Hoehnea 42(4): 749-757, 3 tab., 2 fig., 2015 http://dx.doi.org/10.1590/2236-8906-18/RAD/2015

Anomalous scales of *Tillandsia usneoides* (L.) L. (Bromeliaceae) exposed in the Metropolitan Region of Campinas, SP, Brazil as air pollution markers

Patrícia Giampaoli[1,2], Natalie do Valle Capelli[1], Armando Reis Tavares[1], Francine Faia Fernandes[1], Marisa Domingos[1] e Edenise Segala Alves[1]

Received: 4.05.2015; accepted: 2.10.2015

ABSTRACT - (Anomalous scales of *Tillandsia usneoides* (L.) L. (Bromeliaceae) exposed in the Metropolitan Region of Campinas, São Paulo State, Brazil, as air pollution markers). *Tillandsia usneoides* is an epiphytic bromeliad that has been used as a universal bioindicator. The species accumulates metals and presents foliar scale variations when exposed to air pollutants. This study aimed to use the variations in foliar scales as microscopic markers of pollutant effects in the Metropolitan Region of Campinas (MRC), São Paulo State, Brazil. *T. usneoides* plants were exposed for 12 weeks during dry and wet seasons, totaling four exposures, at five sites in the MRC. Samples were selected before each exposure for initial evaluation of the plants (T0). Leaf fragments were fixed in glutaraldehyde and total scale density and anomalous scale percentage were evaluated. Plants exposed in the MRC showed anomalies and changes in number of subperipheral cells of leaf scales. When compared to T0, *T. usneoides* presented higher total scale density and anomalous scale percentage at disturbed sites, thus the species can be used as a pollution bioindicator for MRC.
Keywords: bromeliad, air pollutants, trichomes, bioindicator

RESUMO - (Escamas anômalas em *Tillandsia usneoides* (L.) L. (Bromeliaceae) expostas na Região Metropolitana de Campinas, SP, Brasil, como marcadoras da poluição aérea). A bromélia epífita *Tillandsia usneoides* vem sendo utilizada como bioindicadora universal por acumular altas concentrações de metais e apresentar alterações nas escamas foliares quando expostas a ambientes poluídos. Neste estudo objetivou-se utilizar alterações morfológicas nas escamas foliares como marcadores dos efeitos de poluentes aéreos na Região Metropolitana de Campinas (RMC), SP, Brasil. *T. usneoides* foi exposta em cinco pontos da RMC durante quatro exposições de 12 semanas, abrangendo duas exposições na estação úmida e duas na seca. Antes de cada exposição, foram selecionadas amostras para avaliação de condições iniciais das plantas (T0). Fragmentos de folhas foram fixados em glutaraldeído e avaliados quanto a densidade total de escamas e porcentagem de escamas anômalas. As plantas expostas na RMC apresentaram anomalias e alteração no número de células subperiféricas das escamas foliares. As plantas expostas em PA apresentaram maior densidade total de escamas e em PC maior porcentagem de escamas anômalas, quando comparadas às plantas T0. *T. usneoides* apresentou respostas relacionadas aos estresses ambientais, com maior presença de escamas anômalas e maior densidade total de escamas em plantas expostas em regiões antropizadas, portanto a espécie pode ser utlizada como bioindicadora da poluição na RMC.
Palavras-chave: bromélia, poluentes atmosféricos, tricomas, bioindicador

Introduction

The Metropolitan Region of Campinas (MRC) is composed of 20 cities and accounts for approximately 15% of São Paulo State total population. The region economic growth initiated in the sixties with the implementation of large urban industrial complexes (Gutjahr 2004), as the city of Paulínia, which has a significant amount of mainly chemical and petrochemical industries (Tresmondi & Tomaz 2004). The MRC has been affected by high levels of primary air pollutants originated from road traffic and, is responsible for over 80% of CO, NOx and hydrocarbon emissions, and develops industrial activities that emit about 70% of the particulate matter present in the region (Ueda & Tomaz 2011).

Air pollution levels are determined by quantifying polluting substances in the air. The main air pollutants

1. Instituto de Botânica, Postal Code 68041, 0161-970 São Paulo, São Paulo State, Brazil
2. Corresponding author: patpaoli83@gmail.com

are carbon monoxide (CO), sulphur dioxide (SO_2), nitrogen oxides (NO_x), particulate matter (PM) and ozone (O_3) (CETESB 2013). Pollutants generated in the MRC and those carried by the south-southwest wind from the Metropolitan Region of São Paulo (MRSP) affect air quality in the region (Boian & Andrade 2012) and impact the existing vegetation of fragments of the seasonal semideciduous forest (Nascimento *et al.* 2006, Cielo-Filho & Santin 2002).

Many plant species have been identified as pollution monitors such as: *Tibouchina pulchra* Cogn. (Melastomataceae) in Cubatão industrial complex (Domingos *et al.* 1998; Domingos *et al*. 2000); *Psidium guajava* "Paluma" in urban regions as ozone bioindicator (Furlan *et al.* 2007, Sandre *et al.* 2014, Assis *et al.* 2015) and the sensitive cultivar of tobacco, *Nicotiana tabacum* "Bel W3", in Europe and Brazil as atmospheric ozone indicator (Klumpp *et al*. 2006; Sant'Anna *et al.* 2008; Esposito 2008, Pedroso & Alves 2015). Plants of the Bromeliaceae family have also been evaluated as biomonitor, especially *Aechmea blanchetiana* (Giampaoli *et al.* 2012), *Aechmea coelestis* (K. Koch) E. Morren, *Nidularium* cf. *inocentii* (Schult. f.) L.B. Smith, *Nidularium krisgreeniae* Leme and *Vriesea carinata* Wawra (Elias *et al.* 2008).

