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RESEARCH ARTICLE

Differential responses of C_3 and CAM native Brazilian plant species to a SO_2- and SPM_{Fe}-contaminated Restinga

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Abstract Aiming to evaluate responses in terms of growth rates, physiological parameters, and degree of sensitivity to SO_2 and SPM_{Fe} in *Eugenia uniflora* L. (Myrtaceae, a C_3 species) and *Clusia hilariana* Schlecht (Clusiaceae, a CAM species); saplings were exposed to emissions from a pelletizing factory for 7 months. The species were distributed along a transect (200, 500, 800, 1400, and 1700 m away from the emission source), and analyses were performed after 71, 118, and 211 days of exposure to the pollutants. *E. uniflora* received higher superficial deposition of particulate iron. The highest total iron foliar contents were observed 200 m away from the emission source in both plant species, while the highest total sulfur foliar contents were observed 200 m away in *C. hilariana* and 800 m away in *E. uniflora*. *E. uniflora* presented decreased values of height growth rate, number of necrotic leaves, chlorophyll analysis (SPAD index) and transpiration, in relation to the distances from the emission source. *C. hilariana* showed decreased values of height growth rate, number of leaves, number of necrotic leaves, total ionic permeability, stomatal conductance, transpiration, net CO_2 assimilation, and total dry matter, in relation to distances from the emission source. In relation to the days of exposure, both species presented increased number of necrotic leaves and foliar phytotoxicity index, and decreased values in the chlorophyll analysis. The two native plant species, both of which occur in the Brazilian Restinga, showed damage when exposed to emissions from an iron ore pelletizing factory. *C. hilariana* was considered the most sensitive species due to the decreased values in a higher number of variables after exposition.

Keywords *Eugenia uniflora* · *Clusia hilariana* · Native plants · Pollution · Sensitive species · Symptomatology

Introduction

Brazil is the second largest producer of iron (Fe) ore worldwide (Instituto Brasileiro de Mineração–IBRAM 2011), and an increasing activity has been observed in mining and processing industries of this metal (Kuki et al. 2009).

Located at the Brazilian coast, the Restinga can be defined as coastal areas constituted by Quaternary sandy deposits of marine origin and dunes built over these deposits by wind

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action (Araujo and Maciel 1998). This biome presents a great variety of communities with typical fauna and flora (Freire 1990; Rocha et al. 2003; Assis et al. 2004), which are subjected to abiotic adversities such as high temperatures, dry periods, constant wind, high salinity, and nutrient scarcity. This makes the structure and function of the Restinga different from those of any other environment (Scarano 2002).

However, this coastal biome is being impacted by the emission of pollutants from mining industries, which causes alterations in the structure, dynamics, and diversity of the exposed vegetation (Silva et al. 2006; Kuki et al. 2008, 2009; Pereira et al. 2009).

On the coast of Espírito Santo state, in the district of Ubu, there is an iron pelletizing factory which processes ore for exportation. This factory has records of suspended particulate matter emission levels that are considered to be below the air quality secondary standard allowed in Brazil (Lopes et al. 2002; CONAMA 1990). Nevertheless, some plant species can be sensitive to levels of air pollution that are regarded as low and may present symptoms, which would qualify them as bioindicators of air quality (De Temmerman et al. 2004).

Pelletizing factories are responsible for increasing atmospheric levels of several pollutants, especially sulfur dioxide (SO_2) and iron suspended particulate matter (SPM_{Fe}) (Lopes et al. 2002; Grantz et al. 2003).

Sulfur dioxide is one of the six major air pollutants and can be absorbed by roots or through stomata, the latter by means of photosynthesis and respiration (Manninen and Huttunen 2000; Zhang et al. 2013). Its absorption by plants causes different responses depending on the species and on environmental conditions. Studies have reported damage to the photosynthetic apparatus (Swanepoel et al. 2007), stomatal density (Haworth et al. 2012), and carbon fixation efficiency (Chung et al. 2011).

Suspended particulate matter is composed of a mixture of several pollutants and contains particles of different sizes, origins, and chemical compositions. The dispersion dynamics of trace elements such as Fe may vary with different factors such as air temperature, chimney height, wind frequency and intensity, precipitation, and the presence of suspended water (marine aerosol) in the atmosphere (Grantz et al. 2003). In the ecosystem, particulate deposition can alter nutrient cycling, thus inhibiting their absorption; reducing growth, flowering, and fruiting; and altering species diversity due to local elimination of the most sensitive species, among other effects (Silva et al. 2006; Kuki et al. 2008).

Along with such problem, in coastal environments, the marine aerosol can adhere to suspended particles. Depending on its size, particulate deposition will either precipitate in the vicinity of the factory or be dispersed for miles inland. The high sodium and chloride concentration in the marine aerosol is extremely toxic to plants, and when adhered to particulate iron, they may produce leaf necrosis. Thus, this may also contribute to the elimination of local species that are intolerant to salt spray (Grantz et al. 2003).

The species *Eugenia uniflora* L. (Myrtaceae) and *Clusia hilariana* Schlecht (Clusiaceae) are sensitive to some air pollutants (Silva et al. 2005; Alves et al. 2008) and are indicated for active biomonitoring studies of air quality (Alves et al. 2008; Pereira et al. 2009; Neves et al. 2009).

E. uniflora and *C. hilariana* have distinct photosynthetic metabolisms, namely C_3 and crassulacean acid metabolism (CAM), respectively. Unlike plants with C_3 metabolism, CAM plants close their stomata during the day, and only at night, when their stomata are open, does atmospheric CO_2 fixation occur, by the activity of the phosphoenolpyruvate carboxylase enzyme (Cushman and Bohnert 1997). Furthermore, the two species also present different anatomical features and metabolic pathways, which can determine their differential susceptibility to pollutants (Silva et al. 2005). Thus, we believed that *E. uniflora* and *C. hilariana* would present different responses to a SO_2- and SPM_{Fe}-contaminated environment.

