

Tillandsia recurvata L. as a bioindicator of sulfur atmospheric pollution

CORINA GRACIANO^{1,✉}, L. V. FERNÁNDEZ¹ & D. O. CALDIZ^{1,2}

¹ Instituto de Fisiología Vegetal (INFIVE), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, La Plata, ARGENTINA.

² Present address: McCain S.A., Ruta 226 Km. 61,5, B7620EMA, Balcarce, Buenos Aires, ARGENTINA

ABSTRACT. *Tillandsia recurvata* L. is an epiphyte that absorbs nutrients from the air, so it could be used as a bioindicator of atmospheric sulfur pollution. In order to test this idea, *Tillandsia recurvata* samples were seasonally collected for two years at three sites of La Plata, Buenos Aires Province, Argentina, in a suburban park and in a rural area 60 km away from the city. Macro- and microscopic observations were carried out and chlorophyll and sulfur concentrations of the tissue were measured to evaluate if this epiphyte shows morphological or physiological damage in the most polluted sites or if it accumulates sulfur in the tissues when sulfur in the air increases. No micro- or macroscopic damage was observed, and chlorophyll content seemed not to be related with pollution level. Samples from the rural site always had a lower sulfur content than those from the urban and suburban sites. In conclusion, *Tillandsia recurvata* tolerates the local level of sulfur contamination without showing morphological damage and could be a suitable bioindicator because of its capacity to accumulate sulfur. [Keywords: *Tillandsia recurvata*, sulfur dioxide, atmospheric pollution, bioindicator.]

RESUMEN. *Tillandsia recurvata* L. como un bioindicador de contaminación atmosférica por azufre: *Tillandsia recurvata* L. es una planta epífita muy abundante en el arbolado urbano de la ciudad de La Plata (provincia de Buenos Aires, Argentina). Como absorbe agua y nutrientes minerales de la atmósfera, puede ser un buen indicador de la contaminación atmosférica. Los organismos indicadores pueden serlo por mostrar modificaciones morfológicas macro o microscópicas, o bien por acumular el contaminante en los tejidos al ser expuestos a mayores concentraciones. Para examinar la posibilidad de usar esta epífita como una especie indicadora de la contaminación por azufre, se recolectaron individuos de *Tillandsia recurvata* durante 2 años en tres sitios del casco urbano de La Plata, en un sitio suburbano ubicado en un gran parque al norte de La Plata y en un sitio rural a 60 km al sur de la ciudad. Se realizaron observaciones macro y microscópicas, se determinó la concentración de clorofila y la concentración de azufre en los tejidos vegetales. No se encontraron modificaciones morfológicas macro ni microscópicas que indiquen daños en las plantas expuestas a mayores niveles de contaminación. La concentración de clorofila no parece estar relacionada con el nivel de contaminación. En todas las fechas de muestreo, la concentración de azufre en los tejidos fue menor en las plantas provenientes del sitio rural, habiéndose encontrado variaciones para cada sitio entre las fechas de muestreo. Se concluye que *Tillandsia recurvata* tolera los niveles de contaminación existentes en La Plata sin mostrar modificaciones morfológicas y que, por su habilidad de acumular azufre en los tejidos, puede ser utilizada como indicadora de contaminación atmosférica por azufre. [Palabras claves: *Tillandsia recurvata*, dióxido de azufre, contaminación atmosférica, bioindicador.]

✉ INFIVE, FCAyF, Univ. Nac. de La Plata; Diag
113 y 61, C.C. 327, 1900 La Plata, ARGENTINA.
invpapa@ceres.agro.unlp.edu.ar

Recibido: 22 marzo 2002; Revisado: 9 septiembre 2002
Aceptado: 12 septiembre 2002

INTRODUCTION

Urban pollution with nitrous oxide, sulfur dioxide and carbon monoxide is an increasingly important problem in many areas of the world (Goldish 1991). Sulfur dioxide and nitrogen oxides mainly derive from fossil fuel combustion (Unsworth et al. 1994) and industrial plants, such as chemical works and metal smelting plants, that also release sulfhydic acid and hydrogen fluoride into the atmosphere (Taiz & Zeiger 1991). The level of atmospheric pollution can be assessed by direct or indirect methods. Direct methods are not frequently used in Argentina, probably because of their high cost. Moreover, the few data available for La Plata (e.g., for atmospheric sulfur content) show high variability, probably due to changes in wind speed and direction and the stability of the pollution source (i.e., emissions from traffic or industry). In this case, indirect methods can be an effective alternative, for example using organisms with the ability to accumulate specific pollutants. Bioaccumulation rate depends on exposure to the pollutant, weather conditions and the organism's ability for uptake (Goldish 1991). Plants used as bioindicators are classified in two groups: those whose vitality or aspect are clearly affected by the pollutant, and those that do not show any pattern of visible modification. The former are sensitive species that react to environmental changes through evident macroscopic or microscopic damage and therefore can be used to obtain information in situ through observations of external symptoms such as chlorosis, necrosis or growth inhibition. The latter seem to be more resistant and can accumulate a large amount of toxic substances before showing external modifications (Strehl & Lobo 1989). Epiphytic plants could be appropriate to assess atmospheric pollution because they absorb nutrients mostly from the air (Holoubek

et al. 2000), so soil and water pollution do not affect the results. Lichens are often used as bioindicators of urban pollution, due to their sensitivity to sulfur and heavy metals. González & Pignata (1994), Levin & Pignata (1995) and Carreras et al. (1998) used different lichen species as indicators of urban pollution by transplanting them to the study area and harvesting the material three months later. Bennett & Wetmore (1999) found that corticolous lichens were more enriched in heavy metals than the terricolous species and that species differ strongly in their sensitivity. Freitas et al. (1999) identified five pollution sources in Portugal using the lichen *Parmelia sulcata*, and Owczarek et al. (1999), in Italy, confirmed the efficiency of fruticose and foliose lichens as bioindicators. Pyatt et al. (1999) found that *Tillandsia usneoides* and *Parmotermia praesorediosum* effectively accumulate heavy metals and sulfur from the atmosphere. Mesophytic species have also been used to evaluate atmospheric pollution. Canas et al. (1997) used *Ligustrum lucidum* to build a contamination index of urban air pollution in Argentina. Alquini et al. (1996) found that pollution near a factory affected leaf anatomy in *Sechium edule*.

