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## EFFECTS OF OZONE AND SULFUR DIOXIDE ON FOUR EPIPHYTIC BROMELIADS

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BENZING D. H., ARDITTI J., NYMAN L. P., TEMPLE P. J. and BENNETT J. P. *Effects of ozone and sulfur dioxide on four epiphytic bromeliads*. ENVIRONMENTAL AND EXPERIMENTAL BOTANY 32, 25–32, 1992.—Plants of *Tillandsia balbisiana*, *T. paucifolia*, *T. recurvata* and *T. utriculata* exposed to 0.15, 0.30 or 0.45 ppm O<sub>3</sub> or to 0.30, 0.60 or 1.20 ppm SO<sub>2</sub> for 6 hr or sequentially to 0.30 ppm O<sub>3</sub> (2 hr), 0.30 ppm O<sub>3</sub> plus 0.60 ppm SO<sub>2</sub> (2 hr) and 0.60 ppm SO<sub>2</sub> (2 hr) did not exhibit visible injury. Fumigations also had no effect on foliar conductance or on ΔH<sup>+</sup> associated with Crassulacean acid metabolism. Characteristics responsible for plant tolerance to short exposures to these two gases probably included low stomatal conductance, the insulating indumentum of absorbing foliar hairs and inherently slow metabolism. Reasons for postulating that *Tillandsia* is a better indicator for technological metals and certain other pollutants than for brief exposures to O<sub>3</sub> and SO<sub>2</sub> are discussed. We also conclude that epiphytic *Tillandsia* spp. offer advantages over lichens for air quality assessments under appropriate conditions. As vascular plants they exhibit sensitive, easily measured responses to stress and can be transplanted with ease to areas where neither they nor lichens occur naturally.

### INTRODUCTION

THE responses of two orchids with Crassulacean acid metabolism (CAM) to brief exposures to O<sub>3</sub> and SO<sub>2</sub> were described in an earlier report.<sup>(9)</sup> Four bromeliads from the same south Florida forests received identical treatments. Although all six subjects grow on trees under similar conditions both bromeliads and orchids were tested because they absorb moisture and nutrients through foliage or aerial roots, respectively, and may therefore differ in vulnerability to air contaminants. Our immediate objective, as in the experiments with the orchids,<sup>(9)</sup> was to establish threshold

exposures for injury and physiological effects from exposures to O<sub>3</sub> and SO<sub>2</sub>. More broadly, we wished to determine whether epiphytic bromeliads could replace lichens for air quality assessment in Florida National Parks located in areas undergoing rapid urban and industrial development. Certain tropical regions, including parts of extreme south Florida, support few suitable lichens, but epiphytic bromeliads are abundant or can be transplanted to desired monitoring sites. Moreover, vascular plants provide sensitive and easily quantified indicators of stress (e.g. stomatal conductance) that have no counterparts in lower plants.

## MATERIAL AND METHODS

*Tillandsia balbisiana*, *T. paucifolia*, *T. recurvata* and *T. utriculata* were collected in the Everglades National Park and adjacent swamp forests in January 1985. Plants were maintained in a charcoal-air-filtered greenhouse at 30–35°C day and about 25°C night temperatures, 70–75% relative humidity, and natural illumination prior to, during and after exposures. These temperatures approximate those that prevail in south Florida except when masses of colder air occasionally move into the area during winter months.

Exposures to O<sub>3</sub> and SO<sub>2</sub> were conducted at the University of California, Riverside in continuously stirred tank reactor (CTSR) exposure chambers 1.3 m in diameter and 1.36 m high covered with mil FEP Teflon film according to the procedures described in NYMAN *et al.*<sup>(9)</sup>

Three plants of each species were placed in a single chamber and exposed to 0.15, 0.3 or 0.45 ppm O<sub>3</sub> for 6 hr or to 0.3, 0.6 or 1.2 ppm SO<sub>2</sub> for 6 hr from 2100 to 0300 hr, an interval that began while stomatal conductance was relatively high and continued through a period of rapid acidification (Figs 1, 2). These exposures were repeated three times on different dates. Five additional plants of each species were subjected to sequential exposure to O<sub>3</sub> and SO<sub>2</sub>. These subjects were first treated with O<sub>3</sub> alone at 0.30 ppm for

2 hr, then to O<sub>3</sub> at 0.30 ppm plus 0.6 ppm SO<sub>2</sub> for 2 hr, and finally to 0.60 ppm SO<sub>2</sub> for 2 hr. The exposures were replicated four times in four different chambers on the same date. Gas mixtures were selected to represent the midpoints of O<sub>3</sub> and SO<sub>2</sub> concentrations applied when these agents were used alone.

