed to become colonized, whereas inoculations at other times of the year are successful. Research was conducted to find optimal, minimum, maximum, and thermal inactivation temperatures. This fungus had an optimum growth temperature of 24 C, a minimum growth temperature of about 2 C, and a maximum growth temperature of about 32 C (Gooding et al., 1966). Spores were heated in water, much as in this study, and spores were also heated on sterile wood discs (Ross, 1969). Ross felt that heating spores on pine discs more closely resembled nature. Conidia could survive 42% longer at 45 C in water than when on pine discs, but basidiospores could live 34% longer on discs than in water.

Heating mycelial discs in water reveals only an approximation of the time it takes to kill 100% of the mycelia in naturally infected tissue. The leaf discs used in my work probably represent a more realistic approximation of what would be found if naturally infected

tissues were used because: 1) the fungus is naturally found in plant material, 2) even though the test was run in water, leaves do not submerge as well as agar discs, indicating air pockets, and 3) conidia are present in fruiting bodies which were not a component in either the conidia nor agar discs in water experiments. GYEA agar discs had an average of 88% living mycelia after 5 min exposure to 45 C, 51% after 10 min, and 0% after 15 min. It was noted that at 10 min, there was a 38% increase in survival of leaf discs over agar discs, but after only 5 min, close to a 100% decrease in survival of spores taken from an autoclaved dogwood leaf on amended PDA verses spores from oatmeal agar plates.

Erbaugh and Windham (1992) reported the results of a small study involving the heat treatment of *Cornus florida* leaves. Viable conidia were noted on leaves kept at 25 C (77 F) for 96 h. Viable conidia were also noted after 96 h at 40 C (104 F), but on fewer leaves, and 48 h at 45 C (113 F) resulted in even fewer leaves with viable conidia. Apparently, dogwood seedlings can withstand temperatures of 45 C for 96 h without damage (Erbaugh and Windham, 1992). This study not only sheds light on the thermal treatment of plant material, but on the natural, *in vivo* conditions affecting conidial viability. It appears that *D. destructiva* conidia can withstand periods as high as 45 C (113 F). Obviously no 48-h period ever remains at 45 C, indicating that conidia can probably withstand daily summertime temperatures.

With such a difference in time this fungus survived in Erbaugh and Windham's study versus my study, it is assumed that the above group did not subject plants to heated water as I have. These

contradictions, as well as what medium conidia were taken from, will have to be explored before valid conclusions can be drawn about regional thermal inactivation of the fungus, as well as thermal inactivation for the purpose of obtaining pathogen free plant material.

