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Does an urban environment affect leaf structure of *Eugenia uniflora* L. (Myrtaceae)?

Laís de Almeida Bezerra[1], Cátia Henriques Callado[1, 2] and Maura Da Cunha[3*]

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ABSTRACT

External factors can interfere with the structure and biological activity of plants. Nevertheless, the susceptibility of plants to specific environmental conditions varies, which raises many questions about the behavior of medicinal plants when grown in urban areas. This study aims to detect possible changes induced by exposure of *Eugenia uniflora* L. to an urban environment, with emphasis on variation in external and internal leaf structure and differences in the production of its main metabolites. We compared leaves of *E. uniflora* cultivated in forest and urban sites and analyzed them for structural plasticity and characteristics indicative of stress in the urban environment. The leaves of the urban site revealed necrosis and reddish spots, higher stomatal density, smaller stomata and more numerous crystals and secretory glands, as well as evident storage of starch, lipids, and mucilage. The presence of numerous crystals had a high plasticity index and great potential as anatomical marker for evaluating the effects of the urban environment. Visual symptoms and anatomical changes were efficient at diagnosing stress in *E. uniflora*, while characteristics of the urban site, such as temperature, heat and pollution, are thought to be responsible for the observed variation and may influence your medicinal characteristic.

Keywords: atmospheric pollution, Brazilian cherry, environmental change, histochemistry, Pitangueira, urban environment

Introduction

Urban environments alter the soil and natural vegetation cover, resulting in significant impacts on local climate such as the formation of heat islands. This occurs because surfaces of urban areas are characterized by high impermeability and thermal properties favorable to energy storage and heat release. As a consequence, this phenomenon influences air quality and environmental and human health (Cosgrove & Berkelhammer 2018).

The city of Rio de Janeiro, located in southeastern Brazil, is the fourth largest metropolis in Latin America in terms of demographic density and economic and industrial development. In addition, the Metropolitan Region of Rio de Janeiro has the highest rate of urbanization in the country, reaching 97.3 % (IBGE 2015). Paradoxically, the city possesses the largest urban forest on the planet (INEA 2015), which includes preserved areas and vegetation in good condition. Oliveira *et al.* (2017) documented heat islands in central areas of the city of Rio de Janeiro with a ~ 5 °C lower temperature in protected regions.

1 Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, 20550-900, Rio de Janeiro, RJ, Brazil
2 Laboratório de Anatomia Vegetal, Departamento de Biologia Vegetal, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, 20550-013, Rio de Janeiro, RJ, Brazil
3 Laboratório de Biologia Celular e Tecidual, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013-602, Campos dos Goytacazes, RJ, Brazil

* Corresponding author: maurauenf@gmail.com

Rates of atmospheric pollution are high in the urban center of the city of Rio de Janeiro (INEA 2017). Mutagenic and genotoxic activities associated with air pollutants, such as polycyclic aromatic hydrocarbons (PAH) were found along Avenida Brazil, the main urban road of Rio de Janeiro (Rainho *et al.* 2013).

The diversity of reported responses in plants indicates that environmental factors can promote variation in functional and structural characteristics of individuals in urban environments (Rai 2016; Vasconcellos *et al.* 2017). Among plant responses to pollutants can be observed changes at biochemical, microscopic and macroscopic levels (Prusty *et al.* 2005). Nevertheless, the degree of tolerance of a plant to a particular pollutant is defined by different levels of change.

Structural analyses are enlightening for studies of stress (Sant'anna-Santos *et al.* 2012). The resistance of leaves to a particular condition is associated with variables such as hairiness, cell wall thickness, pattern of epicuticular wax deposition, and pollutant penetration via the cuticle and stomata, among others (Dickison 2000). In addition, the urban environment presents a combination of drought and heat, which activates specific physiological and molecular responses. These responses, in turn, lead to changes in plant metabolism that mitigate the damaging effects of the combination of stressors (Zandalinas *et al.* 2017).

Eugenia uniflora (Myrtaceae), popularly known as Pitangueira, Suriname cherry, Brazilian cherry, or Cayenne cherry, is a native plant of the Restinga, an ecosystem associated with the Atlantic Forest. The species is widely cultivated in Brazil due to its tasty fruits and medicinal leaves (Lorenzi 2002). In folk medicine, the plant is used to treat various diseases. The infusion of its leaves has been used as an anti-rheumatic and antihypertensive, while its alcoholic extract is used to treat bronchitis, coughs, fevers, anxiety, hypertension, and diseases caused by worms (Queiroz *et al.* 2015). Studies investigating the pharmacological basis of the popular use of *E. uniflora* have shown that the crude aqueous extract prepared with leaves causes inhibition of gastrointestinal transport in cases of disorders, hypotensive and vasodilatory effects, and weak diuretic activity (Queiroz *et al.* 2015). Research over the last decade on the species' potential as environmental bioindicator has revealed physiological and structural changes in response to urban conditions and/or exposure to atmospheric pollutants (Alves *et al.* 2008; Silva *et al.* 2015; 2017).

In general, the secretory structures and secondary metabolites produced by plants are directly related to their medicinal properties. However, external factors may interfere with these structures and, consequently, the medicinal-biological activity of the plant (Okem *et al.* 2015). Air pollution, derived from automobile traffic in the city of São Paulo, the second largest metropolis in Latin America, was found to directly influence the absorption of chemical elements by plants, with the levels of these elements exceeding values recommended for consumption (Amato-Lourenco *et al.* 2016).

Given the scenario described above, we aimed to analyze the leaves of *E. uniflora* collected in the city of Rio de Janeiro, with the objective of detecting changes induced by exposure to the urban environment. Thus, we investigated whether the leaves of *E. uniflora* found in forest and urban environments (1) vary in the external and internal structure; (2) differ in the production of the main metabolites secreted by their secretory structures; and (3) exhibit significant structural plasticity. Data regarding potential bioindicators for urban environments will be provided for the anatomical characteristics of these plants.