As the impacts of atmospheric pollution are poorly studied in tropical ecosystems, the aim of this study was to evaluate the responses in terms of growth and physiological parameters, and the degree of sensitivity of *E. uniflora* and *C. hilariana* to the atmospheric pollutants SO_2 and SPM_{Fe}.

Material and methods

Studied species

The chosen species for the study were *E. uniflora* L. (Myrtaceae) and *C. hilariana* Schlecht. (Clusiaceae), due to their representativeness and frequency in the Restinga of Paulo César Vinha State Park (PCVSP); to the ease of their cultivation; and to their phenological, morphological, and physiological traits.

E. uniflora L. (Myrtaceae) is a shrub ca. 3 m high with C_3 metabolism and occurs both in the backshore and in the Myrtaceae forest formations, on the Setiba Restinga. It possesses opposite phyllotaxis and simple, glossy, glabrous leaves. Its fruit is widely commercially exploited due to its sweet and tangy taste (Neves and Donato 1989).

C. hilariana Schlecht (Clusiaceae) is a tree with CAM metabolism (Franco 1989) that is widely distributed along the Setiba Restinga, especially in the open *Clusia* scrub, where its individuals predominate, forming bushes. It possesses opposite phyllotaxis and its leaves are thick and waxy. It is commercially exploited as an ornamental species and for the extraction of wood and resin, the latter for medicinal purposes (Schneider 1985).

Characterization of the studied area

The studied area belongs to the industrial complex of a pelletizing factory located in the municipality of Anchieta, on the coast of Espírito Santo state, at coordinates 20° 46′ 21.0″ S and 40° 34′ 52.3″ W (Fig. 1).

Climatic conditions were monitored at the meteorological station installed at the same line of the exposure transect, where wind speed and direction, rainfall, and ambient temperature were registered. Data were recorded on a daily basis, measurements being taken from 12:00 AM to 11:59 PM.

Concentrations of emitted SO_2 and SPM, which were obtained at the station located 1700 m away from the emission source on the same transect of the studied area, were determined with a Tri-Gas equipment (Tri-Gas 1/110 V—Energética Indústria e Comércio LTDA, Brazil) and a Hi-Vol air sampler (AGVPTS1—Energética Indústria e Comércio LTDA, Brazil), respectively. Sulfur dioxide concentrations were determined by the hydrogen peroxide method.

Cultivation conditions and plant exposure

Saplings of both species ca. 4 months old provided by the PCVSP nursery garden were standardized according to height, number of leaves, and phytosanitary status. After being transferred to 2-kg plastic pots filled with a mixture of sand, soil, and humus (3:1:1), plants were put in an automatic irrigation system, in which the pots were connected through a wick to water reservoirs that guaranteed the maintenance of soil humidity at an adequate level.

Plants were subjected to the action of the gaseous and particulate emissions from the pelletizing factory for 7 months, under field conditions, from August 14, 2001 to March 12, 2002. They were placed in standardized stations according to