Studies have shown that structural markers allow evaluating the effects of pollutants in plants (Vollenweider *et al.* 2003). Changes in the stellate trichome morphology due to environmental agents have been described in the nickel-accumulating species *Alyssum corsicum* Rob. *ex* Gren. & Godr., when exposed to high concentrations of the metal (Babaoğlu Aydas *et al*. 2013). Trichomes of *Ligustrum japonicum* Thunb. and *Olea europaea* L. lost the original form when exposed to high concentrations of CO and SO_2 from automobile emissions (Abdallah & Mejnum 2013).

The genus *Tillandsia* is found throughout Latin America and has epiphytic habits, living independently of ground in trees or inert substrates and presenting absorbing trichomes on the entire leaf surface (Brighigna *et al*. 1997). Trichomes, also called scales (Benzing *et al.* 1992), protect the plant from UV radiation by reflecting most of the incoming light, absorb moisture from the air, retain dust and prevent the stomata from losing excessive water (Laboy-Nieves *et al.* 2010). Several studies have confirmed that *Tillandsia* genus plants are great bioindicators of gaseous pollutants (Benzing *et al*. 1992, Figueiredo *et al.* 2001, 2004, 2007) and are highly capable of accumulating many metals (Calasans & Malm 1997, Amado-Filho *et al.* 2002).

T. usneoides is an atmospheric epiphyte that depends exclusively on the atmosphere for survival (Brighigna *et al.* 1997). It extracts mineral nutrients and water from the rain and moisture in the air (Benzing *et al.* 1976), thus the species is considered a universal accumulator (Arndt & Schweizer 1991) and is used for metal biomonitoring. However, Alves *et al.* (2008) reported the presence of anomalies in *T. usneoides* scales exposed to polluted areas of São Paulo city, suggesting that anomalous scales are an alternative bioindicator parameter.

The study aimed to detect changes in the scales of *Tillandsia usneoides* exposed to disturbed environments, providing additional information about its use as air quality bioindicator, and generate information about the impact of air pollutants on the atmosphere of the Metropolitan Region of Campinas (MRC).

Material and methods

Study area and plant material

The MRC is located in an intertropical zone with predominantly humid subtropical climate, characterized by two distinct seasons: wet period with high temperatures and dry period with mild temperatures and less rainfall (Rolim *et al.* 2007). Between seasons, exist significant differences on the intensity and direction of the winds, which are predominantly from Southeast to Northwest (Domingos *et al.* 2015). The local climate is defined as prevalent Cfa (humid subtropical zone with dry winter and hot summer) according to Köppen classification (Alvarez *et al.* 2014) or B1rB'4a type according to Thornthwaite's classification, which considers topography and altitude as direct influence on climatic elements (Rolim *et al.* 2007).

Plants of *Tillandsia usneoides* (L.) L. were obtained from a commercial producer located in Cordeirópolis, an agricultural area in the region, and held for six months in a greenhouse at the Botanical Institute of São Paulo, for acclimatization before exposures started.

Five sites were selected in the 10-km radius from the industrial complex of Paulínia (figure 1): Campinas (CA) -22°49'22.65"S-47°06'17.38"W; Jaguariúna (JA) -22°43'3.78"S-47°01'50.71"W; Holambra (HO) -22°39'48.25"S-47°06'26.71"W, Paulínia (PA) -22°44'19.11"S-47°07'40.34"W; Paulínia Downtown

(PC) - 22°46'13.4"S-47°09'25.3"W. From June 2011 to April 2013, five samples of *T. usneoides*, weighing approximately 50 g (fresh matter), were exposed at each sites, in four exposure periods of 12 weeks each, including two wet and two dry periods, (Expo A - 06/22/2011 to 09/14/2011; Expo B - 12/13/2011 to 02/29/2012; Expo C - 05/23/2012 to 08/14/2012; Expo D - 11/06/2012 to 01/23/2013). The samples remained suspended in strands of nylon under a PVC supporter (1.60 alt. × 1.0 w: × 2.0 m lenght) with 50% shade protection and weekly irrigation with deionized water. The plants were replaced by new ones (acclimated) after the end of each exposure. At the beginning of each exposure, three samples were selected from the acclimated plants for the evaluation of the initial state (time zero-T0).

Concentrations of O_3, SO_2, NO_2 and PM_{10} and values of climatic variables were obtained from monitoring stations of CETESB (PC-Paulínia Center and PA-Paulínia) and Center of Meteorological and Climatic Research Applied to Agriculture (Cepagri, UNICAMP). At the other study sites and in the greenhouse, passive SO_2, NO_2, and O_3 (except in the greenhouse) samplers were used (Ogawa 2001, Machado *et al.* 2008, Allen *et al.* 2010), and evaluated weekly (table 1).