Tillandsia recurvata L. (Bromeliaceae) is an epiphyte that has reduced roots, and uses specialized foliar trichomes to absorb minerals and water from rain and canopy runoff as it washes over foliage (Benzing 1973; Benzing & Renfrow 1974; Benzing & Davidson 1979), so it depends on aerial rather than terrestrial sources of nutrients (Benzing et al. 1992). This epiphyte is present upon its hosts even with high levels of contamination (Strehl & Lobo 1989). The latter authors found that urban pollution of Porto Alegre city (Brazil) reduced the number of leaves and flowers, and fruit length in this species. On the other hand, Benzing et al. (1992) showed that plants of *Tillandsia recurvata* exposed to different ozone and sulfur dioxide concentrations did not show

visible injury. They suggested that this epiphyte has advantages over the lichens as a biological indicator of air quality, such as abundance in certain regions and sensitive and easily quantified indicators of stress (e.g., stomatal conductance). Strehl & Arndt (1989) exposed *Tillandsia recurvata* plants to different sulfur dioxide concentrations in chambers and did not find internal or external symptoms of injury that could indicate sensitivity to this gas. Nevertheless, they found that this epiphyte accumulated sulfur during the first days after the treatment.

Hence, the purpose of this paper is to evaluate if different pollution levels in rural, suburban or urban locations affect *Tillandsia recurvata*. Macro and microscopic observations were carried out and chlorophyll and tissue sulfur concentrations were determined to evaluate

if *Tillandsia recurvata* could be a suitable species to assess sulfur dioxide contamination.

STUDY AREA

La Plata city (34°58'S; 57°54'W; 19 m.a.s.l.) has an intensive traffic, with almost 180000 registered motorvehicles. As low sulfur fuel is not used in Argentina, traffic emits high quantities of sulfur. The city is also surrounded by an industrial belt with a range of factories as sources of pollution. As these factories are situated between the city and the Río de la Plata river, and because winds are mainly from E, SE and ES, emissions from these factories often move towards the city. As far as is known, the highest level of sulfur dioxide found near the industrial area have not surpassed the limits established

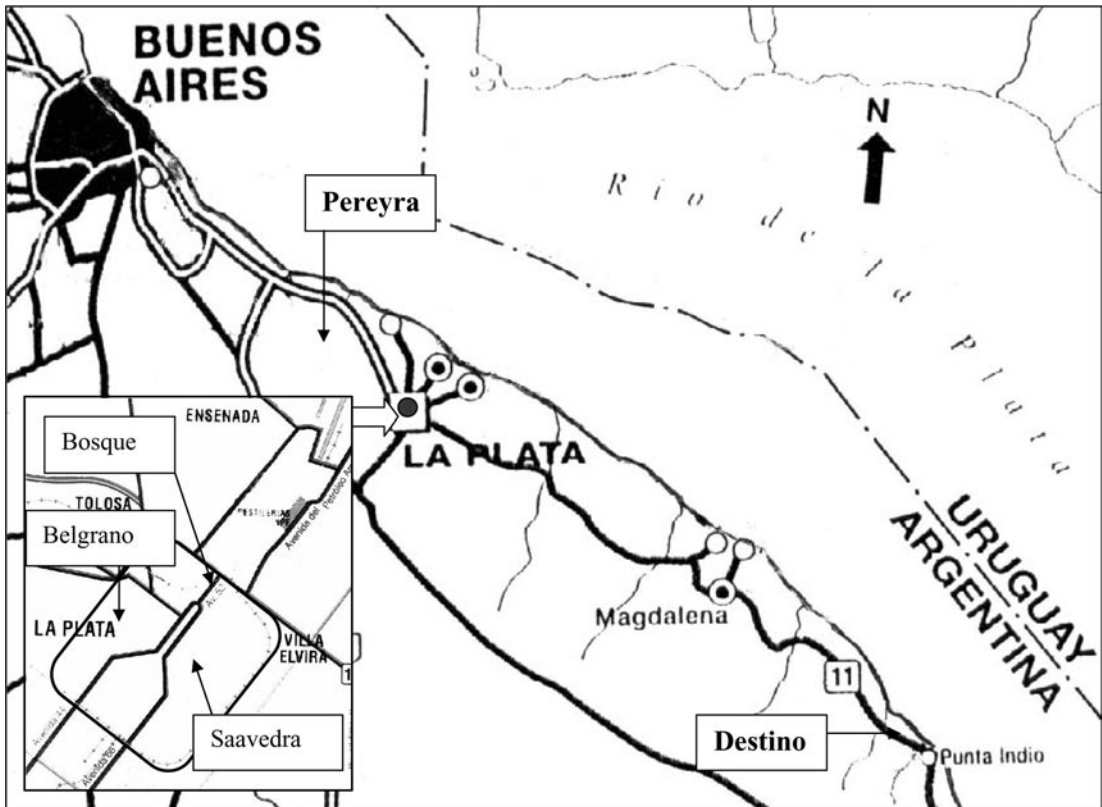


Figure 1. Location of the five sampling sites in Buenos Aires Province where *Tillandsia recurvata* individuals were collected.

Figura 1. Ubicación geográfica de los cinco sitios de muestreo de la provincia de Buenos Aires en donde se colectaron individuos de *Tillandsia recurvata*.

by provincial regulations of 300 ppb in 60 min and 30 ppb as a monthly mean (Provincial Decree 1601/95). However, no systematic measurements of air pollution have been made in La Plata, with only some data taken in different places and dates, and with different methods, since 1977. These data show high variability in each place, probably related to variations in wind speed and direction, and emission at the source. The town council of La Plata took data from 1977 to 1980 in five points of the city, but only monthly means are available for that period. In October 1998, Commissioning Argentina S.A. measured atmospheric sulfur levels during 8 days in Berisso, a city close to La Plata. There are some other sporadic data taken by the Secretaría de Política Ambiental in 1999 at one point, and others taken by the town council of La Plata in 2001 in three points of the city. Overall, official measurements were not systematic, and sampling points were not maintained along the time.