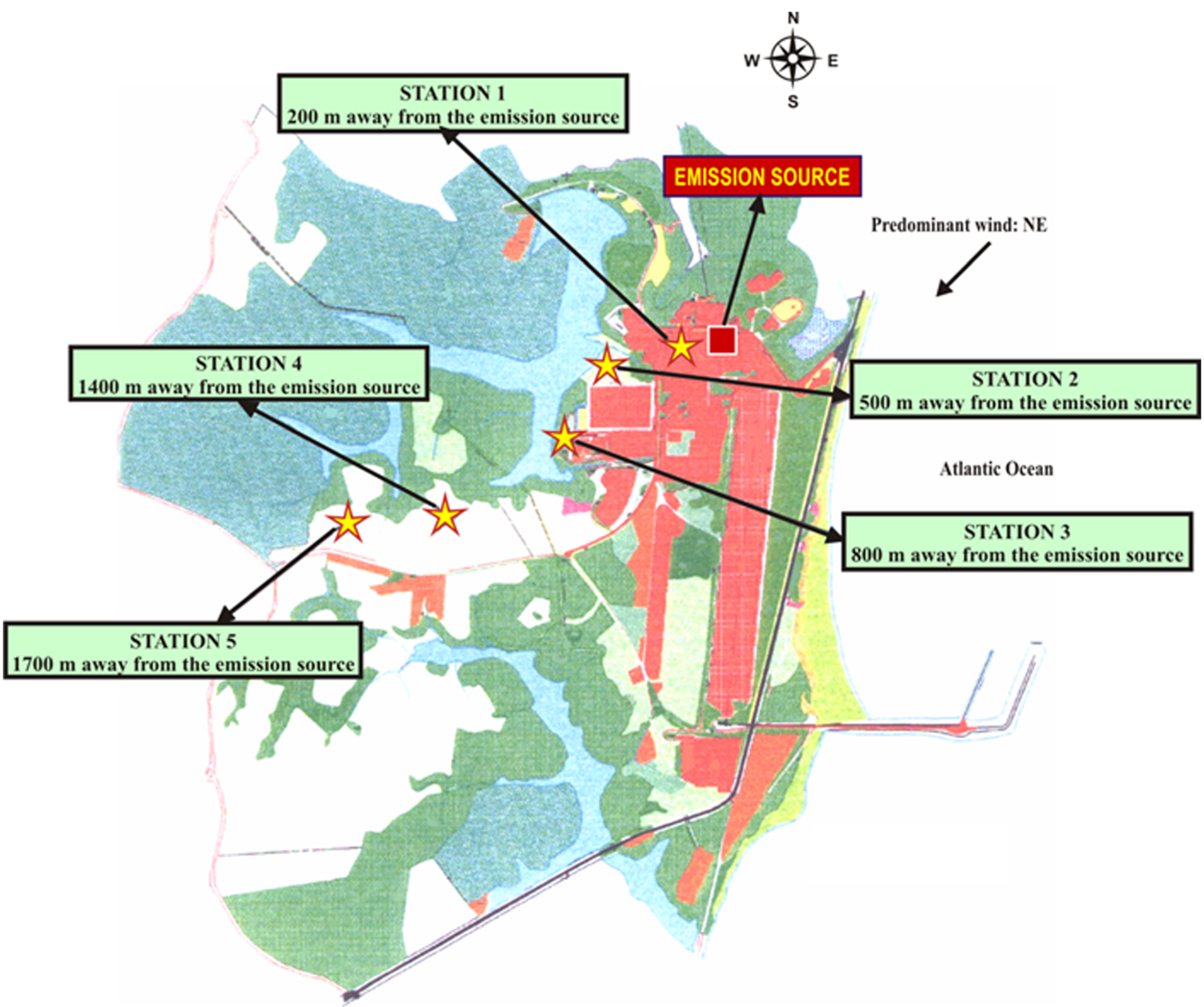

Fig. 1 Map indicating the region of the pelletization factory and the stations of active biomonitoring

the international standards for air quality control using bioindicators (Arndt and Schlutler 1985). The stations were distributed along a transect, which was determined according to the predominant wind direction (NE) in the region and placed 200 m (20° 46′ 25.0″ S and 40° 34′ 58.9″ W), 500 m (20° 46′ 28.0″ S and 40° 35′ 10.1″ W), 800 m (20° 46′ 35.8″ S and 40° 35′ 14.9″ W), 1400 m (20° 46′ 51.2″ S and 40° 35′ 29.8″ W), and 1700 m (20° 46′ 42.2″ S and 40° 35′ 47.3″ W) away from the emission source. This distribution had been previously established by a statistical study coupled to a sulfur dioxide dispersion modeling (Queiroz 1998), which was performed with an Industrial Source Complex Short Term simulator (ISCST3, Environmental Protect Agency—EPA, USA). The location of each station was determined with global positioning system (GPS).

Throughout the experiment, four evaluations were performed, regarding height, number of leaves, total chlorophyll content (SPAD index), number of necrotic leaves, and necrosis-based foliar phytotoxicity index. Measurements were taken at the beginning of the experiment and after 71, 118, and 211 days of exposure. Simultaneously with the last evaluation, measurements of gas exchange, membrane permeability, iron deposition on leaves, iron and sulfate quantification in plant matter, and total dry matter were performed.

Deposition of particulate matter on leaf surface

In order to determine particulate deposition on leaves, three leaves from all *E. uniflora* plants and two leaves from all *C. hilariana* plants were randomly chosen and individually washed with 20 ml of distilled water in pre-weighed glass flasks. The collected percolate was set aside at room temperature to allow deposition of the solid particulate matter on the bottom of the flasks. After 48 h, the flasks were carefully placed into a non-ventilated oven at 40 °C until complete water evaporation. After cooling, flasks were reweighed. Each washed leaf had its area measured by a plan meter (Delta MK2—Delta Devices Ltd, England). The amount of particulate matter deposited on the leaf was expressed as milligrams per square millimeter of leaf area (Prusty et al. 2005).

Iron and sulfur quantification in leaf dry matter

To determine iron and sulfur amounts in plant matter, 15 leaves of *E. uniflora* and 5 leaves of *C. hilariana* were rinsed with running water, then with deionized water, and dried with filter paper. After oven-drying at 75 °C, samples were ground through a 1-mm-mesh sieve. Iron content was determined by atomic absorption spectrophotometry and sulfate content by inductively coupled plasma (ICP). Measurements were taken in composite samples.

Growth analysis

In the two studied species among all distances from the emission source, the following parameters were analyzed: growth rate, number of leaves, and dry matter weight. Growth rate was obtained by the difference between the height values at the end of the exposition and the ones at the beginning. Total dry matter weight was obtained by drying roots and shoots in a drying oven with forced ventilation, until constant weight.

Foliar phytotoxicity

All leaves presenting necroses were quantified. Foliar phytotoxicity was determined by attributing grades to the percentage of necrotic leaf area, according to the following scale: 0—no necrosis, 1—with necrosis in up to 25 % of the leaf area, 2—with necrosis in 26 to 50 % of the leaf area, 3—with necrosis in 51 to 75 % of the leaf area, and 4—with necrosis in 76 to 100 % of the leaf area, as proposed by Bustamante et al. (1993). A general sampling was performed with the most representative adult leaves per pot.

Chlorophyll content (SPAD index)

The relative leaf greenness was measured by the nondestructive chlorophyll meter SPAD (model 501, Minolta, Japan). Each repetition was represented by the mean value of four measurements taken on adult leaves, located on the third (*C. hilariana*) and fourth (*E. uniflora*) nodes. All measured leaves were adult and non-senescent. All noninvasive measurements were performed on a leaf region whose distance from the tip was equivalent to one third of the entire leaf length.