Preparation of anatomical slides

Entire fragments of *T. usneoides* were submerged in 2.5% glutaraldehyde and, after remaining 12 hours in vacuum desiccator, were refrigerated at approximately 5 °C for further anatomical study. The fragments were washed in phosphate buffer Sorensen 0.067 M, pH 7, and transferred to 70% alcohol. The third apical parts of the leaves were cut

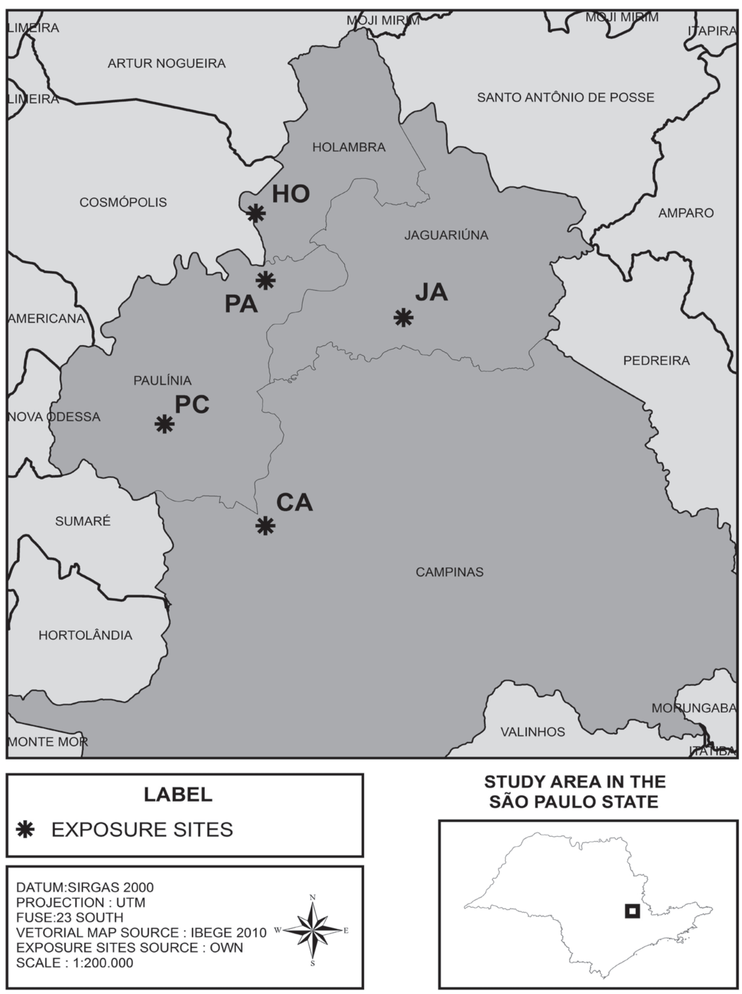

Figure 1. Map distribution of exposure sites of *Tillandsia usneoides* plants in Metropolitan Region of Campinas (MRC), São Paulo State, Brazil.

lengthwise freehand with steel blades and immersed in a maceration solution of hydrogen peroxide 30 vol and glacial acetic acid 1:1, remained in an oven at 60 °C for 12 hours and subsequently washed with distilled water. The fragments were stained with 0.05% toluidine blue (Feder & O'Brien 1968) and assembled in 50% glycerin. Three blades were produced per plant sample.

Images of the scales were captured by Olympus microscope (BX53) equipped with Olympus camera Q color 5 and analyzed with the aid of Image Pro Express 6.3 software. For each exposure site and exposure period, 15 fields were assessed per blade (scales mm^{-2}) and the total scale density and anomalous scale percentage (number of anomalous scales in relation to the total number of scales) were estimated in 1,080 fields.

Statistical analysis

The data were submitted to analysis of variance (ANOVA on ranks) and the averages were compared according to the Kruskal-Wallis method to establish differences between sites, and the Man-Whitman method was used to establish differences between dry and wet exposure periods. For multiple comparisons between the periods and the sites, the Dunm test ($p < 0.05$) was applied. The Spearman correlation test ($p < 0.05$) determined possible associations between pollutant concentration variations and the analyzed parameters (total density of scales and percentage of anomalous scales).

Results and Discussion

The environmental data obtained in PC, when compared with those provided by other monitoring stations, showed little variation in the concentrations of pollutants and climatic parameters, with the exception of precipitation. Therefore, the monitoring station at PC appropriately characterizes the study region and can be used as a reference to the correlation analysis (table 1). Studies conducted in the region (Tresmondi & Tomaz 2004, Boian & Andrade 2012, Bulbovas *et al.* 2015) also showed little spatial variation in air pollution levels in the MRC, confirming the representativeness of the monitoring station in Paulínia Center for the study area.

The surface of *Tillandsia usneoides* leaves is covered with scales that, in frontal view, consist of a central disc formed by four cells surrounded by two sets of cells: the first consists of eight slender cells, called pericentral cells, and the second has sixteen cells and is called subperipheral. Externally, there are empty, elongated, thin-walled and radially arranged peripheral cells, forming a shield that is called wing (figure 2a-f). The scale wings (figure 2a) exhibit a confluent layering, forming a space between them and the epidermal surface (Benzing *et al.* 1976, Souza & Neves 1996, Proença & Sajo 2007).