Stomatal conductance was measured with a LI-COR Steady-State Porometer (Model LI-1600, Licor, Inc., Lincoln, NB) equipped with a narrow (1 cm<sup>-2</sup>) aperture. Two weeks before the experiments abaxial leaf surfaces of five *T. utriculata* plants were monitored consecutively at 3-hr intervals for 48 hr to determine conductance rhythms. Conductance was also recorded 1 hr prior to, at midpoint, immediately after and 12 hr subsequent to each 6-hr fumigation period. Foliage of the other three species was too narrow to fit the porometer cuvette.

Tissue assayed for acid content was collected with a razor blade or cork borer at 3-hr intervals and frozen with dry ice. Samples were ground, extracted with boiling water and titrated to pH 7.5 with 0.01 N NaOH.

Immediately following exposure, 12, 24, 28 hr, 2, 12 weeks and 18 months later unsacrificed plants that had been returned to the greenhouse were inspected for visible injury. Foliar surfaces were also examined by scanning electron microscopy.

## RESULTS

In the greenhouse all four bromeliads exhibited fluctuations in titratable acidity ( $\Delta H^+$ ) that varied somewhat in timing and intensity (Fig. 2A,B), but were characteristic of CAM-type plants. *Tillandsia balbisiana*, *T. paucifolia* and *T. recurvata* foliage began to acidify at about 2200 hr. *Tillandsia utriculata* reached this stage somewhat later. Acid accumulated by *Tillandsia recurvata* was utilized (decarboxylated) several hours before the other species exhausted reserves. Maximum acidity ( $H_{max}^+$ ) ranged between about 25 and 35  $\mu\text{eq/GFW}$  among the four taxa.

A minimum number of plants were sacrificed for the acid determinations in order to preserve enough plants for the long term observations. Nevertheless, there was no evidence that any of the three concentrations of O<sub>3</sub> influenced the

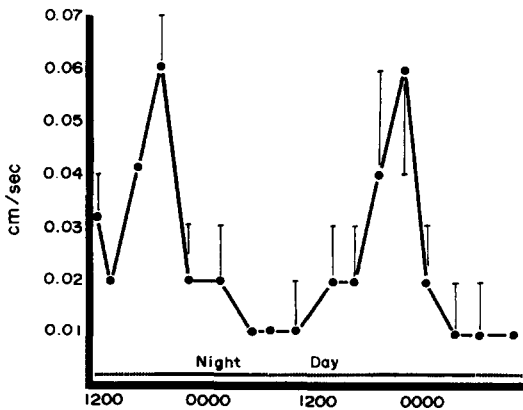


FIG. 1. Diurnal pattern of stomatal conductance (cm/sec) in *Tillandsia utriculata*; means of five plants  $\pm$  standard error of the mean (S.E.M.). Points without error bars had S.E.M. = 0.