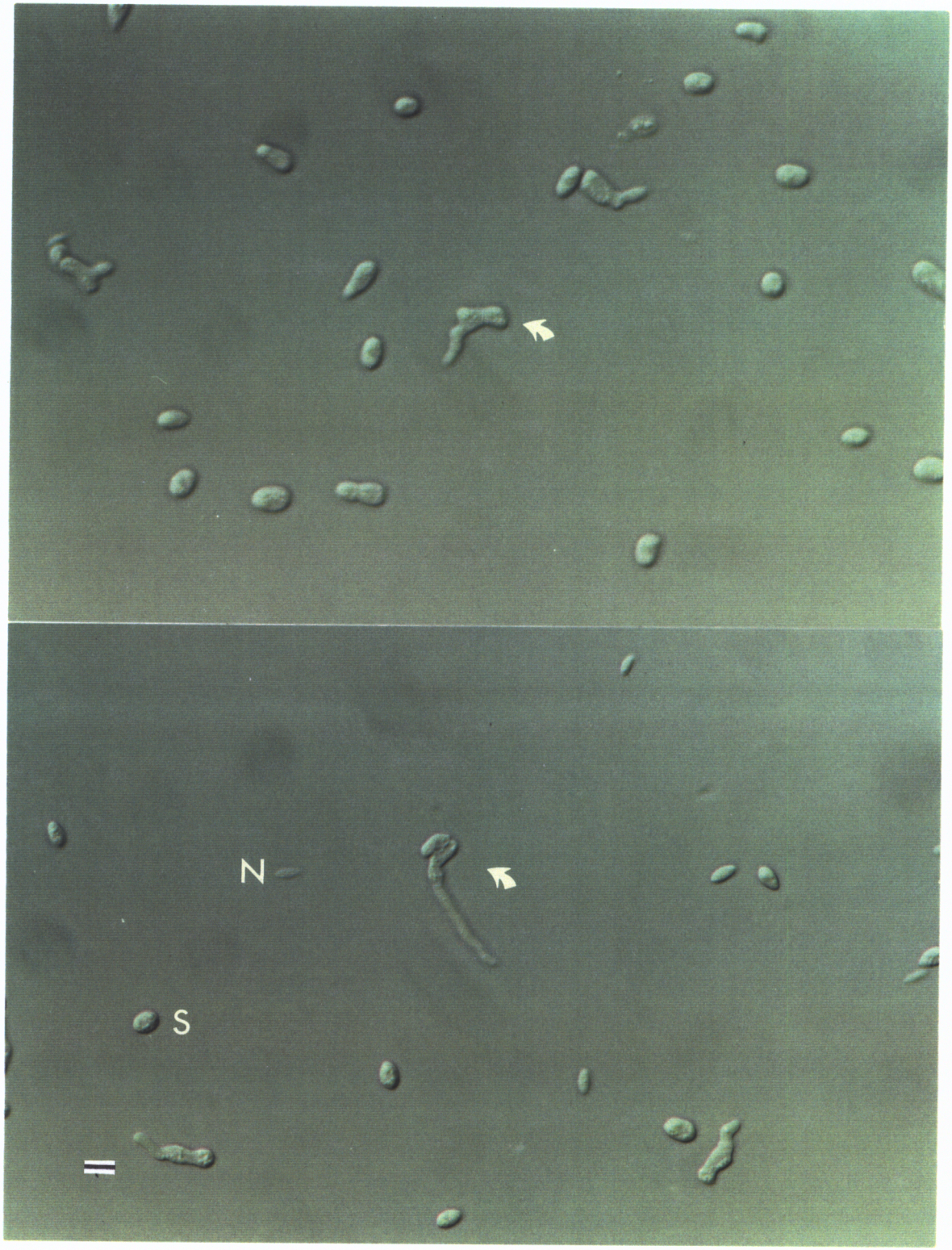


**Fig. 2-1.** The habit of *Discula destructiva* conidia taken from oatmeal agar plates. Note germinating irregular conidia, arrows, "sphere", S, and non-germinated spore, N. Bar = 10 μm.