Evaluation of gas exchange

Stomatal conductance (g_s), transpiration (E), and net CO_2 assimilation (A) were evaluated in adult leaves from the third node, with an infrared gas analyzer (IRGA; model LCA-2, Analytical Development Co. Ltda, Hoddesdon, England) with PLC-B chambers (model ADC, BioScientific Limited, Herts, England) between 7:00 and 10:00 AM in *E. uniflora* and between 9:00 and 11:00 PM in *C. hilariana*, due to the crassulacean acid metabolism of the latter (Liebig et al. 2001).

Membrane permeability

Total ionic permeability was determined in 15 leaf discs of *E. uniflora* and in 10 discs of *C. hilariana* (5 mm diameter, each), due to the higher leaf blade thickness of the latter. The leaf discs, which had been previously weighed and rinsed, were placed in a recipient with deionized water for 4 h, and the initial electrical conductivity of the solution (i) was then measured. After this, the solutions were transferred to an oven at 90 °C for 2 h, in order to provoke cell membrane rupture. After cooling, the final electrical conductivity of the solution (f) was measured. Membrane permeability was calculated by the formula $i/(i+f)\times 100$, as proposed by Tarhanen et al. (1999).

Statistical analysis

The experimental design was completely randomized, and each species was evaluated separately since they have completely different physiologies. For each species, two factors were set. The first one was the station, with five levels (200, 500, 800, 1400, and 1700 m away from the emission source). The second one was the time of exposure (0, 71, 118, and 211 days). Data were transformed whenever they did not present homogeneity of variance nor normal distribution and were then submitted to analysis of variance. Whenever a factor or the interaction between factors was significant by F test at 5 % probability, means were submitted to Tukey's test at 5 % probability for comparison.

Results

Characterization of the studied area

The climate of the studied region is type Aw; tropical hot and wet with two dry months (Köppen 1918). During the experimental period, northeast wind predominated in all months, with an average maximum speed of 1.9 $m\cdot s^{-1}$. Air temperature along the experimental period ranged from 20.71 to 25.62 °C, with the average value of 23.4 °C. Precipitation ranged from 53.9 to 201.4 mm per month, the heaviest rainfall being registered in November. The average for the experimental period was 88.7 mm.

Regarding the amount of total suspended particulates, only in February did the values exceed the secondary standard (66.8 $\mu g\cdot m^{-3}$, for a standard of 60 $\mu g\cdot m^{-3}$) (Fig. 2a). As for sulfur dioxide, the emission values remained below the secondary standard along the entire exposure period (Fig. 2b).

Deposition of particulate matter on leaf surface

No difference regarding deposited particulate matter per leaf area in relation to distances from the emission source was detected in either species (Fig. 3a).

Iron and sulfur quantification in leaf dry matter

Iron and sulfur quantification in plant matter was performed using composite samples, which were obtained by gathering samples from repetitions of a same station. Iron amounts in leaf dry matter ranged between 895 and 273 $mg\cdot kg^{-1}$ and between 596 and 93 $mg\cdot kg^{-1}$ in *E. uniflora* and *C. hilariana*, respectively (Fig. 3b). Sulfur foliar contents in *E. uniflora* ranged between 764 and 151 $mg\cdot kg^{-1}$, while in *C. hilariana*, it ranged between 2762 and 146 $mg\cdot Kg^{-1}$ (Fig. 3c).

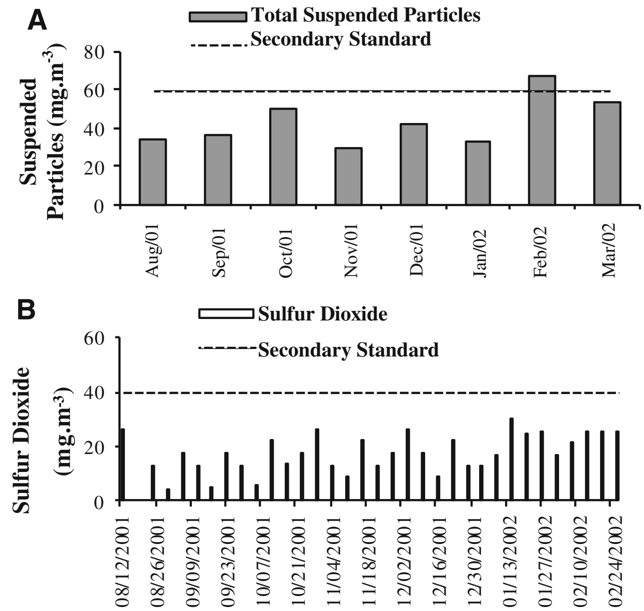

Fig. 2 Emissions from the mining industry. **a** Total suspended particles and **b** sulfur dioxide. Secondary standard according to CONAMA resolution, March 1990

Growth analysis

Emissions from the factory interfered with growth of neither species along the analyzed distances (data not shown). *E. uniflora* plants showed the highest height growth rates at stations 200, 500, and 1700 m away from the emission source (Table 3), while *C. hilariana* plants showed the highest growth rates at 500, 800, and 1700 m away from the emission source (Table 4).

In *E. uniflora*, the number of leaves per pot increased along with the collection dates (Table 1). However, no significant difference was observed in the number of leaves in relation to distances from the emission source (Table 3). *C. hilariana* showed no difference along the days of exposure (Table 2), but there was a significant decrease in relation to distances from the emission source (Table 4).

In *E. uniflora*, there was no difference in total dry matter in relation to distances from the emission source (Table 3). In *C. hilariana*, the highest dry matter production occurred in the nearest station to the factory (Table 4).