The anomalies observed in the scales of plants exposed in the MRC were: change in the number of cells in the subperipheral layer, less (figure 2c) or more (figure 2d) than 16 cells and addition of cells between the subperipheral layer of the disc and the wing (figure 2e). Changes were also observed in cell shapes in different layers, resulting in fully deformed scales (figure 2f). According to Cardoso-Gustavson *et al.* (2015), changes in the number of cells of *T. usneoides* scales exposed to pollutants are related to occurrence of mutagenic processes. The authors found that, during the development of scales, endoreduplication cycles could occur in the nucleus without cell division, resulting in reduced number of subperipheral cells with large nucleus.

Plants exposed in PC and PA showed a significant increase in the density of total scales in the dry period and showed no significant variation in the wet period, when compared to T0 plants. The highest percentage of anomalous scales was observed in plants exposed at PC in the wet period, compared with the T0 plants and did not vary significantly in the dry period.

Total scale density and anomalous scale percentage were significantly different comparing the dry and wet exposure periods at each site. Plants exposed in PA presented higher scale density and anomalous scale percentage in the dry period. At the other sites, plants exposed in the wet period had higher scale density and anomalous scale percentage than those exposed in the dry period. The positive association of the percentage of anomalous scales with moisture is related to greater interaction of scales and pollutants in the presence of water, which facilitates diffusion of pollutants, increasing acidity in intercellular spaces (Larcher 2000) and that is intensified in *T. usneoides* since the species is an atmospheric bromeliad (Benzing *et al.* 1976).

Alves *et al.* (2008) observed a significant increase in the total density of scales in *T. usneoides* exposed at sites in São Paulo city after 24 weeks, compared with the control material. The studied sites were a park with high concentrations of O_3 and an area of intense traffic, with high concentrations of SO_2. In plants of *Pueraria lobata* Will, Sharma *et al.* (1980)

Table 1. Environmental data of Metropolitan Region of Campinas (MRC). Average data from CETESB monitoring stations (Paulínia Downtown and Campinas) combined with values of local passive samplers. EA and EC: dry periods; EB and ED: wet periods; PC (data from Paulínia-Downtown CETESB monitoring station).

Environmental Data	PM_{10}	NO_2	O_3	SO_2	RAD	RH	TEMP.	PRECIP.
Exposition	($\mu g\ m^{-3}$)				$W\ m^{-2}\ s^{-1}$	(%)	(°C)	(mm)
				Metropolitan Region of Campinas				
EA	58.82	31.12	49.90	7.52	153.46	66.59	19.57	69.83
EB	28.72	18.36	72.44	3.91	281.25	73.17	25.49	507.02
EC	39.17	33.09	49.09	6.58	128.17	79.32	26.93	187.49
ED	26.25	22.28	36.69	5.76	171.31	91.23	23.86	460.63
				Paulínia - Downtown				
EA	52.30	31.21	49.37	7.34	180.40	65.40	19.43	35.9
EB	34.53	18.68	76.79	3.35	269.22	68.79	24.32	226.8
EC	37.25	36.04	59.54	6.58	-	76.51	31.86	68.3
ED	24.39	18.43	58.54	6.97	-	82.02	24.22	155.2

also observed higher frequency of trichomes due to increased amount of pollutants. *Quercus ilex* L. exposed to high CO_2 concentrations presented higher trichome density (Paoletti *et al.* 2007); although some authors do not consider CO_2 as a pollutant (Larcher 2000).

JA, HO and CA are under greater agricultural influence, while PA and PC are highly influenced by urban and industrial pollution sources. Higher exposure to pollutants from sources such as SO_2 and PM_{10} can justify the increase of total scale density at the sites, as well as increased anomalous scales in PC.

In this study, the total scale density and anomalous scale percentage were larger than those observed by Alves *et al.* (2008), and the plants exposed in plants at PC presented twice as many anomalous scales as the authors observed in the same species.

The statistical analysis showed higher percentage of anomalous scales in plants exposed at Paulínia (PC); therefore, with the aid of the Spearman correlation test, we established the relationship between the anomalous scale percentage, total scale density of plants exposed in Paulínia and environmental parameters (table 2). The percentage of anomalous scales in plants exposed in PC correlated negatively with SO_2 and PM_{10} concentrations and positively with relative humidity (RH), whereas, total scale density showed no significant correlation with any of the environmental parameters. These results showed that the increasing concentrations of PM_{10} and SO_2 in the region did not influence anomalies of the scales. Cardoso-Gustavson *et al.* (2015) did not observe significant correlation between the formation of anomalous scales in plants of *T. usneoides* and the presence of trace metals, such as Ba, Fe and Cu, normally present in PM_{10}, suggesting that anomalies would be related to other air pollutants from vehicular source, as NO_2 and SO_2.

The plants exposed in Paulínia presented the highest percentages of anomalous scales (table 3), differing significantly from T0, but showed no significant correlation with pollutants from urban sources. However, Alves *et al.* (2008) observed relationship between anomalous scale increase in *T. usneoides* plants and the presence of pollutants in places with high levels of O_3 in the MRSP, when compared to the rural environment. O_3 and SO_2 promote little visible damage in the epiphytic bromeliads with CAM photosynthesis, when compared to C3 plants, due to the presence of thick cell walls and the crown of dead cells (wings of scale) which covers the leaf surface, protects the stomata, promotes greater tolerance to pollutants and functions as primary physical barrier (Benzing *et al.* 1992).