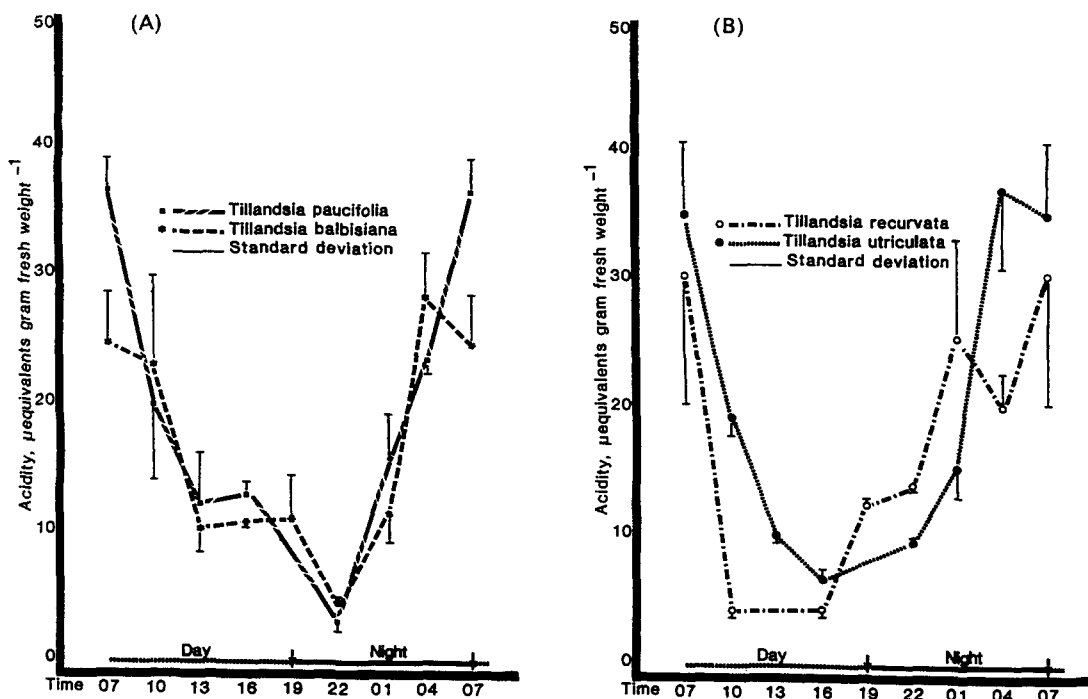


Fig. 2. Acidification of foliage of four *Tillandsia* species in the greenhouse: (A) diurnal rhythms ( $\Delta H^+$ ) in *T. balbisiana* and *T. paucifolia*; (B) diurnal rhythms in *T. recurvata* and *T. utriculata*.

intensity of CAM in any of the tested bromeliads (Fig. 3). No dosage effect was apparent, i.e. acidification generally varied without regard to the severity of the treatment. Moreover,  $H^+$  in the leaves of *T. recurvata* controls at 0700 hr was about three times that exhibited in the greenhouse. Acidification also occurred without discernible trends during exposures to  $SO_2$  (Fig. 3). Treatment with 1.2 ppm  $SO_2$  seemed to enhance acid accumulation by *T. utriculata*, but how much, if any, of this increase represented  $SO_2$  dissolved in cell sap in this most severe of the treatments is not known. Equally inconsistent in their relationships to control values were the magnitudes of acid stores in plants exposed to combined  $O_3$  and  $SO_2$  (Fig. 3). In this instance,  $H^+$  in *T. recurvata* was well below that measured in controls. An explanation for at least part of the variability recorded in these runs is provided in the discussion.

Stomatal conductance in *T. utriculata* was greatest in the evening and early morning (Fig.

1), when it was about five to ten times lower than that of a typical  $C_3$  crop plant,<sup>(14)</sup> and lowest at about midday. An analysis of variance indicated that conductance was not affected ( $P \leq 0.05$ ) by exposures to  $O_3$  or  $SO_2$  (data not shown).

No injuries were apparent immediately after the fumigations nor did any necrosis or abnormal growth responses develop or flower abortion occur during the subsequent 18 months.

## DISCUSSION

*Tillandsia* spp., particularly the xerophytic taxa including those tested in this study, possess characteristics that could increase sensitivity to air quality above that of most vascular plants.<sup>(5)</sup> Absorptive functions no longer performed by much reduced root systems have shifted to shoots equipped with specialized trichomes<sup>(4)</sup> (Figs 4, 5). Tightly overlapping leaf bases of *T. utriculata* form tanks in which falling debris and precipitation,