Materials and methods

Study sites

The study took place in 2017 at two sites of Atlantic Forest in the city of Rio de Janeiro, Brazil: (1) an urban site, located at Fundação Oswaldo Cruz Manguinhos (-22.878639, -43.246621), on the fringes of Avenida Brasil, the main urban road in the city, which presents several already-established cytotoxic and mutagenic variables (Rainho *et al.* 2013; INEA 2017); and (2) a forest site, located at Fundação Oswaldo Cruz Mata Atlântica (-22.939889, -43.404424), on the edge of Parque Estadual da Pedra Branca, a 12,500-hectare forest that represents the largest urban forest on the planet (INEA 2015) (Fig. 1). Table 1 shows the mean annual precipitation for the period of 1995 to 2016 and for 2017; and the values maximum, mean and minimum temperatures for 2017. Meteorological data were obtained from the nearest meteorological stations of the Sistema de Alerta Rio (Rio de Janeiro) to each of the study sites — Estação Metodológica de São Cristóvão for the urban site, and Estação Meteorológica Rio Centro (Rio de Janeiro) for the forest site.

Table 1. Mean annual precipitation for 1995–2016 and annual precipitation for 2017, and mean temperatures for 2017 at the urban study site and the forest study site in the city of Rio de Janeiro, Brazil.

Sites / Periods	Cumulative annual rainfall (mm)		Mean temperatures (°C) for 2017		
	Average: 1995-2016	2017	Maximum	Mean	Minimum
Urban	988.5	666.8	36.01	25.21	19.25
Forest	1.250.30	1.084.00	36.9	24.9	18.35

Source: Sistema de Alerta Rio of the City of Rio de Janeiro.

Sampling, preparation, and analysis of botanical material

Adult leaves exposed to the sun were sampled from four specimens of *E. uniflora* at each of the two study sites. Leaf area (LA) was determined using 10 leaves of each specimen, which were collected, scanned and measured for leaf area using Image-Pro Express 6.0 software. To determine leaf mass per unit area (LMA) and succulence (SUC), ten leaf discs (0.5 mm) were removed from each individual. The disks were hydrated in distilled water for 24 hours and then measured for saturated mass using a digital scale (0.0001 g) and hydrated leaf thickness (HLT) (mm) using a digital caliper (±0.01 mm). Hydrated leaf discs were then placed in an oven at 55 °C for 72 hours and weighed to obtain dry mass (DLM). These values were used to calculate SUC (gm^{-2}), as the difference between the saturated (SLM) and dry mass divided by disc area, and LMA (gm^{-2}) (Kluge & Ting 1978). Density values (DEN) ($mg\ cm^{-3}$) were determined from the ratio between disc dry mass per area and hydrated leaf thickness: DEN = LMA/HLT (Witkowski & Lamont 1991).

For anatomical analysis, samples from the median region of leaves of the third node were fixed in aqueous solution of 2.5 % glutaraldehyde, 4.0 % formaldehyde and 0.05 M sodium cacodylate buffer at pH 7.2, dehydrated in an ascending alcoholic series and included in Historesin® (Feder & O'Brien 1968). The samples were sectioned with a rotating microtome at 8-9µm thick, stained with Toluidine O-Blue (O'Brien *et al.* 1964), mounted on Entellan®, and observed under an Olympus BX 41 light microscope. Images were obtained with the aid of a Q Collor R3 video camera coupled to the microscope.