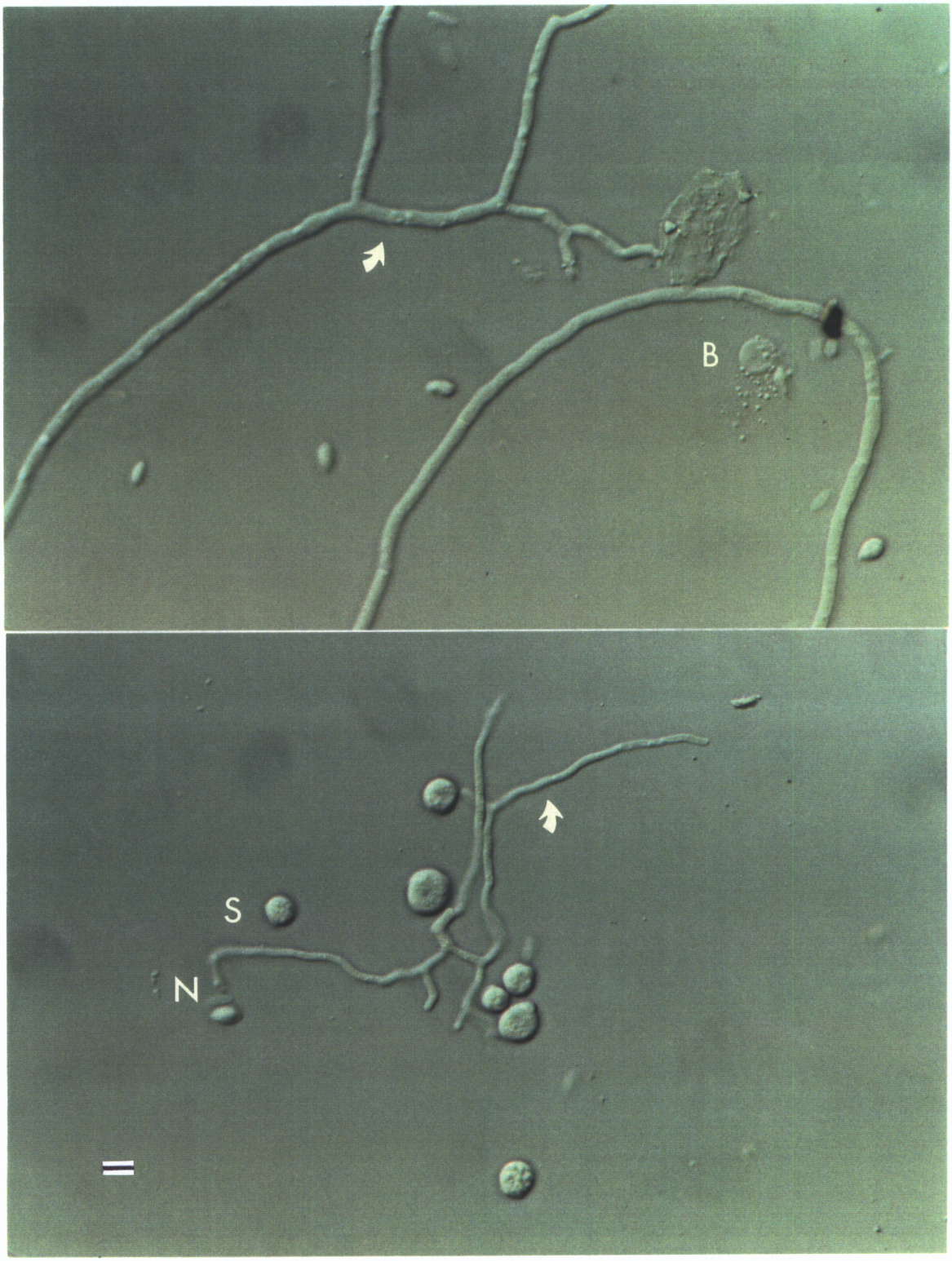


**Fig 2-2.** The habit of *Discula destructiva* conidia taken from oatmeal agar plates. Note mycelium, arrows, "sphere", S, burst "sphere", B, and non-germinated spore, N. Bar = 10 μm.

Table 2-1. Isolate code, sources, dates of collection, tissue type, host *Cornus* species, and isolate type of *Discula destructiva* isolates used in this study.

| Isolate Code | Source | Collection Date | Collection State | Tissue | *Cornus* species | Type |
|---|---|---|---|---|---|---|
| DA 11 | S.C. Redlin[A] | -[B] | WA[C] | living leaf | *nuttallii* | I |
| DA 14 | S.C. Redlin | - | MD | overwintered leaf | *florida* | I |
| DA 17 | J.B. Crozier | 7/92 | MA | living leaf | *florida* | I |
| LTB | J.B. Crozier | 5/93 | TN | living twig | *florida* | I |
| DTD | J.B. Crozier | 5/93 | TN | living twig | *florida* | I |
| LTE | J.B. Crozier | 5/93 | TN | living twig | *florida* | I |
| RRP | R.W. Roncadori[D] | 89 | GA | living twig | *florida* | I |
| A2 | R.W. Roncadori | 89 | NC | - | *florida* | II |

[A]Scott C. Redlin, USDA-ARS, PSI, SBML, Rm. 331, Bldg. 011A , BARC-west, 10300 Baltimore Avenue, Beltsville MD. 20705-2350

[B]Indicates information not known

[C]Washington, Maryland, Massachusetts, Tennessee, Georgia, North Carolina

[D]R. W. Roncadori, Department of Plant Pathology, University of Georgia, Athens, GA, 30602-7274.

Table 2-2. Radial growth of six *Discula destructiva* isolates (mm) after seven days at specified temperature. Means of three tests; each average represents nine observations.