Foliar phytotoxicity

Leaf necrosis was the main visible symptom observed in the plants. The highest number of necrotic leaves was detected in

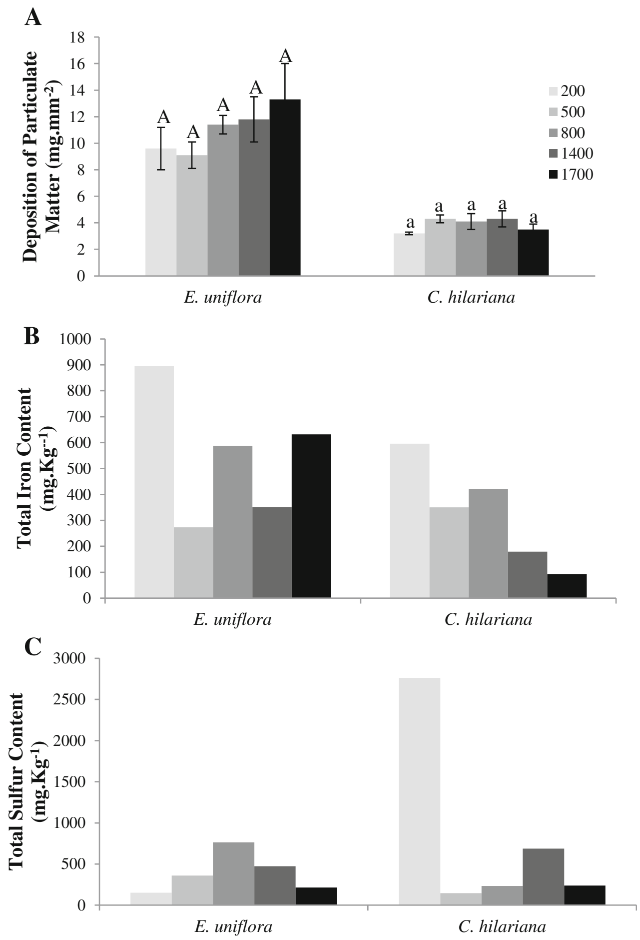

Fig. 3 Deposition of particulate matter per leaf area (**a**), total iron content (**b**), and total sulfur content (**c**) in leaves of *Eugenia uniflora* and *Clusia hilariana* at different distances from the emission source (200, 500, 800, 1400, and 1700 m). *Upper case letters* compare *Eugenia uniflora* and *lower case letters* compare *Clusia hilariana*. Means followed by the same letter do not differ by Tukey's test at 5 % probability. *Error bars* indicate the standard error

the last data collection in *E. uniflora* and from the penultimate collection in C. hilariana (Tables 1 and 2). Reduction in relation to distances from the emission source was observed at 500 m in *E. uniflora* and at 200 m in *C. hilariana* (Tables 3 and 4). In *E. uniflora*, there was interaction between evaluation dates and the analyzed stations (Fig. 4). Plants located 1400 m away from the emission source showed a higher number of necrotic leaves from October. In December, plants located 800, 1400, and 1700 m away showed the highest number of necrotic leaves. In the last evaluation date, all stations showed the highest number of necrotic leaves. Plants located at the nearest station to the emission source became necrotic only by the time of the last evaluation date (Fig. 4).

From the second collection date, the percentage of necrotic leaf area (necrosis-based foliar phytotoxicity index) in both species was higher than in the first collection and did not change on the subsequent dates (Tables 1 and 2). From the second collection, there was a high percentage of necrotic leaf area in *E. uniflora* (Tables 1 and 2). The distance to the emission source did not influence the percentage of necrotic leaves on the studied species (Tables 3 and 4). In *E. uniflora*, the interaction between collection dates and distances was significant, the highest percentages of necrotic leaves being registered at 800 and 1700 m away, in February (Fig. 4).

Chlorophyll content (SPAD index)

Chlorophyll concentration decreased gradually along with the evaluation dates in both species (Tables 1 and 2). Only in *E. uniflora* where there was a significant difference on total chlorophyll content among the evaluated distances, the highest average being registered 800 m away from the emission source (Table 3).

Evaluation of gas exchange

Stomatal conductance in *E. uniflora* did not change along with distances from the emission source, while in *C. hilariana*, there was a decrease from 800 m (Tables 3 and 4).

On both studied species, a decreasing tendency on transpiration rates was observed in relation to distances from the emission source (Tables 3 and 4).

Net CO_2 assimilation in relation to distances from the emission source did not vary in *E. uniflora*, while in *C. hilariana*, the lowest values were observed on the stations located 800 and 1400 m away (Tables 3 and 4).

When comparing gas exchange between the plants with the highest and lowest foliar iron amounts, a significant reduction was observed on photosynthesis values in *E. uniflora*. In

Table 1 Parameters evaluated in *Eugenia uniflora* on the days of exposure to the emissions from the industry. Data represent the average between the five distances from the emission source

Days of exposure (days)	0	71	118	211
Number of leaves (*n*)	29.07±0.49C	63.82±0.23AB	73.06±0.28A	89.66±0.35A
Number of necrotic leaves (*n*)	1.17±0.52D	2.95±0.64C	9.21±0.65B	32.77±0.36A
Foliar phytotoxicity index (%)	0.76±0.21B	1.23±0.33A	1.17±0.18A	1.50±0.18A
Chlorophyll (SPAD index)	41.00±0.19A	38.01±0.12A	31.49±0.16B	25.83±0.22C

Values are means followed by mean test ± SD (*n*=3). Means followed by the same letter in the lines do not differ by Tukey's test at 5 % probability

Table 2 Parameters evaluated in *Clusia hilariana* on the days of exposure to the emissions from the industry. Data represent the average between the five distances from the emission source

Days of exposure (days)	0	71	118	211
Number of leaves (*n*)	11.31±0.24a	11.50±0.28a	12.00±0.18a	11.26±0.23a
Number of necrotic leaves (*n*)	0.52±0.38c	3.90±0.56b	4.23±0.53a	5.47±0.30a
Foliar phytotoxicity index (%)	0.38±0.26b	1.30±0.22a	1.13±0.13a	1.51±0.21a
Chlorophyll (SPAD index)	65.26±5.31a	53.34±8.72bc	56.89±6.97b	50.22±8.71c

Values are means followed by mean test ± SD (n=4). Means followed by the same letter in the lines do not differ by Tukey's test at 5 % probability

C. hilariana, both stomatal conductance and transpiration increased with increasing iron contents (Table 5).

Nonlinear correlation coefficients between foliar sulfur contents and parameters g_s and A were estimated in *C. hilariana* as 98 and 88 %, respectively (Fig. 5).