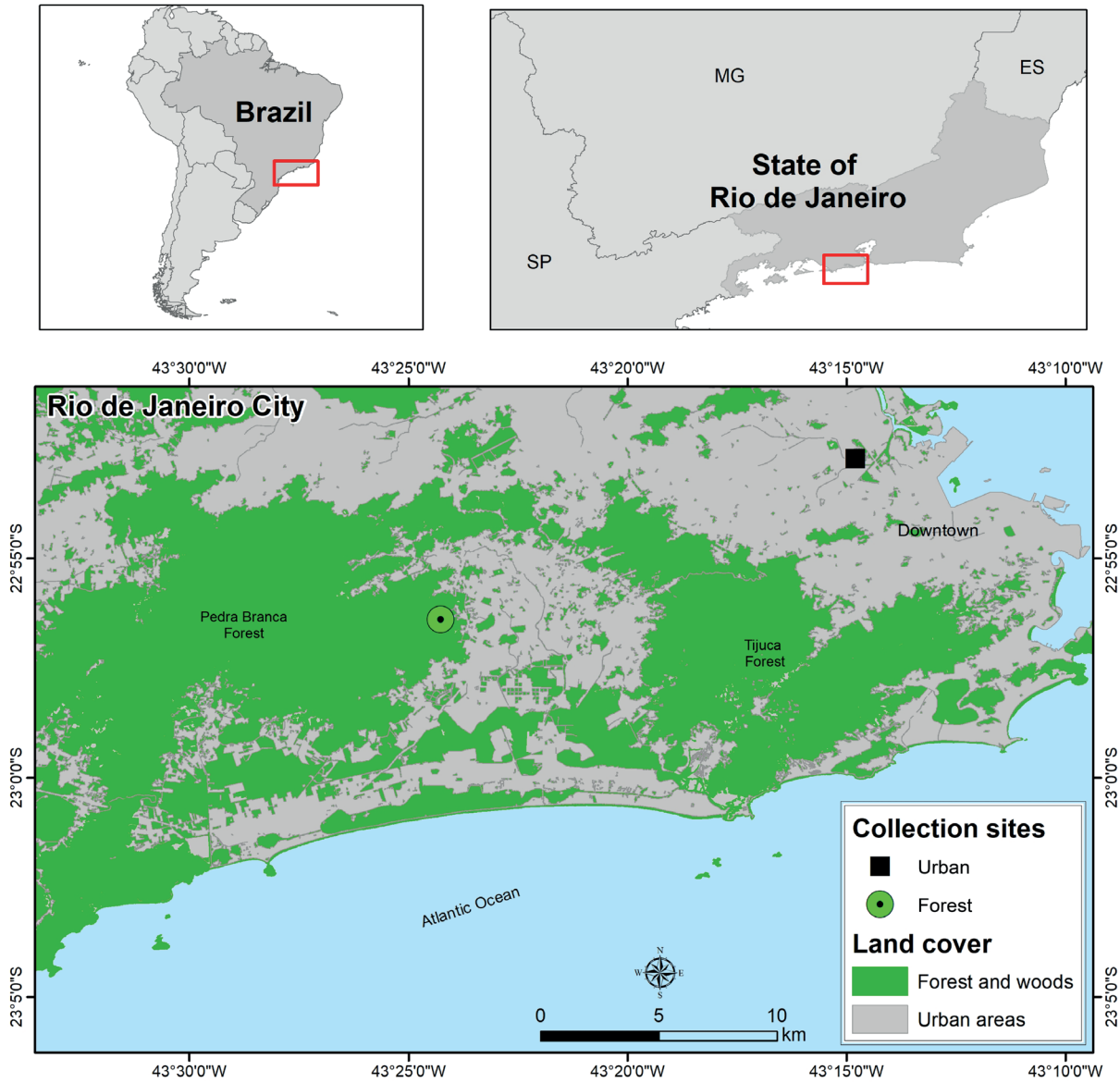

Figure 1. Map highlighting the study sites in the city of Rio de Janeiro, Brazil.

The frequency and distribution of glands and crystals were measured on 10 diaphanized leaves (Strittmatter 1973), obtained from the third node of each of the selected individual plants. Leaves from plants of the forest site were left in 50 % sodium hypochlorite for 12-hours longer than leaves from the urban site to achieve total bleaching.

Ultrastructure and stomatal frequency were evaluated using samples from the median area of leaves, which were dehydrated in an alcoholic series and submitted to the critical point drying with liquid CO_2 using a Bal-Tec CPD 030 Critical Point Dryer. The resulting dry fragments were adhered to supports with carbon tape and covered with a 20 nm layer of gold (Bal-Tec SCD 050 Sputter Coater). Images were obtained by scanning electron microscopy (SEM) with a ZEISS - DSEM 962 at a voltage of 25 kV.

In twenty-five fields of each leaf were examined to evaluate the following leaf variables: adaxial and abaxial epidermis thickness (µm), palisade parenchyma thickness (µm), spongy parenchyma thickness (µm), leaf blade thickness (µm), stomata length (µm), and frequency of stomata, crystals and secretory glands per mm^2. Analyses were performed using Image-Pro Express 6.0 digital image processing system.

The main classes of chemical compounds present in the leaf blade and in the secretion of secretory structures were investigated in cross-sections made by freehand of recently collected material or material included in Historesin®. The following histochemical tests were performed: Sudan IV (Pearse 1980); Ruthenium Red; Ferric chloride III and Lugol (Johansen 1940). Standard control procedures were performed simultaneously.

Phenotypic plasticity indices were calculated for quantitative data (PPI; Valladares *et al.* 2000). Characteristics with PPI values ≥ 0.6 were considered phenotypically plastic, as established by Vasconcellos *et al.* (2017).

All quantitative results were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene tests, respectively. The parametric results for the two sites were compared using Student's t-test, while non-parametric results were compared using the Mann-Whitney test, at a significance level of 95 %. Statistical tests were performed with STATISTICA 7.0 software (StatSoft, Inc., USA).

Results

The quantitative parameters in the *E. uniflora* leaves evaluated were LA, SLM, DLM, HLT, LMA, SUC, and DEN and did not differ significantly between study sites (Tab. 2). *Eugenia uniflora* leaves are glabrous, with a uniseriate epidermis (Fig. 2A-B). The mesophyll is dorsiventral (Fig. 2A-B), with one or two cell layers of palisade parenchyma and six to ten layers of spongy parenchyma. Calcium oxalate crystals are present throughout the mesophyll (Fig. 2A-D) and central vein (Fig. 2E-F). With respect to stomata, the leaves are hypostomatic (Fig. 2G-H). Secretory glands are present in the mesophyll, where they are positioned close to the adaxial

Table 2. Measured parameters of *Eugenia uniflora* at the forest and urban site and the results of the statistical tests; Student t (t) and Mann-Whitney (U).

Parameters	Forest site				Urban Site				t	U	p
	Min.	Medium	Max.	DP	Min.	Medium	Max.	DP			
LA (cm^2)	4.351	9.638	18.833	± 3.467	3.637	8.322	15.868	± 2.861	1.270		0.251
SLM (mg)	0.4	7.3	6.2	± 0.8	3.6	5.7	7.4	± 0.9		6.000000	0.564
DLM (mg)	0.1	1.9	2.7	±0.3	0.9	0.2	2.9	± 0.5	-0.402		0.702
HLT (mm)	0.19	0.280	0.39	± 0.048	0.2	0.2805	0.39	± 0.048	-0.026		0.980
SUC (g/m^2)	17.834	68.758	76.43	± 114.2	20.382	47.484	64.968	± 10.44		2.000000	0.083
LMA (g/m^2)	14.013	23.726	34.39	± 4.49	11.465	25.032	36.943	± 6.42	-0.402		0.702
DEN (mg/mm^3)	52.262	87.170	127.39	± 21.2	33.720	91.141	139.521	± 25.06	-0.342		0.744
Thickness of leaf blade (µm)	166.320	235.705	318.28	± 35.81	132.663	237.696	317.250	± 43.88	-0.172		0.869
Thickness of palisade parenchyma (µm)	32.979	51.025	72.58	± 8.91	35.477	71.996	98.400	± 16.89		3.000000	0.149
Thickness of lacunar parenchyma (µm)	91.846	155.642	210.75	± 27.85	74.915	139.664	191.410	± 28.23	1.329		0.232
Thickness of the adaxial epidermis (µm)	9.801	14.612	18.81	± 1.908	8.477	14.015	18.280	± 1.72	2.373		0.055
Thickness of the abaxial epidermis (µm)	8.742	15.080	23.07	± 3.55	8.216	11.841	16.469	± 1.50		3.000000	0.149
Frequency of secretory glands ($units/mm^2$)	0	1.167	4	± 1.114	1	2.583	4	± 1.083	-3.300		0.016 *
Frequency of crystals ($units/mm^2$)	0	2.25	4	± 1.602	5	10.083	18	± 3.52		0.000000	0.0209 *
Frequency of stomata ($units/mm^2$)	88	105	120	± 16.09	152	181.75	216	± 26.78	-5.108		0.002 *
Length of stomata (µm)	1.203	1.558	2.006	± 0.211	1.032	1.327	1.805	± 0.172	3.778		0.009 *