| | Temperature C +/- 0.2 C | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolate | 1 | 10 | 15 | 20 | 22 | 24 | 28 | 30 | 35 |
| DA 11 | | | | | | | | | |
| X = | 0.2[A] | 16.2 | 33.9 | 41.8 | 40.2 | 37.6 | 0.2 | 0[B] | -[C] |
| SE = | | 1.4 | 1.1 | 1.2 | 1.3 | 1.4 | | | |
| DA 14 | | | | | | | | | |
| X = | 0.2 | 17.8 | 32.4 | 44.9 | 45.7 | 40.0 | 0.2 | 0 | - |
| SE = | | 0.8 | 1.5 | 1.0 | 0.3 | 0.9 | | | |
| DA 17 | | | | | | | | | |
| X = | 0.2 | 11.9 | 24.1 | 27.5 | 26.7 | 18.2 | 0.2 | 0 | - |
| SE = | | 0.7 | 1.3 | 1.2 | 1.8 | 1.1 | | | |
| LTB | | | | | | | | | |
| X = | 0.2 | 11.9 | 33.3 | 41.8 | 40.6 | 32.0 | 0.2 | 0 | - |
| SE = | | 1.0 | 0.7 | 1.0 | 0.9 | 1.4 | | | |
| DTD | | | | | | | | | |
| X = | 0.2 | 19.5 | 33.4 | 39.5 | 41.4 | 33.4 | 0.2 | 0 | - |
| SE = | | 1.6 | 0.7 | 0.6 | 1.0 | 1.6 | | | |
| LTE | | | | | | | | | |
| X = | 0.2 | 20.4 | 34.1 | 44.5 | 43.0 | 37.6 | 0.2 | 0 | - |
| SE = | | 1.8 | 0.5 | 0.7 | 1.8 | 1.9 | | | |

[A]Indicates growth was noted, but too little to measure accurately. Regrowth was noted when moved to 23 C.

[B]Indicates no growth was noted initially, but regrowth was noted when moved to 23 C.

[C]Indicates no growth was noted, and no regrowth was noted when moved to 23 C.

Table 2-3. Percentage of ten, 4-mm GYEA *Discula destructiva* mycelial discs that produced mycelia after exposure to 45 C for specified time.

| Exposure (Min) | Isolate code | | | | | | Average for 6 isolates |
|---|---|---|---|---|---|---|---|
| | 11 | 14 | 17 | LTB | DTD | LTE | |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 5 | 90 | 70 | 100 | 90 | 100 | 80 | 88 |
| 10 | 60 | 30 | 70 | 50 | 30 | 55 | 51 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0[A] | 0 | 0 | 0 | 0 | 0 | 0 |
| 10[B] | 83 | 83 | 100 | 100 | 67 | 100 | 89 |

[A]Represents average of three mycelium discs.

[B]Represents average of 6 4-mm leaf discs taken from a sterilized dogwood leaf on amended PDA plates.

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**APPENDIX I:** Literature review for "Effects of Acidic Fog on Leaf Surface Anatomy of *Cornus florida* and *Cornus kousa* Seedlings."

**LITERATURE REVIEW**

Over the last 35 years we have seen an overall decrease in the pH of precipitation; in some cases pH levels between 2.0 and 3.5 have been measured (Ferenbaugh, 1976). Rainwater, containing dissolved carbon dioxide, normally has a pH of 5.5, but with the increasing pollution of the atmosphere with industrial fumes and automobile exhaust, sulfuric and nitric acids have become a common component of precipitation (Evans et al., 1977, Evans and Curry, 1979, Ferenbaugh, 1976). Prior to 1976, there were several review articles discussing the deleterious effects of acid rain on biological systems, and most of the experimental work reported concerned itself mostly with gross effects of acid precipitation, one as early as 1915 (Ferenbaugh, 1976, Holms, Franklin, and Gould, 1915). Prior to 1976, no detailed work of the physiological effects of acid rain on flowering plants had been reported (Ferenbaugh, 1976).

Ferenbaugh (1976) studied the effects of sulfuric acid solutions (pHs 5.5, 4.5, 3.5, 3.0, 2.5, 2.0, and 1.5) on *Phaseolus vulgaris*. At pH levels below 2.5, plants were shorter, bushy, and had smaller leaves with leaf roll. At pHs of 2.0 and 1.5, large portions of the leaves became necrotic, and primary leaves often abcised prematurely. Leaves of acid-treated plants had a smaller cell size and less intercellular space, most notably in plants at the 2.5 or lower level. There was drastic reduction in starch granules size, and epidermal cells and bundle sheath parenchyma treated at a pH of 2.5 were acidified below pH 4.0. Chlorophyll content of leaves was

significantly lower at pH 2.0, but this was found to be due to the large areas of necrosis as a result of the acid treatment. Both the increase in respiration and photosynthesis observed were highly significant based on statistical analysis; the rate of photosynthesis increased much more than the rate of respiration. There was a significant decrease in both average sugar concentration and average starch concentration, as well as a drop in biomass. It should be mentioned that there was no evidence that the sulfate portion of the sulfuric acid solution was causing inhibition of photosynthesis. Even though sulfate has been shown to be an inhibitor of photosynthesis, Ferenbaugh believed that this had no effect since the control plants dipped into hydrochloric acid showed the same effect. He also tested *Chenopodium quinoa* and *Hordeum vulgare*, but they showed no immediate discernable response to the acid treatment.