Membrane permeability

Significant difference on total ionic permeability of cell membranes in relation to distances from the emission source was observed only in *C. hilariana*, the highest values being detected on plants located 500 m away from the emission source, followed by plants located 200 m away (Tables 3 and 4).

Discussion

Data on SPM_{Fe} and SO_2 emissions along the 7 months throughout which the present experiment was conducted proved that the pelletizing factory was responsible for the increased levels of these pollutants in the atmosphere, which, in February, exceeded the secondary standard for deposition of suspended particles. Regarding sulfur dioxide, the emission values were acceptable according to CONAMA resolution 03/1990 (National Council for the Environment, the Brazilian agency that legislates over the National Environmental Policy) (CONAMA 1990). The emissions from the factory could be confirmed by the foliar iron deposition and by the quantification of iron and sulfur contents on the *E. uniflora* and *C. hilariana* plants exposed at different distances from the emission source.

C. hilariana leaves are extremely waxy (Schneider 1985; Silva et al. 2005). Epicuticular wax features might have contributed to the lower iron retention on the leaf of this species, in relation to *E. uniflora*. The plaque-shaped epicuticular wax visualized by Silva et al. (2005) might have favored the non-adherence of iron particles to the leaves. Some specific features of the epicuticular wax, such as its chemical composition and the structure of its layers, might be related to its potential for capturing atmospheric particulates (Kaupp et al. 2000; Jouraeva et al. 2002; Dzierżanowski et al 2011). Along with the epicuticular wax, other factors, such as precipitation and wind, may have been capable of reducing foliar iron deposition, as verified with other pollutants (MacLean et al. 1989).

The total iron content found on *E. uniflora* and *C. hilariana* leaves after 7 months of exposure to particulate deposition exceeded the needs of crop plants cited by Larcher (1995) and Dobermann and Fairhurst (2000). In *C. hilariana*, on

Table 3 Parameters evaluated in *Eugenia uniflora* at different distances from the emission source. Data represent the average between the four evaluation dates

	E. uniflora				
	200 m	500 m	800 m	1400 m	1700 m
Height growth rate (m/7 months)	0.014AB±0.004	0.013AB±0.001	0.004C±0.003	0.007BC±0.002	0.015A±0.004
Number of leaves	67.89A±0.38	58.66A±0.59	54.04A±0.58	69.05A±0.55	48.68A±0.61
Total dry matter (MS) (g)	13.21A±2.36	12.96A±2.15	10.43A±2.22	11.68A±0.43	14.12A±2.55
Number of necrotic leaves	7.08A±1.01	4.64B±1.18	6.92A±1.17	8.39A±1.18	5.42A±1.44
Foliar phytotoxicity index (%)	1.35A±0.27	1.16A±0.30	1.15A±0.14	1.04A±0.22	1.08A±0.30
Chlorophyll (SPAD index)	32.42AB±0.23	33.02AB±0.26	41.67A±0.19	30.17B±0.27	31.65AB±0.19
Stomatal conductance (g_s) ($mol \cdot m^{-2} \cdot s^{-1}$)	0.18A±0.04	0.25A±0.04	0.13A±0.01	0.22A±0.13	0.13A±0.01
Transpiration (E) ($mol \cdot m^{-2} \cdot s^{-1}$)	0.93AB±0.33	1.39A±0.17	0.82AB±0.07	0.97AB±0.40	0.53B±0.04
Net CO_2 assimilation (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.48A±0.14	0.44A±0.12	0.31A±0.12	0.27A±0.08	0.40A±0.06
Total ionic permeability (%)	13.26A±2.87	13.66A±5.50	11.89A±1.55	12.09A±1.23	11.41A±1.99

Values are means ± SD (n=3). Means followed by the same letter in the lines do not differ by Tukey's test at 5 % probability

Table 4 Parameters evaluated in *Clusia hilariana* at different distances from the emission source. Data represent the average between the four evaluation dates

	C. hilariana				
	200	500	800	1400	1700
Height growth rate (m/7 months)	0.020B±0.005	0.053A±0.01	0.056A±0.03	0.029AB±0.002	0.056A±0.01
Number of leaves	11.78A±0.16	12.48A±0.18	12.48A±0.20	11.70AB±0.26	9.44B±0.25
Total dry matter (MS) (g)	7.49A±0.63	4.83B±0.38	3.92B±1.36	3.83B±0.71	3.98B±0.48
Number of necrotic leaves	2.36B±0.65	4.04A±0.7	3.12A±0.69	3.72A±0.79	3.02A±0.72
Foliar phytotoxicity index (%)	1.05A±0.28	1.26A±0.24	0.85A±0.18	0.87A±0.26	1.24A±0.38
Chlorophyll (SPAD index)	55.23A±7.88	54.22A±6.52	60.81A±8.27	56.22A±8.62	55.64A±13.51
Stomatal conductance (g_s) (mol·m^{-2}·s^{-1})	0.10A±0.02	0.13A±0.03	0.05B±0.02	0.05B±0.02	0.04B±0.01
Transpiration (E) (mol·m^{-2}·s^{-1})	0.29A±0.10	0.35A±0.05	0.24AB±0.08	0.17AB±0.12	0.09B±0.03
Net CO_2 assimilation (A) (μmol·m^{-2}·s^{-1})	0.55AB±0.28	0.81A±0.28	0.34B±0.07	0.31B±0.05	0.38AB±0.18
Total ionic permeability (%)	21.05AB±3.64	28.15A±2.15	14.10B±2.32	17.86B±1.58	17.56B±1.68