Note: values referring to the minimum value (Min.) - average value (Medium) - maximum value (Max) ± standard deviation (DP). * Represents a significant difference with a 95 % confidence interval (p). LA: Leaf area; SLM: Saturated leaf mass; DLM: Dry Leaf mass; HLT: Hydrated leaf thickness; SUC: Succulence; LMA: Leaf mass per unit area; DEN: Density.

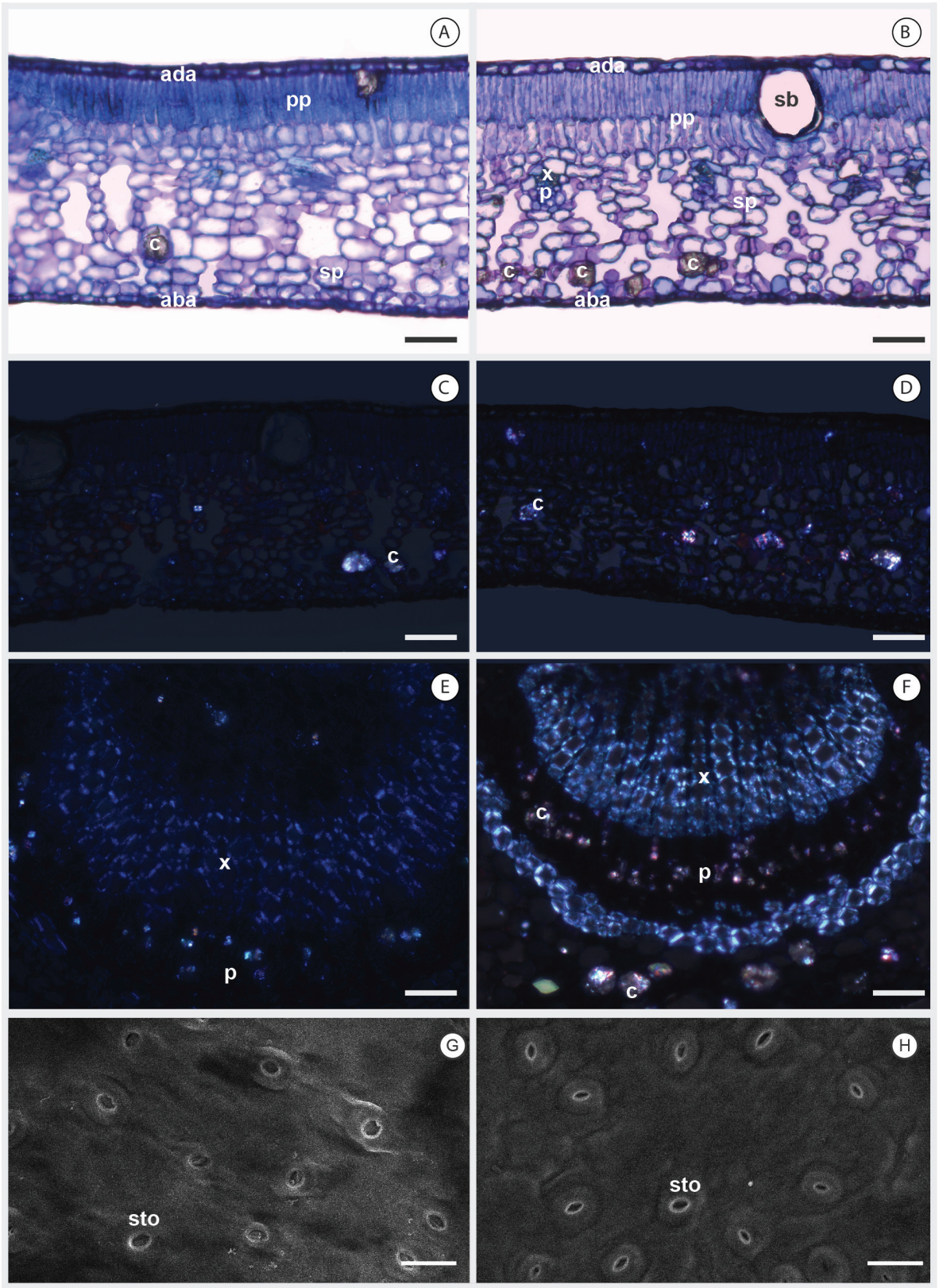

Figure 2. Cross-sections of leaves of *Eugenia uniflora* from the forest site (**A**, **C** and **E**) and from the urban site (**B**, **D**, **F**). **AD.** leaf blade. **EF.** central vein**. CF.** analysis under polarized light, highlighting the presence of crystals. **G-H.** Abaxial face of leaves of *Eugenia uniflora* from the forest site (**G**) and from the urban site (**H**), under scanning electron microscopy. pp: palisade parenchyma; sp: spongy parenchyma; ada: adaxial epidermis; aba: abaxial epidermis; c: crystal; x: xylem; p: phloem; sb: secretory glands; sto: stomata. Bar **A-F**= 50 µm**; G-H**= 20 µm.

(Fig. 2B) or abaxial surfaces. The vascular system of the main vein possesses sclerenchyma associated with xylem and phloem.