Evans et al. (1977) studied the effect of simulated acid rain on *Phaseolus vulgaris* and *Helianthus annuus*, and observed plant leaf indumentum responses, as well as whole plant and individual leaf responses. They observed the adaxial surface; previous results had shown that lesions often developed near trichomes and stomata in these species. In *P. vulgaris*, young leaves were not as sensitive to acidic precipitation as mature leaves, but in *H. annuus*, young leaves were injured first. Overall, there was an increase in the total area of leaf injury with repeated exposure to simulated acid rain exposure. Leaf tip necrosis and chlorosis were noted in older plants of *H. annuus* at all pH levels of acid rain solution. Injury was thoroughly described at pH 2.7. Noted were: 1) shallow circular depressions in the leaf surface less than 0.25 mm in diameter, sometimes becoming

slightly chlorotic, resulting from a collapse of the epidermal cells, 2) circular depressions, 0.25-1.00 mm in diameter with chlorosis and some necrosis, resulting from collapse of both epidermal cells and the palisade layer, 3) larger, deeper, 1-2 mm depressions, at times irregular, with chlorosis and necrosis, resulting from a collapse of the epidermal tissue and the palisade layer, and 4) large (greater than 2 mm) irregular lesions, where abaxial necrosis corresponded to adaxial necrosis. These larger lesions seemed to be located near vascular tissue, possibly where there are natural concave areas in the leaf surface. The first three types of lesions were commonly associated with trichomes and stomata.

Evans and Curry (1979) studied the responses of *Glycine max*, *Tradescantia* sp., *Quercus palustris*, and *Pteridium aquilinum* sporophyte foliage to acid rain. In clones of *Tradescantia* sp., 16 rainfalls at 20 minutes each at a pH of 2.3 caused hypertrophy and collapse of mesophyll cells, and lesion formation near trichomes, stomata, and along leaf margins and veins. All other plant material behaved quite similarly.

Hindawi et al. (1980) studied the response *Phaseolus vulgaris* 'Contender' has to acid mist, using 6 weekly, 45 minute exposures at pHs 2.0, 2.5, 3.0, 4.0, and 5.5. Bronze spotting and pitting were noted at pH 3.0.

Acid rain effects on foliar histology of *Artemisia tilesii* was examined by Adams et al. (1984). This plant does not exhibit foliar lesions under acidic field conditions, and appears to be resistant to acid injury in the greenhouse. Sulphuric and nitric acids at a ratio of 3:1 plus a mixture of various other compounds simulating acid rain

were combined and used at pH 2.0, 2.5, 3.0, 3.5, 4.0, and 5.6. Within 12 to 24 hours pitting was noted at pH 3.0, and bifacial necrosis occurred at 2.5 and 2.0. At pH 2.0, damage could be seen even before the droplets had dried. The scanning electron microscope revealed that in plants which exhibited no visible macroscopic damage at pH 3.0, there were in fact one to several collapsed cells in areas on the leaf surface. The SEM indicated that stomata near damaged cells were open, while those surrounded by undamaged cells, as well as stomata in the control plants were closed. Once again, it was noted that lesions in the larger size class, 0.25-1.00 mm in diameter occurred over vascular tissue. Lesions appeared to form both surrounding and not surrounding trichomes.

Bytnerowicz et al. (1986) studied the effects of simulated acid fog on *Phaseolus vulgaris* cv. UI 111. Acid fog in california has been recorded as low as 2.0. Fog can be much more acidic than rain because there is less water in association with the sulphuric or nitric acid (Boris Chevone, personal communication). Bytnerowicz et al. noted that exposures of simulated acid fog greater than pH 2.8 did not cause injury during treatment, but showed signs of damage in the form of 0.1-0.2 mm diameter necrotic lesions on both surfaces one week after exposure. Two days after the 8 hour pH 2.8 treatment, there was still no visible damage with the SEM. Erosion of the cuticle of the leaf surface occurred at pH 2.4. The stomata seemed to be unaffected even in cases where dead cells surrounded the guard cells. Even at pH 2.0 where there was extensive damage, stomata appeared to be functionally intact. Damage was not associated with stomata or trichomes. This group felt that the very small particle size involved allowed

condensation of the fog on both leaf surfaces allowing more liquid to cover the leaf surface before runoff than raindrops would allow.