The city has many urban forests that play an important role counteracting the effect of pollutants. During the last 15 years trees and shrubs in this area have been heavily colonized by *Tillandsia recurvata* (Claver et al. 1983; Caldiz et al. 1993).

METHODS

Tillandsia recurvata individuals were collected from five sites, three located in the urban area, one in a suburban area and the last one in a rural area (Figure 1). Locations were: (1) Paseo del Bosque (Bosque), (2) Plaza Belgrano (Belgrano), and (3) Parque Saavedra (Saavedra), all located in different areas of La Plata city, (4) Parque Pereyra Iraola (Pereyra), a suburban area 10 km north from the city, and (5) Estancia El Destino (Destino), Magdalena (35°22'S; 57°17'W; 22 m.a.s.l.), located in a rural area 60 km south from the city.

Both Destino and La Plata city have similar meteorological conditions (Figure 2). Destino is a farm isolated from urban centres, with a dense forest. The only road to Destino has an annual mean traffic of 203 vehicles per day. Two important roads with intensive traffic (annual mean traffic of 8406 and 32067 vehicles per day; MOSP-BA 2000) cross Pereyra, a park of about 13000 ha extended to the north of La Plata. Bosque, Belgrano and Saavedra are squares located in different areas of the city. Bosque is a park with numerous perennial trees and streets with a moderate amount of traffic (annual mean traffic of 5695 vehicles per day); it is close to a petrochemical industrial belt. Belgrano is a square that has a few deciduous trees and is surrounded by car-crowded streets. Saavedra has a mix of deciduous and evergreen trees and is situated in an area with regular traffic.

During 1997-1999 plants of *Tillandsia recurvata* with at least one flowering stalk were collected in July, December and March in the sites described above. A randomized sample of 60 individuals per site was collected.

Macro and microscopic observations

Possible morphological abnormalities caused by atmospheric pollution were evaluated in 30 plants per site in the first sampling date. Macroscopic observations of foliar aspect and plant vigour were done. Basal leaf length was measured as an indicator of plant size. Observations with a microscope (Photomicroscope Ultraphot, Carl Zeiss, Jena, Germany) were done in semipermanent histological mountings of transversal and longitudinal leaf sections, by using a gelatine-glycerin mounting medium (D'Ambrogio de Argüeso 1986). Observations with a scanning electron microscope were performed by fixing the material in FAA (formaldehyde, glacial acetic acid and ethyl alcohol) and dehydrating in 70%

absolute ethyl alcohol. Plant material was prepared by critical point drying and coated with gold. Trichomes and stomata were observed in leaves, and transversal sections were prepared to observe the bundle sheath.

Chlorophyll concentration

Chlorophyll concentration was determined spectrophotometrically as in Inskeep & Bloom (1985). To avoid differences in light conditions within each sampling site, all samples were collected from the same tree in each site. Samples were collected only from Destino, Pereyra and Belgrano since December 1997. Chlorophyll concentration in Saavedra and Bosque was not measured because it was impossible to take samples always from the same tree. Twenty rep-

lications (15 pieces, 1 cm-long of fresh leaf material each) from each sampling site were weighed and extracted with 5 ml of N,N-Dimethylformamide for 72 h in darkness. Absorbance was read at 647 and 664,5 nm with a spectrophotometer (Spectronic 21, Bausch & Lomb, Rochester, New York, USA). Chlorophyll a, b and total chlorophyll were determined using the equations given by Inskeep & Bloom (1985).

Sulfur content

Tissue sulfur concentration was determined for each site at six sampling dates to test possible seasonal variations due to different amounts of sulfur dioxide in the atmosphere or to differences in metabolic activity of the plant. In each sampling date, a pool of full-expanded leaves was done for each site. Thirty replications per sampling site were analyzed with a turbidimetric method (Blanchar et al. 1965). One-gram aliquots of fresh material from all plants were used for an acid digestion. Sulfur content was determined as BaSO₄ by addition of BaCl₂ to the digested material. Absorbance was read at 420 nm with a spectrophotometer (Spectronic 21, Bausch & Lomb, Rochester, New York, USA). Sulfur content was expressed on a dry weight basis.

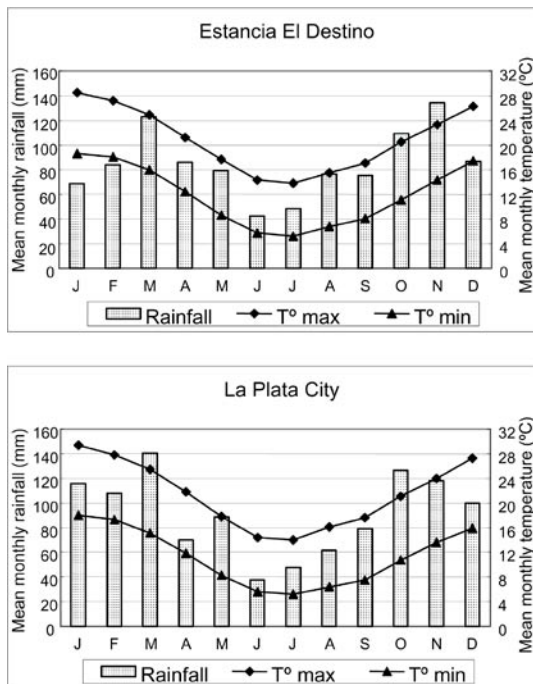


Figure 2. Mean monthly rainfall, and mean monthly maximum and minimum temperatures in Estancia El Destino (Magdalena) and La Plata city, Buenos Aires Province.