The mean leaf size of individuals of *E. uniflora* sampled at the urban site exhibited greater variation than those of the forest site (Tab. 2). Although differences can be identified among the measured parameters, only stomatal frequency, stomatal length, crystal frequency, and secretory glands frequency differed significantly between the two sites (Tab. 2). We highlight the inverse relationship between the frequency and the size of the stomata, with a greater number of stomata to security the decrease in size in the urban site had (Fig. 3).

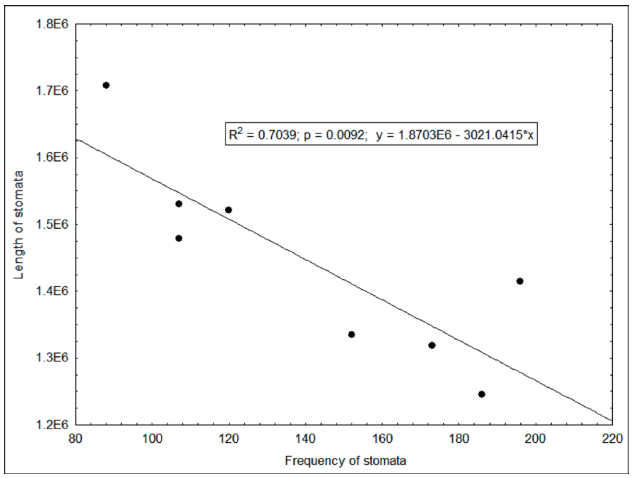

Figure 3. Relationship between stomata length and stomata density in the *Eugenia uniflora* leaves from the two study sites.

Among the specimens of the two study sites, in the leaves of the urban site were more abundant with calcium oxalate crystals (Fig. 4), moreover, there were greater frequencies of secretory glands (Fig. 4A-B) and phenolic compounds. Histochemical tests of the leaves of *E. uniflora* of the urban site revealed an increase in the production of secondary metabolites (Fig. 5). Secretions in the gland of the urban site showed a positive reaction to starch (Fig. 5B), lipid (Fig. 5C) and mucilage (Fig. 5G). In addition, histochemical tests detected the lipid accumulation in the mesophyll for plants of the urban environment (Fig. 5C). In the abaxial face of the central vein of the individuals of the urban site, cells with mucilage accumulation were observed (Fig. 5I).

Leaves *of Eugenia uniflora* of the urban environment exhibited symptoms as necrosis and the sprouting of completely reddish new leaves (Fig. 6A-C). On the abaxial face of some leaves of *E. uniflora* from the urban site, cells full of secondary metabolites were observed surrounding the necrotic region (Fig. 6D-E). These characteristics were not observed in the leaves of the forest site. Presence of cells with phenolic compounds in the xylem parenchyma (Fig. 6F) and on the adaxial side of the vein was observed (Fig. 6G).

Plasticity indices revealed that the parameters with higher phenotypic plasticity (Tab. 3) were: LA, SLM, DLM, SUC, DEN, leaf blade thickness, palisade, and spongy parenchyma thickness, abaxial epidermis thickness, and frequencies of stomata, crystals, and secretory glands.

Table 3. Phenotypic plasticity indices for the evaluated quantitative parameters.

Parameters	Phenotypic Plasticity
LA	0.8
SLM	0.5
DLM	0.6
HLT	0.5
SUC	0.8
LMA	0.7
DEN	0.8
Leaf blade thickness	0.6
Palisade parenchyma thickness	0.7
Spongy parenchyma thickness	0.6
Adaxial epidermis thickness	0.5
Abaxial epidermis thickness	0.6
Frequency of secretory glands	0.8
Frequency of crystals	1.0
Density of stomata	0.6
Stomatal length	0.5

Note: LA: Leaf area; SLM: Saturated Leaf mass; DLM: Dry Leaf mass; HLT: Hydrated Leaf Thickness; SUC: Succulence; LMA: Leaf mass per unit area; DEN: Density.

Discussion

The emission of atmospheric pollutants can affect from isolated individuals to entire populations of several species (Kuki *et al.* 2008). In this sense, the mean leaf size of individuals of *E. uniflora* sampled at the urban site exhibited greater variation than those of the forest site, where some leaves present a more development of the second layer of the palisade parenchyma and shortening of the spongy parenchyma. Variation in the thickness of the palisade parenchyma is usually related to responses of light radiation (Fernandes *et al.* 2014). According to Khosropour *et al.* (2018), the proportion of mesophyll tissue in plants in an urban environment is influenced by chloroplasts used in photosynthesis located in the palisade parenchyma. The spongy parenchyma may be decisive in the number of pollutants that penetrate the leaf interior, due to the number of intercellular spaces, and of stomata allowing the access of the gases. Thus, more palisade cells allow more photosynthesis, and reduce the thickness of the spongy parenchyma, while stomata decrease the absorption of pollution as well as CO_2. Ultimately it is necessary to have a balance between these tissues since decreased CO_2 absorption will limit photosynthesis.

Modifications that lead to an optimal fit between the control of gas exchange and the consequent entry of pollutants through stomata can follow two paths: (1) plants reduce the uptake of pollutants by decreased stomatal density (Kulshreshtha *et al.* 1994); or (2) plants increase stomatal

density (Alves *et al.* 2008; Gostin 2009) and concomitantly reduce stomatal size (Rashidi *et al.* 2012). In the present study, stomatal density and stomatal length differed significantly between the two environments. Individuals of *E. uniflora* that developed in an urban site had a greater number of stomata to counter their decrease in size, allowing an ideal adjustment for the control of gas exchange and decreased pollutant uptake. Bettarini *et al.* (1998) considered changes in the density, distribution, and morphology of stomata as important characteristics for adaptation/tolerance to air pollution. These attributes are often used in environmental biomonitoring using plants (Wuytack *et al.* 2010).

Greater stomatal density may also be related to stress caused by the reduced availability of water and the higher temperatures of the urban environment (Tab. 1). The relationship between stomatal density and water stress has been addressed by several studies with different types of plants (Gan *et al.* 2010; Peterson *et al.* 2012).