In 1989, Anderson et al. induced dogwood anthracnose (*Discula destructiva*) symptoms consistently on leaves pre-treated with HCl at pH 2.8. DA symptoms were otherwise reproduced only very infrequently. They discovered that if they sprayed HCl acidified mist pH 2.8 on days 1 and 3, and sprayed *D. destructiva* conidia in water pH 7 on days 2 and 4, that they obtained consistent leaf spots in 7 days and twig dieback in 14 days. No other combination of sprays was effective. In 1993, Anderson et al. reported a continuation of this work. They applied simulated acid rain from needle points pH 2.5, 3.5, 4.5, and 5.5. The solution used was similar to ambient rain consisting of a 1 N mixture of 70 mequiv. $SO_4^{2-}$ to 30 mequiv. $NO_3^-$. Plants received 10 simulated rain treatments one week apart, and were then moved to a laboratory with low light conditions at approximately 20C for inoculations. On the first day of inoculations, *D. destructiva* conidia at a concentration of $5 \times 10^4$ spores/ml in deionized water, pH 5.8, were applied as a mist, while only deionized water was applied on the second day. On the third day conidia were applied at a concentration of $2.5 \times 10^3$ spores/ml. Plants were kept in clear plastic bags held away from the leaves with bamboo shoots, and were sprayed once a week with deionized water for 28 days at which time all leaves with spots were removed and the percent area affected was calculated. Plants which received simulated rain pH 2.5 had 1-3 mm diameter lesions occupying approximately 6% of the leaf surface. There was statistically significant evidence that with increasing

acidity there was an increase in dogwood anthracnose symptoms. It is of particular interest that there was a significant difference between simulated acid precipitation levels pH 4.5 and 5.5 since 4.7 is the approximate pH of rainfall in this area according to Boris Chevone (personal communication, 1993), and Binkly et al. (1989). Fewer than 3% of the uninoculated seedlings showed symptoms of necrosis. A small percentage of leaves which were not inoculated yielded symptoms of *D. destructiva*, possibly due to careless handling of material. My personal experience would suggest that based on the absence of macroscopically visible evidence of acid damage on naturally occuring leaves, rain or fog events at the higher pH levels (possibly between 4.0 and 5.0 ) could be a contributing factor to this disease.

In 1992, Thornham et al. sprayed dogwood leaves with HCl solutions of various pHs. Scanning fixed material, it was evident that the T shaped trichomes of *Cornus florida* were severely injured, appearing dehydrated, at pH levels of 5 and below. Noting this condition several times, it is our opinion that this is caused by the acid procedure and is not an artifact of fixation. A large basin is created around the base of the injured trichome, possibly allowing the leaching of metabolites. Wood and Bormann (1975) indicates that there is a significant ammount of leaching of potassium, magnesium, and calcium in pinto bean and maple after application of acid precipitation pH 2.3. This same study however showed that there was not a significant ammount of leaching at pH levels 3.0 and 5.0.

K. T. Thornham and I have noted that *D. destructiva* conidia are trapped easily at the base of the trichome (unpublished, 1993), and could be an area of leaching of nutrients and high humidity. No

evidence thus far has shown whether acid precipitation affects the structure or function of stomata in *C. florida*.

No one has determined how *D. destructiva* enters the leaf. Possible points of entry are stomata, trichome bases, or possibly this fungus enters by direct penetration. The closely related fungal genus *Colletotrichum* enters tissue by direct penetration by the use of an appressorium which forms approximately one spore length away from the conidium. Windham and Graham (U. Tennessee, unpublished) have claimed to have seen direct penetration of *D. destructiva* conidia, but this was in no way confirmed through histological work. This group claimed that the germ tube emerged and immediately entered the leaf. *Discula* species do not have appressoria, but it is possible that the tip of the germ tube is capable of penetrating leaf tissue. It is evident that the actual mode of infection remains uncertain.