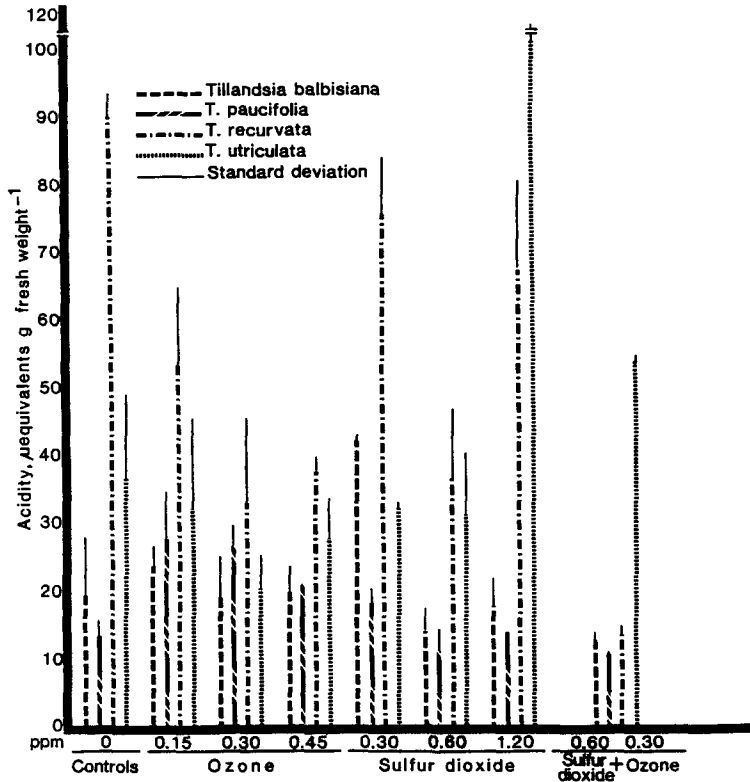


Fig. 3. Effects of O<sub>3</sub> and SO<sub>2</sub> singly and in combination on H<sup>+</sup> following fumigations.

the major sources of nutrient ions for this species, accumulate. The other three so-called atmospheric bromeliads lack shoot impoundments and obtain nutrients from rain and canopy runoff as it washes over foliage.<sup>(5)</sup> Critical to our decision to evaluate these four taxa is their dependence on aerial rather than terrestrial sources of nutrients.

Two aspects of CAM probably influenced the responses of our subjects and those of the two orchids described earlier<sup>(9)</sup> to the tested gases. Crassulacean acid metabolism is part of the complex mechanism that allows these bromeliads and many other epiphytic and terrestrial xerophytes to achieve exceptional water use efficiency and to avoid severe desiccation during prolonged drought.<sup>(14)</sup> This mechanism incorporates generally low stomatal conductances that peak at

night and perhaps also accounted in our treatments for the absence of leaf damage from exposures to concentrations of O<sub>3</sub> and SO<sub>2</sub> that injure some other plants. Further insulating the leaf interior were the thick-walled, dead cells comprising each of the trichome caps that form a continuous layer over the stomata (Figs 4, 5). In addition to these two physical features that slowed penetration of the fumigants, plant vulnerability may have been reduced by the inherently sluggish growth and metabolism associated with CAM.

Variations in ΔH<sup>+</sup> that bore little if any relationship to the type or concentration of the fumigants used in these experiments are inherent to CAM plants.<sup>(14)</sup> Acid rhythms change in intensity depending on previous and immediate growing conditions (e.g. day and night temperature,

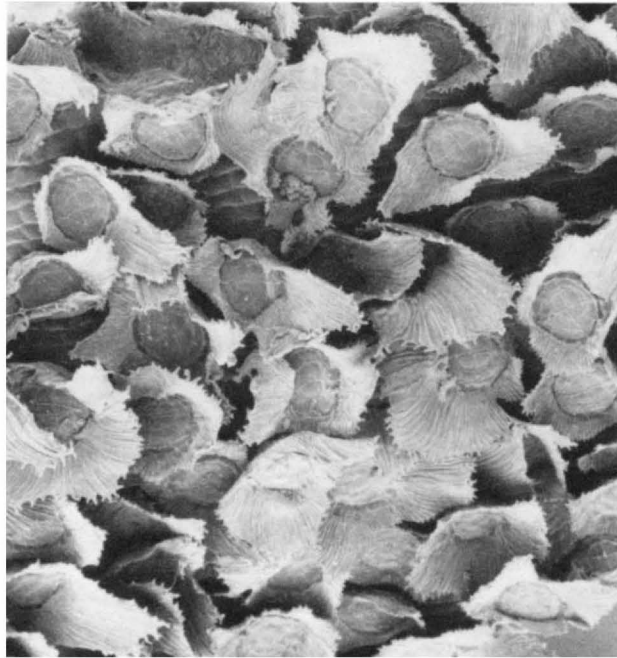


FIG. 5. Absorbing trichomes covering the abaxial leaf surface of *Tillandsia paucifolia* ( $\times 80$ ).