The increase in the amount of these crystals, as happened to the analyzed individuals of the urban site, is a response

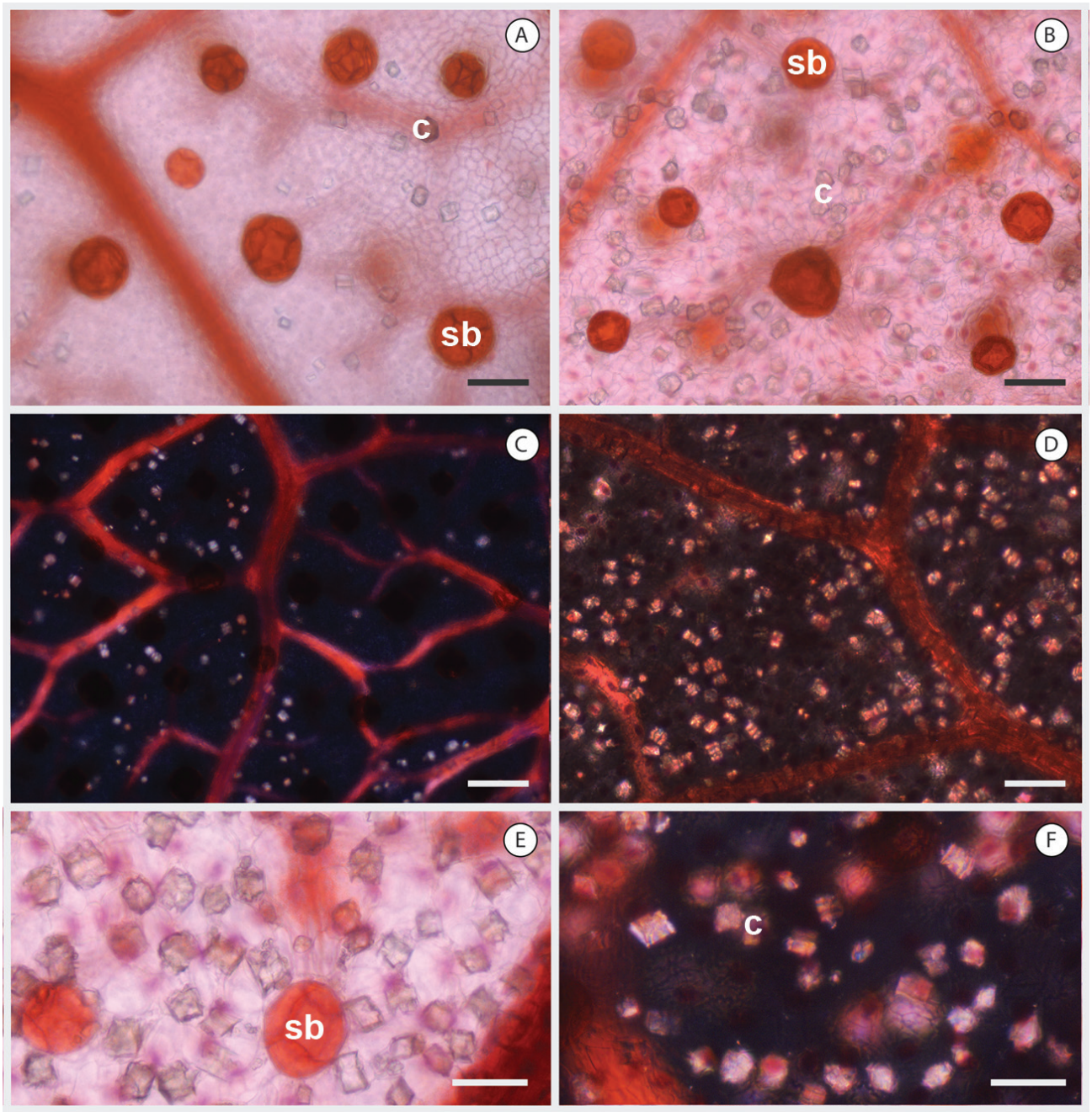

Figure 4. Diaphanous leaves of *Eugenia uniflora* under light microscopy. The images show the difference in crystal density between the two sites and further detail the crystal structure. **A**, **C.** forest site; **B**, **DF.** urban site. **C**, **D**, and **F.** view under polarized light, highlighting the presence of crystals. c: crystal; sb: secretory glands Bars **A-D** = 50 µm; **E-F** = 20 µm.

that has been observed in a variety of species subjected to gaseous pollutants (Alves *et al.* 2008). According to the literature, plants can act as biological filters, removing large amounts of pollutants from the urban environment (Tomaševič *et al.* 2008). This strategy allows plants to maintain ionic equilibrium, which is affected when pollutant load is high, as well as favor the incorporation of heavy metals into oxalate crystals in plant tissues (Choi *et al.* 2001; Tomaševič *et al.* 2008). The pollutants increase the permeability of cell membranes, promoting an influx of Ca^{++} from the apoplast into the cells. Therefore, crystal formation would serve as a defense reaction to encapsulate excess calcium inside the cell (Fink 1991). Oxalate can have negative impacts on the health of an individual who consumes plants containing it since it can cause renal complications (Holmes *et al.* 2001). In fact, more than 75 % of all kidney stones contain calcium oxalate as the major component (Nordin *et al.* 1979).