A recent study of stomatal ontogeny and morphology in beans in relation to appressorium formation in *Uromyces appendiculatus* (Terhune et al., 1991) sheds light on the structure of stomata, and their possible importance in allowing infection. Terhune et al. noted that physical features of leaf surfaces often have important roles in the development of infection structures of powdery mildew, anthracnose, and rust fungi. Topographical features may include depressions located at cell junctions, cuticular wax crystals, trichomes, and stomata (Terhune et al., 1991). The bean rust fungus, *Uromyces appendiculatus*, forms an appressorium directly over the stomatal aperature (Terhune et al., 1991). Through SEM, TEM, and fluorescence microscopy techniques, this group noted specific features of bean stomata. Three apertures were noted, two of which are seen easily

through SEM micrographs. The outer ledge is a raised area with a fine wispy edge composed of cuticle (Terhune et al., 1991). In bean, when the stomata are closed, the outer ledges are pushed together and the wispy cuticular lips stick upward (Terhune et al., 1991). Since cuticle thickness is influenced by environment (Pallardy and Kozlowski, 1980, Thornham, 1992, unpublished), this group feels that environmental conditions may influence lip size as well as other morphological features of stomata (Terhune et al., 1991).

It has long been noted that DA-infected flowering dogwood trees are more commonly found in wooded, deeply shaded areas (Hibben and Daughtrey, 1988). Trees in urban landscapes in full sun tend to show very little or no disease. At the Seventh Regional Dogwood Workshop Erbaugh et al. (1993) reported that droughted and nondroughted flowering dogwood plants grown in 100% ambient light exhibited very little disease progression, while in plants grown in 10% and 2% ambient light, disease progression was much more evident. At 50% ambient light, disease only progressed in drought stressed plants. Less disease in full sun plants may be due to a combination of factors, including less humidity, quicker leaf drying time, and higher ambient temperature. Chellemi and Britton (1992) reported more rapid disease development in understory and interior canopy leaves due to the low evaporative potential in these microclimates. Another possible factor reducing disease incidence in full sun plants could be that full sun flowering dogwoods have a thicker cuticle and waxy layer, possibly protecting the leaf from *D. destructiva* fungal infection. Often flowering dogwoods grown in full sun seem to have thicker, waxy leaves. Using the SEM, K. T. Thornham scanned several

flowering dogwood leaves from full sun trees, and found that full sun leaves were waxier (unpublished data, 1992).

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**APPENDIX II:** Literature review for "Effects of temperature on growth and survival of *Discula destructiva*".

**LITERATURE REVIEW**

From the time dogwood anthracnose (DA), caused by *Discula destructiva* Redlin (Redlin, 1991), was observed in the late 1970's, then called "lower branch dieback", and through the realization that the disease was progressing southward along the Appalachain mountains through the 1980's, it was observed that severely diseased trees were most often found in apparently cooler, wooded, mountainous areas, and disease was rarely noted in apparently hotter areas at lower elevation. For example, by 1992, virtually all of the western mountainous counties in Virginia were classified as having dogwood anthracnose present according to the USDA Forest Service (Knighten and Anderson, 1993). Even though dogwood trees are common throughout most of Virginia, of the eastern counties in virginia, virtually all but two or three are DA free. DA in one or several of the positive eastern counties could have been introductions (wild dogwoods obtained from a DA positive county) rather than natural occurrences. North Carolina, South Carolina, and Georgia have no dogwood anthracnose in the low elevation areas to the east and south-east. Not only does this trend seem to hold on a regional basis, but on a local basis as well. Outplanted trees, trees which are planted in the urban setting or in the suburban yard, tend to show much less disease than trees found in cooler, shaded, wooded areas in the same vicinity, even under what appears to be severe disease pressure.

Foliar lesions noted in outplanted trees often have a restricted appearance, while characteristically rapidly expanding necrotic

lesions are often noted in the infected understory tree. Parham and Windham (1991) conducted a brief study in which they measured the growth rate of foliar lesions in shaded versus outplanted trees. Even though the leaf temperature of outplanted trees was greater than that of shade trees, lesion size progressed at a similar rate in both sun and shade trees. One interesting difference, however, is that sun leaves tended to exhibit purpling of the lesion edge and less acervular formation, indicating a possible inhibition due to a factor such as heat intensity. Parham and Windham (1992) found leaf temperatures of sun leaves were 4-5 C warmer (10 F) than shade leaves. Maximum temperatures were 32 and 27 C (89.6 and 80.6 F) for sun and shade leaves respectively during the duration of the experiment. Lesions were quite restricted in the full sun leaves. Lesions expanded much faster in shade leaves and on the north facing inner canopy shade leaves of full sun plants. Again the dominant lesion type for sun leaves was the purple rimmed type producing fewer acervuli. Erbaugh et al. (1993) noted that at higher light intensities, there was less disease.