Figura 2. Precipitación mensual promedio y temperaturas máxima y mínima promedio en Estancia El Destino (Magdalena) y en la ciudad de La Plata, provincia de Buenos Aires.

Table 1. Mean (\pm SD) basal leaf length of *Tillandsia recurvata* in the five sampling sites in Buenos Aires Province. Means followed by the same letter are not significantly different ($P < 0.05$).

Tabla 1. Longitud promedio (\pm DE) de la hoja basal de *Tillandsia recurvata* en los cinco sitios de muestreo de la provincia de Buenos Aires. Los promedios señalados con la misma letra no difieren significativamente ($P < 0.05$).

	Basal leaf length (cm)
Destino	4.15 \pm 0.71 a
Bosque	3.65 \pm 0.83 b
Belgrano	3.61 \pm 0.63 bc
Saavedra	3.34 \pm 0.67 b
Pereyra	3.11 \pm 0.58 c

Statistical analysis

The Statgraphics Plus 3.0 computer program was used to perform an Analysis of Variance, and averages were compared with the Tukey's Test ($P < 0.05$).

RESULTS AND DISCUSSION

Macro and microscopic observations

Leaf length showed a high variability within each site and no clear relationship was observed between this parameter and sampling site. The longest mean leaf was observed in Destino, and the shortest one was observed in Pereyra (Table 1). No macro or microscopic morphological differences indicating tissue injury due to atmospheric pollution were observed. These results disagree with those of Strehl & Lobo (1989) who found that in the polluted urban environment of Porto Alegre city, *Tillandsia recurvata* had fewer and shorter leaves and inflorescences. These authors also stated that necrosis or chlorosis, and even death of the vegetative parts could occur. Deltoro et al. (1999) suggested that the ability to cope with absorbed SO_2 must play a role in determining the sensitivity of a species to this pollutant.

In all sites, scanning electron microscope observations showed no cellular damage (Figure 3). SO_2 concentrations above 400 ppb depressed photosynthesis in many species but, provided that tissues were not damaged, a rapid recovery was recorded when the pollutant was removed from the environment (Unsworth 1981). More recently, Lenzian & Unsworth (1983) stated that sensitive species such as alfalfa, cucumber, bracken and pine may show visible injury after a few hours exposure to 100–500 ppb SO_2 . In the surroundings of Bosque, continuous atmospheric SO_2 concentrations close to 5 ppb, and peaks of 180 ppb during only a few minutes were recorded by direct

methods (Secretaría de Política Ambiental, Departamento Laboratorio, La Plata, July-September 1997), therefore it is not surprising that plants show no external damage. However, *Vicia faba* plants exposed for 2 h to 175 ppb showed collapsed epidermal cells (Unsworth 1981), and exposure for 2 h to 0.85 and 0.65 ppb SO_2 in small chambers caused leaf necrosis in pea and tomato (Olszyk & Tingey 1985). At Bosque, peak SO_2 concentrations can be just a bit higher, but during a shorter period than in the *Vicia faba* experiment. Moreover, Alquini et al. (1996) reported plasmolysed epidermal and mesophyll cells in leaves of *Sechium edule* plants collected near a calcareous industry, an important source of SO_2 emissions. But Benzing et al. (1992), working with *Tillandsia recurvata*, found no injuries after fumigation with 1.2 ppm SO_2 during 6 h. They postulated that plant vulnerability is reduced by the low stomatal conductance, the insulation produced by the trichome layer and the slow metabolism associated with CAM.

Tillandsia recurvata seems to have the capacity to grow and reproduce normally, without showing internal or external damages, under the local levels of atmospheric pollution.

Chlorophyll concentration

The highest total chlorophyll concentration was 2.488 mg/g DM at Belgrano in July 1998, and the lowest concentration was 0.994 mg/g DM at Destino, in the same sampling date (Table 2). At all sites chlorophyll concentration in *Tillandsia recurvata* was lower than in mesophytic species (García & Galindo 1991; Akhatar et al. 1999), but similar to those found by Martin et al. (1985) in *Tillandsia usneoides*. This may be due to leaf thickness and succulence of these epiphytes. Chlorophyll concentration in *Tillandsia recurvata* seemed not to be related with SO_2 atmospheric pollution.