Values are means ± SD (n=4). Means followed by the same letter in the lines do not differ by Tukey's test at 5 % probability

plants located 1400 m away from the emission source, iron content was within the limits of normality for crop plants, although the plants presented considerable necrosis. Iron contents on plants located 200 m away from the emission source reached 895 and 596 mg·kg^{-1} in *E. uniflora* and *C. hilariana*, respectively, exceeding the toxicity limit stipulated for crop plants, of 500 mg·kg^{-1} (Marschner 1995; Dobermann and Fairhurst 2000). These data suggest that for these native species, the critical level is lower than the one stipulated for crop plants.

E. uniflora seems to demonstrate a capacity for acclimation in the presence of the factory emissions. This could be verified by the production of a higher number of leaves with increasing days of exposure. *C. hilariana*, on the other hand, showed higher number of new leaves on the nearest stations to the emission source. Iron and sulfur, both essential for plants, may have acted as foliar fertilizers on the studied species, thus

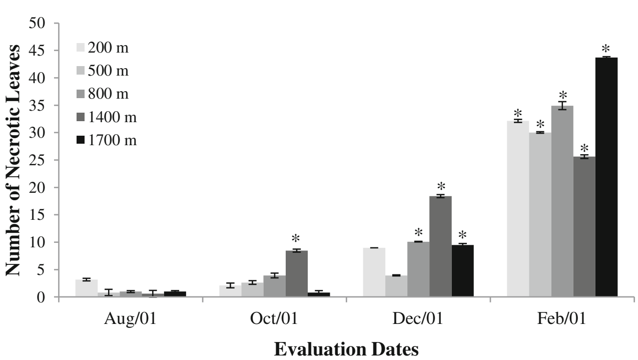

Fig. 4 Interactions between collection dates and distances from the emission source in plants of *Eugenia uniflora* exposed to the depositions from the factory for 7 months, from August 2001 to March 2002, regarding number of necrotic leaves. *Asterisks* indicate significance in the interactions. *Error bars* indicate the standard error

stimulating the production of leaf biomass, as reported to *Pinus ponderosa* by Momen et al. (2002).

Although no difference among the sampled stations could be observed, the height of the factory chimney as well as climatic conditions seem to have produced a protective effect against the deposition of particulate matter on the *C. hilariana* plants located nearest to the chimney, since plants of the station located 200 m away from the emission source presented the highest total dry matter and the lowest number of necrotic leaves. In *E. uniflora*, however, the lowest number of necrotic leaves was found on plants located 500 m away from the emission source. These results are in agreement with those presented by Lopes et al. (2002), according to which saplings of native plants from the Restinga exposed to similar depositions to the ones of the present work showed the highest number of necroses on plants located 800 and 1400 m away from the emission source. According to Grantz et al. (2003), depending on climatic conditions and on topographic features, nanoparticles can remain in the air for days or even months and may be transported from 1000 to 10,000 km away from the emission source.

According to Spiro and Stigliani (2002), the longer the time of exposure to atmospheric pollutants, the higher is the percentage of damage presented by the plants. This was also proved in the present study, with both *E. uniflora* and *C. hilariana*, since the longer was the period of exposure to the factory emissions, the higher were the number of necrotic leaves and the foliar phytotoxicity index found, and the lower were the total chlorophyll contents.

The foliar iron and sulfur amounts found in *C. hilariana* plants located 200 m away from the emission source were responsible for damage to cell membranes, as shown by the high values of total ionic permeability observed in plant samples from that station. The significant increase in electrolyte

Table 5 Comparison between stomatal conductance (g_s), transpiration (E), and photosynthesis (A) between *Eugenia uniflora* and *Clusia hilariana* plants with the highest and lowest values of iron accumulation observed in plant tissues

Iron content	360 mg.k^{-1}			3989 mg.kg^{-1}		
Parameters	g_s	**E**	**A**	g_s	**E**	**A**
E. uniflora	0.19±0.05 A	0.68±0.13 A	0.60*±0.10 A	0.26±0.05 A	0.65±0.08 A	0.30*±0.05 B
Iron content	93 mg.k^{-1}			3092 mg.k^{-1}		
Parameters	g_s	**E**	**A**	g_s	**E**	**A**
C. hilariana	0.04*±0.00 b	0.09**±0.01 b	0.38±0.09 a	0.07*±0.01 a	0.17**±0.02 a	0.42±0.07 a

Upper case letters in the lines compare *E. uniflora* means and lower case letters compare *C. hilariana* means

*Significant at 5 % probability ($p \leq 0.05$); **significant at 1 % probability ($p \leq 0.01$)

leakage suggests that the stress caused by the excess iron, and sulfur may be related to a possible oxidative stress in this species, through formation of reactive oxygen species (ROS), which results in several physiological disturbances such as peroxidation of the phospholipid membrane and damage to plant tissues (Becana et al. 1998; Hänsch and Mendel 2005; Sinha and Saxena 2006; Karuppanapandian et al. 2011).