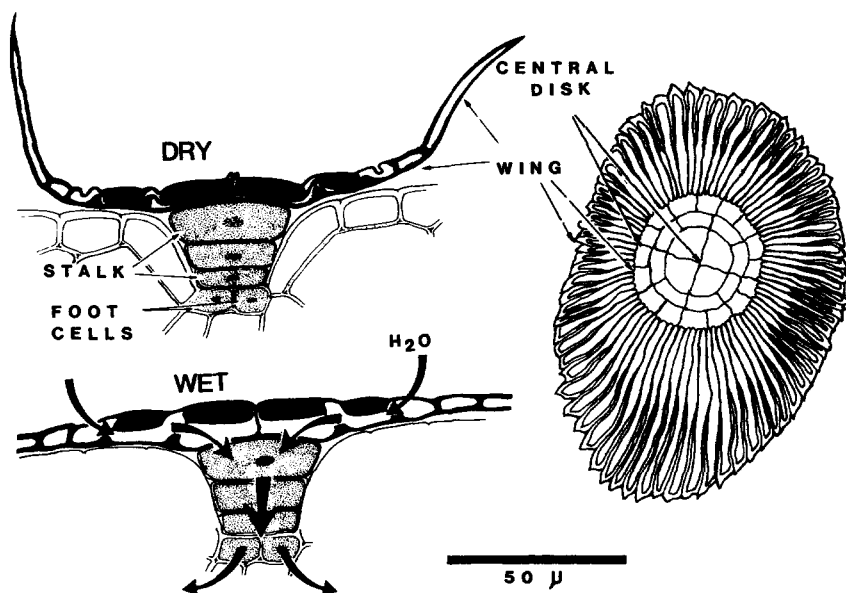


FIG. 4. Foliar trichome of an atmospheric bromeliad illustrating the dry and wet conditions and the pathway of absorption when the leaf surface is moist.

photoperiod, photon flux density) and endogenous factors (e.g. supplies of reserve carbohydrates, moisture status) that vary within populations and can change from day to day for individual plants. Additionally, much of the acid synthesized at night represents refixed, respired CO<sub>2</sub>. Recycling, which occurs while stomata are open or closed, often accounts for >50% of  $\Delta H^+$  in bromeliads and in severely drought-stressed specimens (those in the CAM-idling mode) this value approaches 100%.<sup>(3)</sup> Sufficient for our purposes in this study was confirmation that CAM function continued despite the treatments, i.e. that  $H_{max}^+$  in leaf tissue remained high and  $\Delta H^+$  tracked the normal diurnal pattern.

Additional CAM plants have tolerated relatively short exposures to SO<sub>2</sub> without apparent injury;<sup>(9,10)</sup> others have not. Fumigations for several days at 0.14–0.18 ppm induced considerable leaf necrosis in atmospheric *T. aeranthos*, but whether damage was sustained at the epidermis or deeper was not determined.<sup>(1)</sup> Neither were effects on CAM recorded.

*Tillandsia* spp. have been used to document the presence of a variety of airborne substances. For

instance, SCHRIMPF<sup>(12)</sup> employed *T. recurvata* to monitor a constellation of airborne metals, pesticides and polycyclic aromatic hydrocarbons at two sites in Colombia, South America. A record of lead from automobile exhaust was found in *T. usneoides* (Spanish moss) by MARTINEZ *et al.*<sup>(8)</sup> and ROBINSON *et al.*<sup>(11)</sup> This same bromeliad revealed nickel (Ni) contamination near a battery factory.<sup>(6)</sup> Total sulfur (S) in the foliage of *T. balbisiana*, *T. paucifolia* and *T. utriculata* in south Florida indicated utility for monitoring S-enriched aerial deposition.<sup>(2)</sup> The most comprehensive demonstration that *Tillandsia* spp. are effective reflectors of atmospheric chemistry was presented by SHACKLETTE and CONNOR<sup>(13)</sup> and CONNOR and SHACKLETTE<sup>(7)</sup> who sampled 25 elements in biomass of Spanish moss at 123 sites ranging from Texas to Florida to North Carolina.

Two plant characteristics probably account for the sensitivity of bromeliads to some air pollutants: (1) dependence on ions drawn directly from the atmosphere rather than the ground, and (2) the relatively non-specific nature of the absorption mechanisms located in foliar trichomes. Effective nutrient scavenging is crucial to the

epiphyte to maintain adequate nutrition in tree crown habitats, but these same mechanisms promote abnormal and even toxic accumulations of required (e.g. Cu) and other substances if normal supplies are supplemented.<sup>(5)</sup> As a result, among vascular plants, xerophytic *Tillandsia* is an extraordinary indicator of contamination by technological metals and a variety of other agents that enter leaves via foliar trichomes. However, these epiphytes do not appear to be as sensitive as some other vegetation (e.g. certain crops) to short exposures to several gases that enter through stomata.

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