T. usneoides is a species that accumulates metals in particulate matter, (Calasans & Malm 1997, Figueiredo *et al.* 2007), due to its adsorption on the scales (Amado-Filho *et al.* 2002), which makes it more tolerant to PM_{10}. This assumption has been confirmed by the results obtained in this study, considering that there was negative correlation between anomalous scales and PM_{10}.

The largest emissions of pollutants in the MRC, especially particulate matter, NOx, CO and SO_2, come from industrial and automotive traffic sources (Ueda & Tomaz 2011), which is in accordance with the largest percentages of anomalies in PA and PC (table 3), the

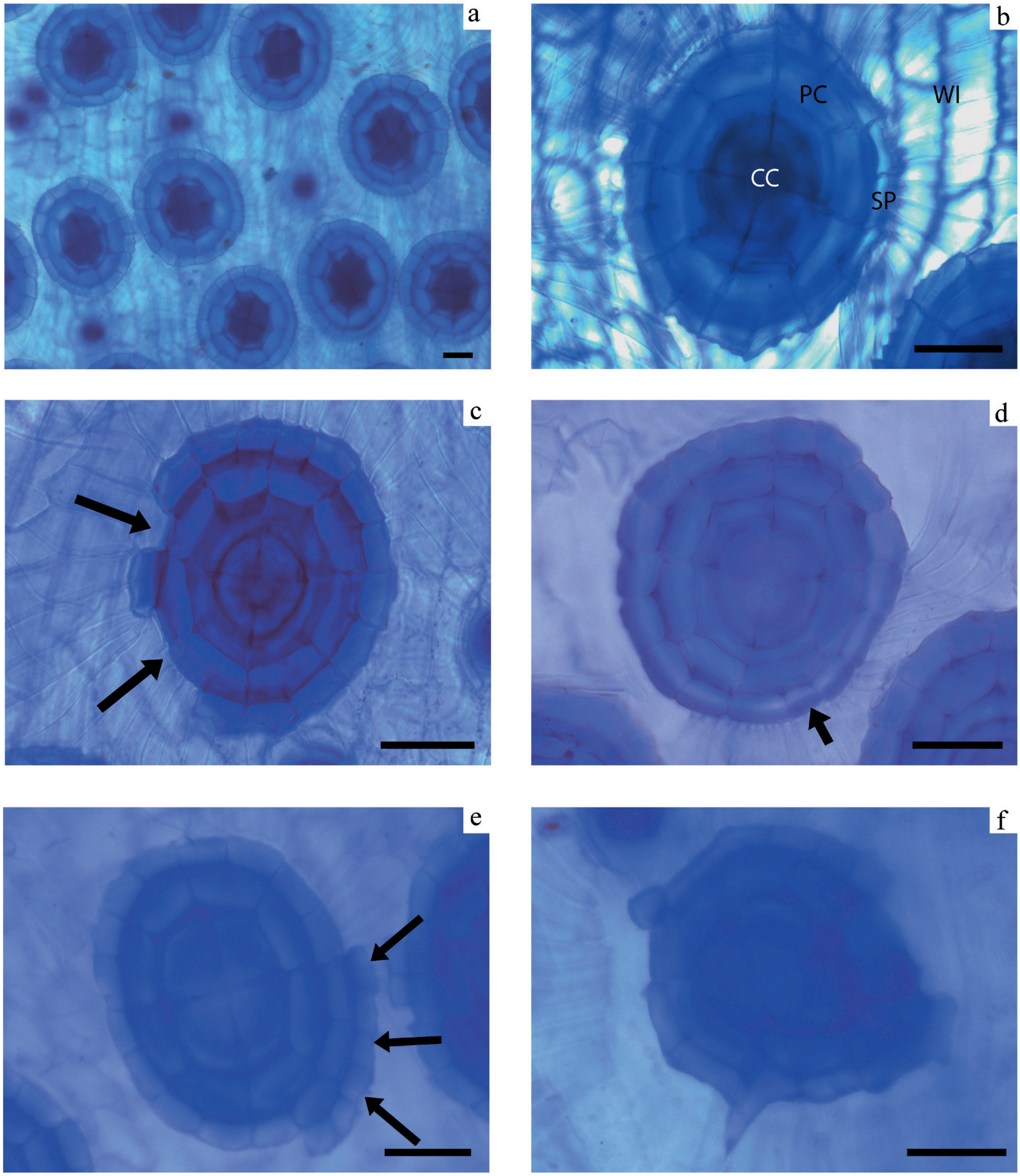

Figure 2. Paradermic surface of *Tillandsia usneoides* leaf, highlighting scales with normal pattern (a-b) and anomalous scales (c-f). a. Epidermis with scales. b. Highlighting scales four central cells (CC) forming a central disc, eight pericentral cells (PC), 16 subperipheral cells (SP) and peripheral layer forming the wings (WI). c. Reduction of three cells in the subperipheral disk (arrows); d. Addition of one cell in subperipheral disk (arrow); e. Addition of six cells between the subperipheral layer and the wing; f. Shape and number of cells of scale modified in all layers, including the central disk. Bars = 20 µm.