As seen from our research, Alves *et al.* (2008) also observed a higher number of stomata and calcium oxalate crystals when evaluating the influence of urban pollution in the city of São Paulo on *E. uniflora*. These authors observed only one layer of palisade parenchyma, which differs from the present study where several leaves of the urban site

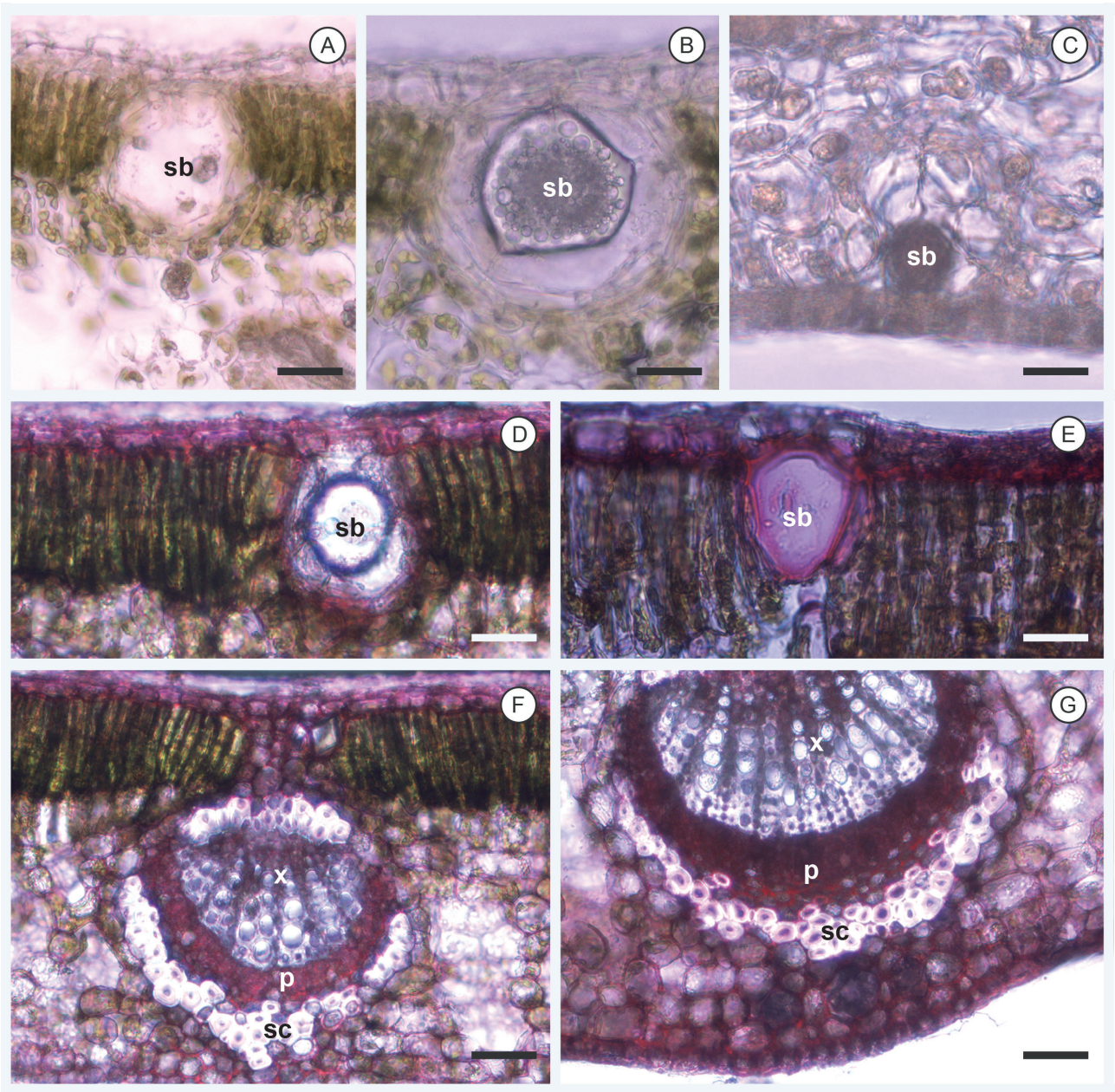

Figure 5. Cross-section of the leaf blade of *Eugenia uniflora* under light microscopy, under different histochemical tests. **A**, **D**, **F.** forest site. **B**, **C**, **E**, **G.** Urban site. Histochemical tests: **AB.** Lugol; **C.** Sudan IV; **D-G.** Ruthenium Red. x: xylem; p: phloem; sb: secretory glands sc: sclerenchyma Bars **A-E** = 20 µm; **F-G** = 50 µm.