Along with low evaporative potential (Chellemi and Britton, 1992), and thick cuticles, it is thought that *D. destructiva* may be intolerant of higher temperatures, resulting in more disease in cooler, shaded, mountainous regions. By understanding the thermal biology of *D. destructiva* we may gain insight into the possibility of discovering geographical and regional boundaries of the disease, as well as begin efforts to conduct thermal treatment of plant material.

Stipes and Ratliff (1991) studied briefly the thermal sensitivity of eight *D. destructiva* isolates from six different states. The

isolates were grown on potato-dextrose agar at seven temperatures, and observations were recorded after seven days. All but one isolate grew at 5 C (41 F), while no isolates grew at 28 or 32 C (82.4 or 89.6 F). These data suggest that this fungus prefers cooler temperatures. Optimum growth was estimated at 20-25 C (68-77 F).

In 1992, Erbaugh and Windham reported the results of a small study involving the heat treatment of *C. florida* leaves. Viable conidia were noted on leaves kept at 25 C (77 F) for 96 hours. Viable conidia were also noted after 96 hours at 40 C (104 F), but on fewer leaves, and 48 hours at 45C (113 F) exhibited fewer leaves with viable conidia. Apparently, dogwood seedlings can withstand temperatures of 45C for 96 hours without damage. This study not only sheds light on thermal treatment of plant material, but on the natural, *in vivo* conditions affecting conidial viability. It appears that *D. destructiva* conidia can withstand brief periods as high as 45 C (113 F). Obviously no 48 hour period ever remains at 45 C, indicating that conidia can probably withstand daily summertime temperatures.

Another devastating pathogen, *Heterobasidion annosum* (*Fomes annosus*) which causes annosum root rot of conifers colonizes freshly cut conifer stumps, and from them enters living trees through root grafts. Less annosum root rot develops in summer cut stands than in winter cut stands (Gooding et al., 1966). Summer-inoculated stumps failed to become colonized, whereas inoculations at other times of the year are successful (Gooding et al., 1966). In studying optimum, cardinal, and thermal inactivation temperatures, this group found optimum, minimum, and maximum growth temperatures to be 24, 2, and 32 C respectively (Gooding et al., 1966). However, there was some

variation among isolates, with some isolates growing equally well at 28 C as others did at 24 C. Many isolates in one report (Persson, 1957) were killed at 38 C in 9 days, whereas Roll-Hansen (1940) found that an isolate was killed at temperatures over 43 C after 2 hours. Ross (1969) placed conidia and basidiospores into capillary tubes, immersed the tubes at 45 C, and cooled the tubes in a 24 C water bath. He also sprayed conidia and basidiospores onto sterile pine discs, and recorded the percent colonization. With no exposure to the 45 C water bath, conidia and basidiospores had approximately 100 percent germination; after 20 minutes, conidia were down to about 70 percent germination, while basidiospores had less than 1 percent germination. Most conidial isolates were inactivated after 120 minutes while basidiospores were inactivated after 30 minutes. Conidia placed onto sterile pine discs were inactivated after 60 minutes. Ross felt that this method closely represented what would occur in the field, whereas the free water technique may not represent true life situations.

Microbiologists, especially those that work with food products, have a system for describing the thermal biology of bacteria (Anonymous, 1968). The thermal death point (TDP) is the lowest temperature at which the organism dies in a ten minute time period. The thermal death time (TDT) is the time it takes an organism to die at a particular temperature. This terminology will be used for ease of understanding and hopefully will be useful if applied to heat treatment of plant material and future studies into the regional differences among isolates.

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**Abiotic Stressors**

**in the**

**Dogwood Anthracnose Complex**

by

James Brooks Crozier

Committee Chairman: R. J. Stipes

Plant Pathology, Physiology, and Weed Science

(ABSTRACT)

Acidic precipitation reportedly enhances disease severity of dogwood anthracnose (DA) caused by *Discula destructiva*, on *Cornus florida*, the flowering dogwood. Seedlings were subjected to acidic fog episodes at pHs 2.5, 3.5, 4.5, and 5.5, using a simulated acidic rain solution. Leaf discs from these and non-treated plants were examined by scanning electron microscopy (SEM). Damage was noted at all pH levels. *Discula destructiva* conidia may germinate at trichome bases where damage may cause the leaching of nutrients. Also, the difference in stomatal damage may account, in part, for differences in disease susceptibility.

Cardinal growth temperatures and response to thermal stress regimes were determined for isolates of *Discula destructiva*. This information may lead to an understanding of possible climatic barriers, and the thermal treatment of plant material.