Table 2. Correlation among pollutant concentrations and environmental data provided by CETESB station-Paulínia Downtown and anomalous scales in leaves of *Tillandsia usneoides* at PC (Paulínia Downtown).

		MP_{10} (µg m^{-3})	NO_2 (µg m^{-3})	O_3 (µg m^{-3})	SO_2 (µg m^{-3})	Temp. (°C)	Precip. (mm)	R.U. (%)
Scales density	R^2	0.0197	-0.11	-0.233	0.233	-0.245	-0.121	-0.0313
	p	0.881	0.402	0.0728	0.0728	0.0593	0.357	0.812
Percentage of anomalous scales	R^2	-0.585	-0.413	-0.0552	0.0552	0.0942	0.153	0.734
	p	< 0.01	< 0.01	0.708	0.708	0.522	0.298	< 0.01

R^2 – correlation coeficient; *p* – statistical significance

Table 3. Density and average percentage of anomalous scales in *Tillandsia usneoides* at different locations and exposure periods (wet or dry). T0: initial time, SP: São Paulo, CA: Campinas, JA: Jaguariúna, HO: Holambra, PA: Paulínia, PC: Paulínia Downtown.

		Density (scales mm^{-2})			Percentage (%)		
Periods	Sites	Mean	Error	Dunn's Test	Mean	Error	Dunn's Test
Dry (EA + EC)	T0	17	± 0.8	Bb	35.5	± 4.6	Aa
	CA	18	± 0.8	Bb	44.0	± 5.4	Aa
	JA	19	± 0.6	Bb	43.0	± 4.0	Aa
	HO	19	± 0.6	Ba	28.1	± 2.6	Ab
	PA	22	± 0.8	Aa	54.3	± 4.6	Aa
	PC	22	± 0.9	Aa	37.9	± 3.6	Ab
Wet (EB + ED)	T0	22	± 0.9	Aa	29.7	± 2.2	Ba
	CA	23	± 0.8	Aa	33.8	± 3.0	ABa
	JA	22	± 0.8	Aa	30.2	± 3.0	ABa
	HO	19	± 0.9	Aa	43.4	± 3.6	ABa
	PA	19	± 0.7	Ab	34.9	± 2.9	ABb
	PC	22	± 0.6	Aa	56.1	± 5.3	Aa

Capital letters compare locations and lowercase exposure periods (dry or wet)

closest points to the industrial complex region. In the study of Alves *et al.* (2008), *T. usneoides* plants were exposed continuously for 8, 16 and 24 weeks; changes in the scales became visible after 8 weeks of exposure. In the present study, the plants were exposed for 12 weeks and no direct relationship between O_3 concentrations and percentage of anomalies was observed. The studied region encompasses different sources of air pollutants and climatic regime comparing with the sites studied by Alves *et al.* (2008) in the city of São Paulo. The difference among dry and wet periods is more pronounced in the MRC than in São Paulo (Alvarez *et al.* 2014), suggesting that the variations in density of the scales and occurrence of anomalies can be induced not only by pollutants, but by weather conditions as well.

Studies carried out with *T. usneoides* in the same cities of the MRC showed low or no impact of air pollutants on physiological (as chlorophyll contents and plant mass loss) or biochemical (antioxidant) activities (Giampaoli *et al.* unpublished data), indicating that the tolerance of the species is related to the primary barrier represented by trichomes, as observed in the density variation and/or structure changes in other species (Moura 2013).

T. usneoides responded to environmental stress with high presence of anomalous scales and total scale density in plants exposed in disturbed regions, mainly at PC and PA.

Acknowledgment

The authors thank the CNPq for scholarships granted to P. Giampaoli (DR), N.V. Capelli (PIBIC), A.R. Tavares, M. Domingos and E.S. Alves (PQ); and to the geographer Marco Aurélio Formentin Serra by provide the map in figure 1.

Literature cited

Abdallah, Y.N. & Mejnun, G.E. 2013. Change of the morpho-anatomical structure of leaves of *Ligustrum japonicum* and *Olea europea* caused by heavy metal pollution. Caspian Journal of Applied Sciences Research 2: 59-67.

Allen, A.G., Cardoso, A.A., Wiatr, A.G., Machado, C.M.D., Paterlini, W.C. & Baker, J. 2010. Influence of intensive agriculture on dry deposition of aerosol nutrients. Journal of Brazilian Chemistry Society 21: 87-97.

Alvares, C.A., Stapez, J.L., Sentelhas, P.C., Gonçalves J.L.M. & Sparovek G. 2014. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22: 711-728.

Alves, E.S., Moura, B.B. & Domingos, M. 2008. Structural analysis of *Tillandsia usneoides* L. exposed to air pollutants in São Paulo City-Brazil. Water, Air and, Soil Pollution 189: 61-68.

Amado-Filho, G.M., Andrade, L.R., Farina, M. & Malm, O. 2002. Hg localization in *Tillandsia usneoides* L. (Bromeliaceae), an atmospheric biomonitor. Atmospheric Environment 36: 881-887.

Arndt, U. & Schweizer, B. 1991. The use of bioindicators for environmental monitoring in tropical and subtropical countries. . *In*: H. Ellenberg, U. Arndt, R. Bretthauer, B. Ruthsatz & L. Steubing (eds.). Biological Monitoring Signals from the Environment. Vieweg, Berlin, pp. 199-259.