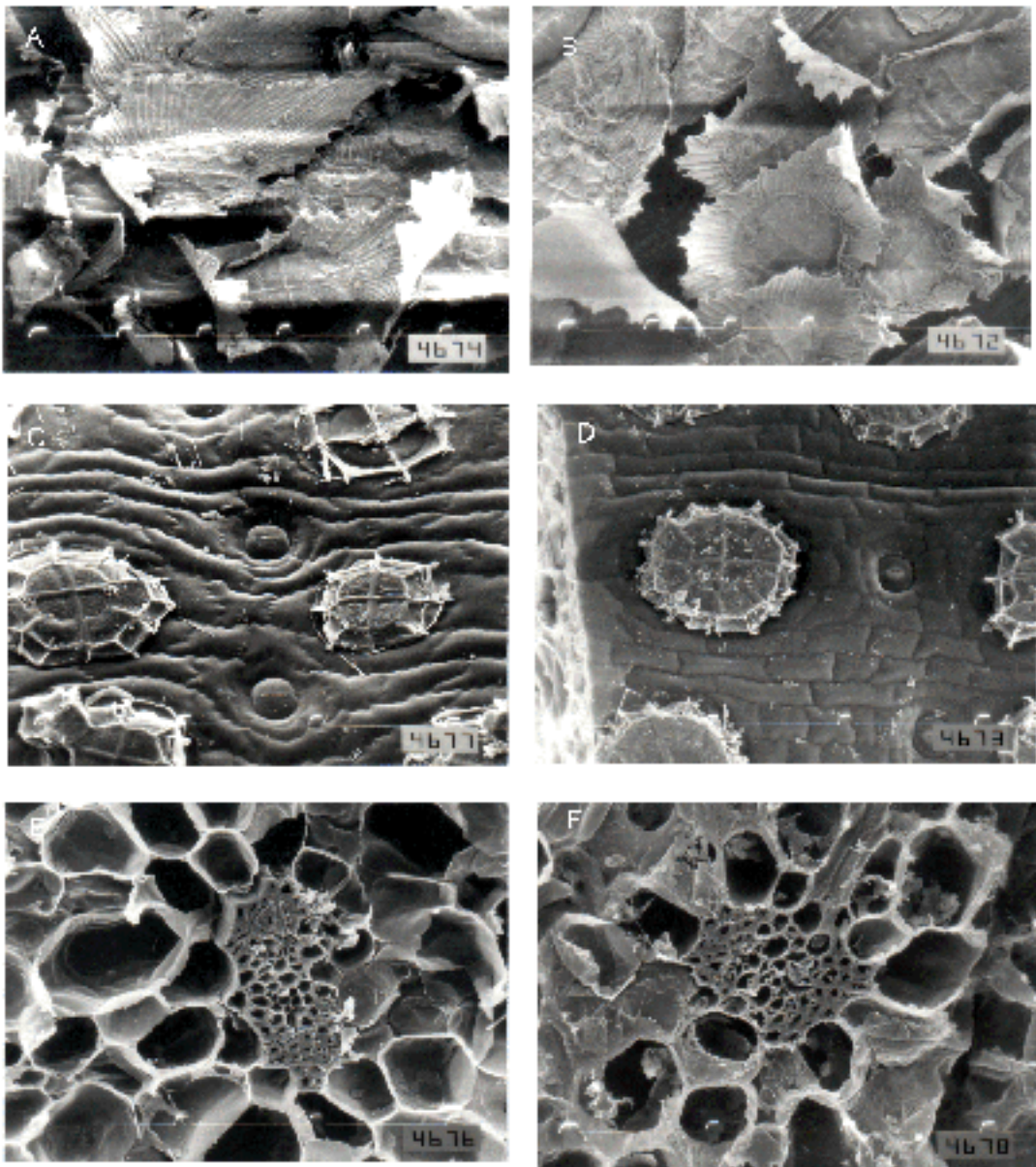


Figure 3. Scanning electron microscope photographs of *Tillandsia recurvata* leaves. (A-B) Intact surface of a leaf, where the appearance of trichomes can be observed (A: Destino, 175x; B: Belgrano, 200x). (C-D) Leaf epidermis, where trichome wings were removed to see stomata and epidermal cells (C: Destino, 350x; D: Belgrano, 350x). (E-F) Leaf cross section, where the bundle sheath can be observed (E: Destino, 350x; F: Belgrano, 350x).

Figura 3. Fotografías de hojas de *Tillandsia recurvata* obtenidas con microscopio electrónico de barrido. (A-B) Superficie intacta de la hoja, donde pueden observarse los tricomas (A: Destino, 175x; B: Belgrano, 200x). (C-D) Epidermis de la hoja, donde las células del ala del tricoma fueron removidas para visualizar los estomas y las células de la epidermis (C: Destino, 350x; D: Belgrano, 350x). (E-F) Sección transversal de hoja donde se pueden observar los haces vasculares (E: Destino, 350x; F: Belgrano, 350x).

Table 2. Mean (\pm SD) chlorophyll b, chlorophyll a and total chlorophyll (mg chlorophyll/g DM) in three of the five sampling sites in Buenos Aires Province at five sampling dates. For each sampling date, means followed by the same letter are not significantly different ($P < 0.05$).

Tabla 2. Concentración promedio (\pm DE) de clorofila b, clorofila a y clorofila total (mg clorofila/g MS) en tres de los cinco sitios de muestreo de la provincia de Buenos Aires en cinco fechas de muestreo. Para cada fecha de muestreo, los promedios señalados con la misma letra no difieren significativamente ($P < 0.05$).

	Dec. 1997	March 1998	July 1998	Dec. 1998	March 1999
Chlorophyll b					
Destino	0.328 \pm c 0.030	0.412 \pm c 0.064	0.337 \pm c 0.055	0.411 \pm c 0.034	0.437 \pm c 0.052
Pereyra	0.689 \pm b 0.042	0.554 \pm a 0.051	0.554 \pm b 0.054	0.597 \pm b 0.076	0.801 \pm a 0.082
Belgrano	0.751 \pm a 0.063	0.523 \pm b 0.042	0.829 \pm a 0.074	0.819 \pm a 0.084	0.604 \pm b 0.050
Chlorophyll a					
Destino	0.675 \pm c 0.053	0.794 \pm c 0.107	0.657 \pm c 0.098	0.704 \pm c 0.059	0.885 \pm b 0.106
Pereyra	1.364 \pm b 0.106	1.157 \pm a 0.112	1.131 \pm b 0.109	1.283 \pm b 0.167	1.334 \pm a 0.095
Belgrano	1.558 \pm a 0.382	1.031 \pm b 0.084	1.660 \pm a 0.139	1.326 \pm a 0.174	0.968 \pm b 0.082
Total					
Destino	1.003 \pm c 0.082	1.205 \pm c 0.196	0.994 \pm c 0.152	1.115 \pm c 0.082	1.322 \pm c 0.157
Pereyra	2.052 \pm b 0.132	1.711 \pm a 0.158	1.685 \pm b 0.161	1.880 \pm b 0.233	2.134 \pm a 0.170
Belgrano	2.308 \pm a 0.195	1.554 \pm b 0.123	2.488 \pm a 0.212	2.144 \pm a 0.252	1.572 \pm b 0.130

In general, chlorophyll concentrations were higher in Belgrano than in Pereyra and Destino, which are more distant from SO₂ sources in the area. Canas et al. (1997) found that in *Ligustrum lucidum* exposed to urban pollution the concentration of photosynthetic pigments correlates negatively with foliar sulfur concentration. This trend was not found in our results. Differences in chlorophyll concentration could be explained by differences in irradiance. Destino always showed lowest concentration probably because this is an open area where *Tillandsia recurvata* plants may receive more light. Higher concentrations were obtained in Pereyra and Belgrano, both sites with evergreen trees and tall buildings surrounding the sampling point. Martin et al. (1985) also found great differences in chlorophyll concentration in *Tillandsia usneoides* plants

exposed to high and low irradiances (1.75 \pm 0.61 mg/g DW, and 4.08 \pm 2.51 mg/g DM, respectively).