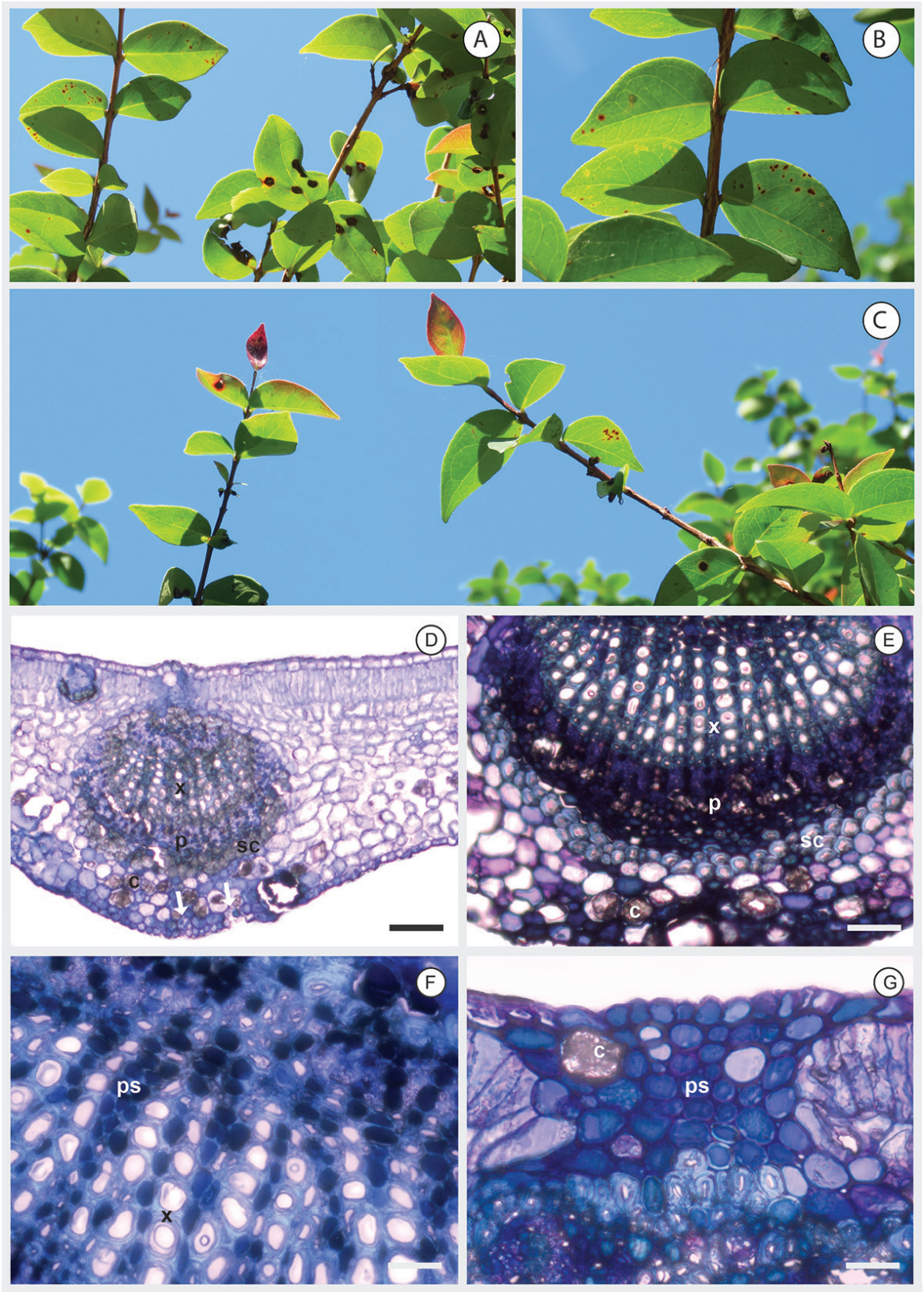

Figure 6. Leaves of different specimens of *Eugenia uniflora* in the urban environment (**A**, **B** and **C**). Cross-section, under light microscopy, stained with toluidine blue (**D**, **E**, **F**, and **G**). Note in different regions of the central vein the presence presenting of cells with phenolic substances and cells with cell death aspect (arrow). x: xylem; p: phloem; sc: sclerenchyma; c: crystal; ps: phenolic substances. Bar **A**= 100 μm; **B** = 50 μm; **CD** = 20 μm.

had a second layer of palisade parenchyma. Another new feature in our study was the observation of increasing the number of secretory glands in urban sites. This data is consistent with the concept of increased development of defenses when plants are subjected to stress conditions, such as pollution (Coley *et al.* 1985). It should be noted that changes in secondary metabolites directly influence the quality of a plant for medicinal purposes (Santos *et al.* 2006). Histochemical tests of the leaves and in the structure secretory of *E. uniflora* of the urban site revealed an increase in the production of secondary metabolites, which corroborates results observed for plants under long-term exposure to atmospheric pollutants (Gostin 2009). Regarding the detection of the lipid accumulation and starch in individuals of the urban site, what can it be a defense response in plants under stress (Berkey *et al.* 2012; Guo *et al.* 2017). The presence of mucopolysaccharides, such as mucilage, in the secretion of secretory glands and in idioblasts present in the central vein of leaves of *E. uniflora* of the urban site may be related to water storage (Souza *et al.* 2015). Furthermore, these substances can protect against the higher temperatures of this site, by functioning as osmoprotectants (Rizhsky et al. 2004).

Visible damage to leaves, such as necrosis and chlorosis, as observed in *E. uniflora* leaves of the urban site, can be induced by particles carried by air, which is indicative of atmospheric pollution (Silva *et al.* 2017). Silva *et al.* (2017) observed that emissions from a pelletizing factory also caused visual damage to *E. uniflora*, including foliar abscission, necrosis and purplish coloration of young leaves. Silva *et al.* (2015), previously also observed that *E. uniflora* individuals cultivated under the same conditions accumulated iron and sulfur in the leaves.

Some leaves from the urban site of the present study exhibited a reddish coloration, indicating the presence of photoprotective pigments, which may be a compensatory mechanism under conditions of environmental stress (Ashrafuzzaman *et al.* 2017). The antioxidant defense system of plants may act by increasing levels of low molecular weight non-enzymatic metabolites such as anthocyanin and other phenolic substances (Sytar *et al.* 2013). This defense system plays a key role in detoxifying cells and can improve tolerance to different types of stress (Gill & Tuteja 2010).

Observation of cells with evidence of cell death in the central vein of urban *E. uniflora* leaves suggests that there is a defense system formed by the accumulation of secondary metabolites that develop a protective barrier to prevent the progression of necrosis toward tissues healthy, as also observed by Sant'anna-Santos *et al.* (2012) and Silva *et al.* (2017). The urban site characteristics of the present study were enough to cause damage to *E. uniflora* leaves, similar to what was found for the influence of pollutants emitted by an iron pellet factory (Silva *et al.* 2017). These findings provide more information for describing *E. uniflora* as a bioindicator species of the environment.

The anatomical traits of *E. uniflora* with phenotypic plasticity indices equal to or greater than 0.6 reinforce the impact of one of the sites on certain leaf traits. The accumulation of crystals showed had a high plasticity index with great potential to be an anatomical bioindicator. In this sense, we can suggestion variable traits in the phenotype of *E. uniflora* that help to evaluate the effects exerted by urban conditions, including interactions with pollutants or climatic conditions (Tripathi & Gautam 2007).

This study highlights the importance of performing more comprehensive studies regarding interference by urban environments with secondary metabolites. As a consequence, this involves the biological activity of plants in the urban environment and their viability for use, such as for medicinal purposes. We conclude that visual and anatomical symptoms were efficient at diagnosing stress in *E. uniflora*. It is believed that the characteristics of the urban site may be responsible for the variation observed. The analyzed alterations, such the increased frequency and decreased size of stomata and the higher frequency of secretory glands and crystals may have contributed to the acclimatization of *E. uniflora* in the urban condition and may be useful for biomonitoring in such environments.

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