Assis, P.I., Alonso, R., Meirelles, S.T. & Moraes, R.M. 2015. DO3SE model applicability and O_3 flux performance compared to AOT40 for an O_3-sensitive tropical tree species (*Psidium guajava* L. 'Paluma'). Environmental Science and Pollution Research 22: 10873-10881.

Babaoğlu Aydas, S.S., Açik, L., Leduc, D., Adigüzel, N., Ellialtioğlu, S.S, Suludere, Z. & Kadioğlu, Y.K. 2013. Localization and distribution of nickel and other elements in *in-vitro* grown *Alyssum corsicum* exhibiting morphological changes in trichomes: initial insights into molecular mechanisms of nickel hyperaccumulation. Turkish Journal of Botany 37: 1115-1124.

Benzing, D.H., Arditti, J., Nyman, L.P. & Temple, P.J. 1992. Effects of ozone and sulfur dioxide on four epiphytic bromeliads. Environmental Experimental Botany 32: 25-32.

Benzing, D.H., Henderson, K., Kessel, B. & Sulak, S. 1976. The absorptive capacities of bromeliad thichomes. American Journal of Botany 63: 330-348.

Boian, C. & Andrade, M.F. 2012. Characterization of ozone transport among Metropolitan Regions. Revista Brasileira de Meteorologia 27: 229-242.

Brighigna, L., Ravanelli, M., Minelli, A. & Ercoli, L. 1997. The use of an epiphyte (*Tillandsia capuy-medusae* Morren) as bioindicator of air pollution in Costa Rica. The Science of the Total Environment 198: 175-180.

Bulbovas, P., Camargo, C.Z.S. & Domingos, M. 2015. Ryegrass cv. Lema and guava cv. Paluma biomonitoring suitability for estimating nutritional contamination risks under seasonal climate in Southeastern Brazil. Ecotoxicology and Environmental Safety 118: 149-157.

Calasans, C.F. & Malm, O. 1997. Elemental mercury contamination survey in a chlor-alkali plant by the use of transplanted Spanish moss *Tillandsia usneoides* (L). The Science of the Total Environment 208: 165-177.

Cardoso-Gustavson, P., Fernandes, F.F., Alves, E.S., Victorio, M.P., Moura, B.B., Domingos, M., Rodrigues, C.A., Ribeiro, A.P., Nievola, C.C. & Figueiredo, A.M.G. 2015. *Tillandsia usneoides*: a successful alternative for biomonitoring changes in air quality due to a new highway in São Paulo, Brazil. Environmental Science and Pollution Research DOI 10.1007/s11356-015-5449-8.

CETESB - Companhia de Tecnologia de Saneamento Ambiental. 2013. Relatório da qualidade do ar no estado de São Paulo. Série Relatório. Secretaria do Meio Ambiente, São Paulo.

Cielo-Filho R.C. & Santin D.A. 2002. Estudo florístico e fitossociológico de um fragmento florestal urbano - Bosque dos Alemães, Campinas, SP. Revista Brasileira de Botânica 25: 291-301.

Domingos, M., Bulbovas, P., Camargo, C.Z.S, Aguiar-Silva, C., Brandão, S.E., Dafré- Martinelli, Dia, A.P., Engela, M.R.G.S, Gagliano, J., Moura, B.B., Alves. E.S., Rinaldi, M.C.S., Gomes, E.P.C., Furlan, C.M. & Figueiredo, A.M.G. 2015. Searching for native tree species and respective potential biomarkers for future assessment of pollution effects on the higly diverse Atlantic Forest in SE-Brazil. Environmental Pollution 202: 85-95.

Domingos, M., Klumpp, A. & Klumpp, G. 1998. Air pollution impact on the Atlantic Forest at the Cubatão region, Brazil. Ciência e Cultura 50: 215-234.

Domingos, M., Lopes, M.I.S. & Struffaldi-De Vuono, Y. 2000. Nutrient cycling disturbance in Atlantic Forest sites affected by air pollution coming from the industrial complex of Cubatão, Southeast Brazil. Brazilian Journal of Botany 23: 77-85.

Elias, C., Fernandes, E.A.N., França, E.J., Bacchi, M.A. & Tagliaferro, F.S. 2008 Native bromeliads as biomonitors of airborne chemical elements in a Brazilian Restinga forest. Journal of Radioanalytical and Nuclear Chemistry 278: 423-427.

Esposito, M.P. 2008. Estudo da relação entre necroses foliares indicadoras de ozônio atmosférico e defesas antioxidativas em folhas de *Nicotiana tabacum* "Bel W3" Dissertação de Mestrado - Instituto de Botânica, São Paulo.

Feder, N. & O'Brien, T.P. 1968. Plant microtechnique: some principles and new methods. American Journal of Botany 55: 123-142.

Figueiredo, A.M.G., Alcalá, A.L., Ticianelli, R.B., Domingos, M. & Saiki. M. 2004. The use of *Tillandsia usneoides* L. as bioindicator of air pollution in São Paulo. Brazilian Journal of Radioanalitical Nuclear Chemistry 259: 59-63.

Figueiredo, A.M.G., Nogueira C.A., Saiki, M. & Domingos, M. 2007. Assessment of atmospheric metallic pollution in the metropolitan region of São Paulo, Brazil, employing *Tillandsia usneoides* L. as biomonitor. Environmental Pollution 145: 279-292.