Sulfur concentration

Large differences in sulfur concentrations between plants from Destino (the rural site) and the other sampling sites in La Plata city were found (Figure 4). The sulfur concentrations are in agreement with those of Levin & Pignata (1995), who found in lichens a mean of 0.78 mg/g DM for the control unpolluted site, 1.35 mg/g DM for a transect with low traffic level, and 2.44 mg/g DM for a transect characterized by intensive traffic.

Environmental factors such as light intensity and quality, temperature, relative humidity, and intercellular CO₂ concentrations define stomatal responses. As SO₂ enters through the stomata,

following the same diffusion pathway as CO₂ (Taiz & Zeiger 1991), seasonal variation in environmental factors may also affect SO₂ uptake and thus internal sulfur content, as shown in Figure 3. For example, in the two sites more distant from SO₂ sources (i.e., Destino and Pereyra), tissue sulfur concentration tended to be highest in summer and lowest during the winter. Mean values (\pm SD) considering the five sampling points and both years are: 1.507 \pm 0.804 mg/g (July), 1.735 \pm 0.843 mg/g (March), and 2.106 \pm 0.757 mg/g (December). Differences between these three values are significant (d.f. = 2, F = 31.598, P = 0.000). These variations should be taken into account when *Tillandsia recurvata* is used as a bioindicator. Moreover, it is highly recommended to take samples always during the same month. Changes in sulfur content may be due to changes in sulfur atmospheric concentration or to changes in assimilation rates. In

some cases, exposure to pollutant gases, particularly SO₂, cause stomatal closure, which protects the leaf from further entry of the pollutant but also curtails photosynthesis (Taiz & Zeiger 1991). Reduction of photosynthesis could affect plant growth and development, as found by Stiles & Martin (1996) in *Tillandsia utriculata*. Under drought stress, this plant had substantially lower nocturnal CO₂ uptake and, after two months of desiccation, net CO₂ exchange was nearly zero, although substantial nocturnal increases in malic acid concentrations were still measurable. Thus, the proportion of CO₂ recycled internally via CAM increased dramatically. This protective mechanism could be used by *Tillandsia recurvata* to limit SO₂ uptake in sites with high SO₂ pollution to avoid tissue damage. Hence, this could explain results by Strehl & Arndt (1989), who found that in *Tillandsia recurvata* plants exposed to a SO₂ concentration of 380-

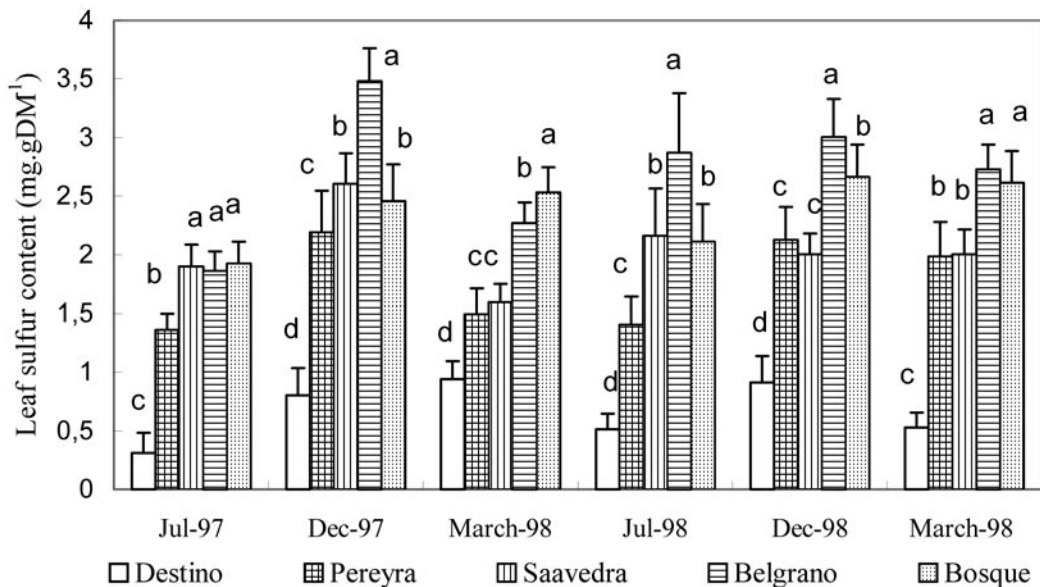


Figure 4. Mean (+ SD) leaf sulfur content in samples of *Tillandsia recurvata* from the five sampling sites in Buenos Aires Province at six sampling dates. For each sampling date, means with the same letter are not significantly different ($P < 0.05$).

Figura 4. Concentración promedio (+ DE) de azufre en tejidos de *Tillandsia recurvata* provenientes de los cinco sitios de muestreo de la provincia de Buenos Aires en seis fechas de muestreo. Para cada fecha de muestreo, los promedios señalados con la misma letra no difieren significativamente ($P < 0.05$).