Figueiredo, A.M.G., Saiki. M., Ticianelli, R.B., Domingos, M., Alves, E.S. & Market, B. 2001. Determination of elements in *Tillandsia usneoides* by neutron activation analysis for environmental biomonitoring. Brazilian Journal of Radioanalytical and Nuclear Chemistry 249: 391-395.

Furlan, C.M., Moraes, R.M., Bulbovas, P., Domingos, M., Salatino, A. & Sanz, M.J. 2007. *Psidium guajava* 'Paluma' (the guava plant) as a new bio-indicator of ozone in the tropics. Environmental Pollution 147: 691-695.

Giampaoli, P., Tresmondi, F., Lima, G.P.P., Kanashiro, S., Alves, E.S., Domingos, M. & Tavares, A.R. 2012. Analysis of tolerance to copper and zinc in *Aechmea blanchetiana* grown *in vitro*. Biologia Plantarum 56: 83-88.

Gutjahr, M. 2004. Avaliação da qualidade do ar em Paulínia-SP. Boletim Goiano de Geografia 24: 55-68.

Klumpp, A., Ansel, W., Klumpp, G., Vergne, P., Sifakis, N., Sanz, M.J., Rasmussen, S., Ro-Poulsen, H., Ribas, A, Peñuelas, J., Kambezidis, H., He, S., Garrec, J.P. & Calatayud, V. 2006. Ozone pollution and ozone biomonitoring in European cities Part II. Ozone-induced plant injury and its relationship with descriptors of ozone pollution. Atmospheric Environment 40: 7437-7448.

Laboy-Nieves, E.N, Goosen M.F.A. & Emmanuel, E. 2010 Environmental and Human Health–Risk Management in Developing Countries. Taylor & Francis Group, London.

Larcher, W. 2000. Ecofisiologia Vegetal. RiMa, São Carlos.

Machado, C.M.D., Cardoso, A.A. & Allen, A.G. 2008. Atmospheric emission of reactive nitrogen during biofuel ethanol production. Environmental. Science Technology 42: 381-385.

Moura, B.B. 2013. Análises estruturais e ultraestruturais em folhas de espécies nativas sob influência de poluentes aéreos. Tese de Doutorado, Instituto de Botânica, São Paulo.

Nascimento, L.F.C., Pereira, L.A.A., Braga, A.L.F., Módolo, M.C.C. & Carvalho Jr, J.A. 2006. Efeitos da poluição atmosférica na saúde infantil em São José dos Campos, SP. Revista de Saúde Pública 40: 77-82.

Ogawa. 2001 Protocol for ozone measurement using the ozone passive sampler badge. Disponível em http://ogawausa.com/wp-content/uploads/2014/04/proozone.pdf (acesso em 12-II-2011).

Paoletti, E., Seufert, G., Rocca, G.D. & Thomsen, H. 2007. Photosynthetic responses to elevated CO_2 and O_3 in *Quercus ilex* leaves at a natural CO_2 spring. Environmental Pollution 147: 516-524.

Pedroso, A.N.V. & Alves, E.S. 2015. Temporal dynamics of the cellular events in tobacco leaves exposed in São Paulo, Brazil, indicate oxidative stress by ozone. Environmental Science Pollution Research 22: 6535-6545.

Proença, S.L. & Sajo, M.D.G. 2007. Anatomia foliar de bromélias ocorrentes em áreas de cerrado do Estado de São Paulo, Brasil. Acta Botanica Brasilica 21: 657-673.

Rolim, G.S., Camargo, M.B.P., Lania, D.G. & Moraes, J.F.L. 2007. Classificação climática de Köppen e de Thornthwaite e sua aplicabilidade na determinação de zonas climáticas para o estado de São Paulo. Bragantia 66: 711-720.

Sandre, A.A., Pina, J.M., Moraes, R.M. & Furlan, C.M. 2014. Anthocyanins and tannins: is the urban air pollution an elicitor factor? Brazilian Journal of Botany 37: 9-18.

Sant'Anna, S.M., Esposito, M.P., Domingos, M. & Souza, S.R. 2008. Suitability of *Nicotiana tabacum* 'Bel W3' for biomonitoring ozone in São Paulo, Brazil. Environmental Pollution 151: 389-394.

Sharma, G.K., Chandler, C. & Salemi, L. 1980. Environmental pollution and leaf cuticular variation in Kudzu (*Pueraria lobata* Willd.). Annals of Botany 45: 77-80.

Souza, R.C.O.S. & Neves, L.J. 1996. Anatomia foliar de quatro espécies de *Tillandsia*. Bromélia 3: 28-39.

Tresmondi, A.C.C.D.L. & Tomaz, E. 2004. Air pollution and the influence of sources on Paulinia (Brazil) and surroundings. International Journal of Environment and Pollution 22: 490-506.

Ueda, A.C. & Tomaz, E. 2011. Inventário de emissão de fontes veiculares da Região Metropolitana de Campinas, São Paulo. Química Nova 34: 1496-1500.

Vollenweider, P., Ottiger, M. & Günthardt-Goerg, M.S. 2003. Validation of leaf ozone symptoms in natural vegetation using microscopical methods. Environmental Pollution 124: 101-118.