480 mg/m³, tissue sulfur concentration increased only during the first days of SO₂ fumigation, remaining constant thereafter (and therefore, no internal or external alterations that could indicate SO₂ sensitivity were seen). Relationships between atmospheric and plant sulfur concentration were also found by Pyatt et al. (1999) in *Tillandsia usneoides*, a species that effectively accumulates sulfur and heavy metals from the atmosphere. As vehicular traffic density is one of the causes of sulfur contamination, in sites near important avenues (e.g., Belgrano) a higher sulfur content in *Tillandsia recurvata* tissues was found (Figure 4). The influence of traffic density on atmospheric SO₂ pollution is demonstrated by the few available data that show a mean of 5.113 ± 3.714 ppb SO₂ for three years (1977–1980) near Belgrano, and 4.335 ± 9.888 ppb in a quieter area near Saavedra, although the highest SO₂ concentration in the air was 7.496 ± 4.988 ppb near petro-chemical industries, close to Bosque (Municipalidad de La Plata 1981). Levin & Pignata (1995) and Carreras et al. (1998) found significant differences in tissue sulfur content in lichens transplanted to a point with high

traffic level, with the lowest values at the control zone with low traffic. Data taken during 2001 at three points of La Plata city indicate that traffic emissions seem to be more stable sources of SO₂, while factory emissions generate more variability in daily SO₂ concentration (Figure 5). It can be observed that the point called Plaza Moreno shows less variation than the other two points (i.e., 1 y 60 and Bosque). Plaza Moreno has heavy traffic and is distant from the factories, 1 y 60 has heavy traffic and is closer to the factories, while Bosque, one of our sampling sites, is close to the oil refineries and has less traffic. The variability found by direct methods could be buffered by using plants that accumulate sulfur over a long time. Plant tissue analysis gives a mean pollutant concentration probably related with the mean air pollution, but peak concentrations, which are important for human health, are not detected with this kind of analysis.

Based on these results, we conclude that *Tillandsia recurvata* accumulates sulfur from the air and tolerates the local level of air contamination without showing morphological damage, and is therefore

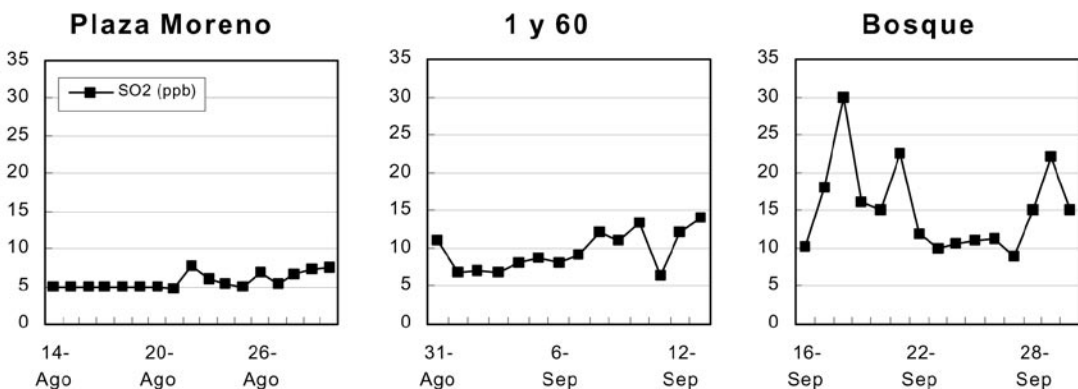


Figure 5. Air sulfur atmospheric concentration (ppb) in three sites of La Plata city, in August and September 2001. Data provided by the Instituto de Medio Ambiente, Municipalidad de La Plata.

Figura 5. Concentración de SO₂ atmosférico (ppb) en tres sitios de la ciudad de La Plata, durante agosto y septiembre de 2001. Datos provistos por el Instituto de Medio Ambiente de la Municipalidad de la Ciudad de La Plata.

suitable as a bioindicator of sulfur contamination. Further research is necessary to establish if it can be used for quantitative or semi-quantitative estimations, depending on whether a close correlation between tissue content and atmospheric SO₂ measured by a direct method can be established.

ACKNOWLEDGEMENTS

We wish to thank the owners and personnel of Estancia El Destino for allowing us access to their premises, to Ing. Agr. D. Giménez for helping with sulfur determinations, and to Dr. J. J. Guiamet for his valuable comments. During 1997-1999 Corina Graciano was supported by a CICPBA fellowship.

REFERENCES

- AKHATAR, MS; EE GOLDSCHMIDT; I JOHN; S RODONI; P MATILE & D GRIERSON. 1999. Altered patterns of senescence and ripening in *gf*, a stay-green mutant of tomato (*Lycopersicon esculentum* Mill.). *J. Exp. Bot.* **50**:1115-1122.
- ALQUINI, Y; AC CUSATIS & A SCHULTZ. 1996. Structural organization of the leaf of *Sechium edule* Sw (Cucurbitaceae) at two environmental conditions. *Arq. Biol. Tecnol.* **39**:651-656.
- BENNETT, JP & CM WETMORE. 1999. Changes in element contents of selected lichens over 11 years in Northern Minnesota, USA. *Environ. Exp. Bot.* **41**:75-82.
- BENZING, DH. 1973. The monocotyledons: their evolution and comparative biology. I. Mineral nutrition and related phenomena in Bromeliaceae and Orchidaceae. *Q. Rev. Biol.* **48**:277-290.
- BENZING, DH; J ARDITTI; LP NYMAN; PJ TEMPLE & JP BENNETT. 1992. Effects of ozone and sulfur dioxide on four epiphytic bromeliads. *Environ. Exp. Bot.* **32**:25-32.
- BENZING, DH & EA DAVIDSON. 1979. Oligotrophic *Tillandsia circinnata* Schlecht (Bromeliaceae): an assessment of its patterns of mineral allocation and reproduction. *Am. J. Bot.* **66**:386-397.
- BENZING, DH & A RENFROW. 1974. The mineral nutrition of Bromeliaceae. *Bot. Gaz.* **135**:281-288.
- BLANCHAR, RW; G REHM & AC CALDWELL. 1965. Sulfur in plant materials by digestion with nitric and perchloric acid. *Soil Sci. Soc. Am. Pro.* **29**:71-73.
- CALDIZ, DO; J BELTRANO; LV FERNÁNDEZ & I ANDÍA. 1993. Survey of *Tillandsia recurvata* L. Preference, abundance and its significance for natural forests. *Forest Ecol. Manag.* **57**: 161-168.
- CANAS, MS; HA CARRERAS; L ORELLANA & ML PIGNATA. 1997. Correlation between environmental conditions and foliar chemical parameters in *Ligustrum lucidum* Ait exposed to urban air pollutants. *J. Environ. Manage.* **49**:167-181.
- CARRERAS, HA; GL GUIDINO & ML PIGNATA. 1998. Comparative biomonitoring of atmospheric quality in five zones of Córdoba city (Argentina) employing the transplanted lichen *Usnea* sp. *Environ. Pollut.* **103**:317-325.
- CLAVER, FK; JR ALANIZ & DO CALDIZ. 1983. *Tillandsia* spp. Epiphytic weeds of trees and bushes. *Forest Ecol. Manag.* **6**:367-372.
- D'AMBROGIO DE ARGÜESO, A. 1986. *Manual de técnicas en histología vegetal*. Ed. Hemisferio Sur. Buenos Aires.
- DEL TORO, VI; C GIMENO; A CALATAYUD & E BARRENO. 1999. Effects of SO₂ fumigations on photosynthetic CO₂ gas exchange, chlorophyll a fluorescence emission and antioxidant enzymes in the lichen *Evernia prunastri* and *Ramalina farinacea*. *Physiol. Plantarum* **105**:648-654.
- FREITAS, MC; MA REIS; LC ALVES & HT WOLTERBEEK. 1999. Distribution in Portugal of some pollutants in the lichen *Parmelia sulcata*. *Environ. Pollut.* **106**:229-235.
- GARCÍA, AL & L GALINDO. 1991. Chlorophyllase activity as biochemical indicator of Mn and Fe deficiencies in *Citrus*. *Photosynthetica* **25**: 351-357.
- GOLDISH, T. 1991. *Air quality*. Lewis Pub.
- GONZÁLEZ, CM & ML PIGNATA. 1994. The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulfur and heavy metals in a transplanted lichen. *Chemistry and Ecology* **9**:105-113.
- HOLOUBEK, I; P KORINEK; Z SEDA; E SCHNEIDEROVÁ; I HOLOUBKOVÁ; A PAČL; J TRISKA; P CUDLÍN & J CASLAVSKY. 2000. The use of mosses and needles to detect persistent organic pollutants at local and regional scales. *Environ. Pollut.* **109**:283-292.
- INSKEEP, WP & PR BLOOM. 1985. Extinction coefficients of chlorophyll a and b in N,N-

- dimethylformamide and 80% acetone. *Plant Physiol.* **77**:483-485.
- LENDZIAN, KJ & MH UNSWORTH. 1983. Ecophysiological effects of atmospheric pollutants. Pp. 465-502 in: OL Lange; PS Nobel; CB Osmond & H Ziegler (eds). *Encyclopaedia of plant physiology. New series. Volume 12D: Physiological plant ecology IV*. Springer Verlag, Berlin.
- LEVIN, AG & ML PIGNATA. 1995. *Ramalina ecklocnii* as a bioindicator of atmospheric pollution in Argentina. *Can. J. Botany* **73**: 1196-1202.
- MARTIN, CE; KW MCLEOD; CA EADES & AF PITZER. 1985. Morphological and physiological responses to irradiance in the CAM epiphyte *Tillandsia usneoides* L. (Bromeliaceae). *Bot. Gaz.* **146**:489-494.
- MOSP-BA. 2000. *Tránsito medio diario anual. Años 1997-1998-1999*. Sub Gerencia de Planificación Vial, Depto. Planeamiento y Programación, Dirección de Vialidad, Ministerio de Obras y Servicios Públicos de la Provincia de Buenos Aires. 22 pp.
- MUNICIPALIDAD DE LA PLATA. 1981. *Informe de progreso: control ambiental*. Municipalidad de La Plata, La Plata. 3 pp.
- OLSZYK, DM & DT TINGEY. 1985. Metabolic basis for injury to plants from combinations of O₃ and SO₂. *Plant Physiol.* **77**:935-939.
- OWCZAREK, M; M SPADONI; A DEMARCO & C DESIMONE. 1999. Lichens as indicators of air pollution in urban and rural sites of Rieti (Central Italy). *Fresen. Environ. Bull.* **8**:288-295.
- PYATT, FB; JP GRATTAN; D LACY; AJ PYATT & MRD SEAWARD. 1999. Comparative effectiveness of *Tillandsia usneoides* L. and *Pramoterma praesorediosum* (Nyl.) Hale as bio-indicators of atmospheric pollution in Louisiana (USA). *Water Air Soil Poll.* **111**: 317-326.
- STILES, KC & CE MARTIN. 1996. Effects of drought stress on CO₂ exchange and water relations in the CAM epiphyte *Tillandsia utriculata* (Bromeliaceae). *J. Plant Physiol.* **149**:721-728.
- STREHL, T & U ARNDT. 1989. Alteracoes apresentadas por *Tillandsia aeranthos* e *T. recurvata* (Bromeliaceae) expostas ao HF e SO₂. *Iheringia, Ser. Bot.* **39**:3-17.
- STREHL, T & EA LOBO. 1989. Analysis of the morphological characters of *Tillandsia aeranthos* (Loisel) L. B. Smith and *T. recurvata* (L.) L. (Bromeliaceae) as bioindicators of the urban pollution in Porto Alegre city, Southern Brazil. *Aquilo, Ser. Bot.* **27**:19-27.
- TAIZ, L & E ZEIGER. 1991. *Plant physiology*. Benjamin/Cummings Pub. Co. Redwood City.
- UNSWORTH, MH. 1981. Air pollution and plant productivity. Pp. 293-306 in: CB Johnson (ed). *Physiological processes limiting plant productivity*. Butterworths. London.
- UNSWORTH, MH; JJ COLLS & GE SANDERS. 1994. Air pollutants as constraints for crop yields. Pp. 467-486 in: KJ Boote; JM Bennett; TR Sinclair & GM Paulsen (eds). *Physiology and determination of crop yield*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. Madison.