The decreased SPAD index in relation to days of exposure in both studied species indicates that two processes may have been affected: chlorophyll synthesis and/or its degradation. The synthesis may have probably been reduced due to the iron deposited on the leaf, which causes shading of the organ, limits incident radiation, and increases foliar temperature (Pereira et al. 2009). Chlorophyll degradation, on the other hand, could be the consequence of oxidative stress produced by the excess free iron ions, after iron absorption by the leaf (Jeong and Guerinot 2009; Jucoski et al. 2013).

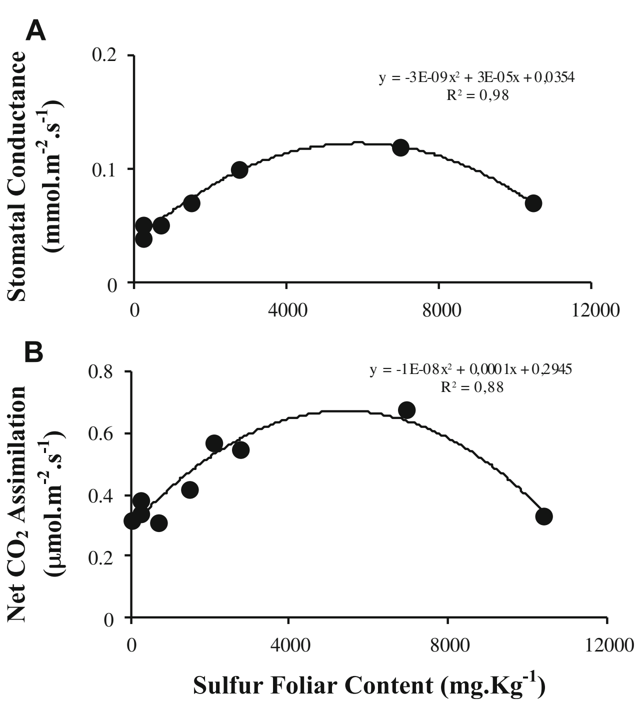

Fig. 5 Gas exchange according to sulfur foliar content, in *Clusia hilariana* exposed to particulate deposition of metallic iron for 7 months. **a** Stomatal conductance (g_s) and **b** net CO_2 assimilation (A)

The decrease in g_s, E, and A occurred at the largest distances from the emission source, especially in *C. hilariana*, which suggests a protective effect regarding deposition of particulate matter from the chimney on the nearest plants to the emission source. Chimney height, wind speed, and the presence of marine aerosol can favor the deposition of particulate iron at larger distances from the emission source (Grantz et al. 2003).

The obstruction of stomatal pores (Rocha et al. 2014) and the decreased incident luminous radiation by SPM_{Fe} may have been responsible for the decreased g_s and E in *C. hilariana*. The reduction on A, on the other hand, was the consequence of such stomata obstruction and of the decreased g_s and E, which may have possibly also decreased the carboxylation capacity on this species. Similar results were observed by Pereira et al. (2009), according to which the increased leaf temperature, which is caused mainly by the decreased incident luminous radiation, and the decreased conductance may be the explanation for alterations in gas exchange parameters in *C. hilariana* plants exposed to SPM_{Fe}.

Plants that accumulated the highest iron amounts in their leaves seem to have presented higher damage to their stomata. In *E. uniflora*, this fact could be verified due to decreased photosynthesis (A), although no variation in g_s and E was observed. In *C. hilariana*, the increase in g_s and E in plants with higher iron amounts did not reflect in an increased A. In that case, it seems that an alteration on the capacity of stomatal movements has occurred. In rice plants subjected to excess iron, the responsible factor for decreased photosynthetic parameters was stomatal limitation (Pereira et al. 2013).

C. hilariana accumulated ca. 10 $g \cdot kg^{-1}$ of sulfur in plant matter, a much higher amount than the one found by Klumpp et al. (1998), who exposed plants of *Psidium guajava* and *Psidium cattleianum* on several sites of the industrial complex of Cubatão city (São Paulo state, Brazil).

The correlation coefficients between foliar sulfur and g_s and between foliar sulfur and *A* in *C. hilariana* suggest an effect of SO_2 on stomatal conductance and net CO_2 assimilation in this species. According to Rennenberg et al. (1996a, b), since plants that present crassulacean acid metabolism keep stomata open at night, they get exposed to a higher amount of gaseous air pollutants. During the day, the high temperatures allow a greater dissipation of gaseous emissions, conversely to what happens at night, when the lower temperatures hamper the diffusion of gases to the environment, rendering leaves to be exposed for a longer period of time to the gaseous pollutants. Thus, CAM plants show higher sensitivity to SO_2 and this may be related to the diurnal differences in cellular capacity for SO_2 detoxification (Swanepoel et al. 2007). These factors may have been the reason for the greater damage caused by SO_2 exposure to *C. hilariana* than the one caused to *E. uniflora*.

Conclusions

The two species showed different strategies when exposed to the pollutants. *E. uniflora* presented decreased values of height growth rate, number of necrotic leaves, chlorophyll analysis (SPAD index), and transpiration, in relation to the distances from the emission source. *C. hilariana* presented decreased values of height growth rate, number of leaves, number of necrotic leaves, total ionic permeability, stomatal conductance, transpiration, net CO_2 assimilation, and total dry matter, in relation to the distances from the emission source. In relation to days of exposure, both species presented increased values of number of necrotic leaves and foliar phytotoxicity index, and decreased values in the chlorophyll analysis. *E. uniflora* seems to demonstrate a capacity for acclimation in the presence of the stressing agent, producing a higher number of new leaves with increasing days of exposure to the pollutants.

The native plant species that occur in the Brazilian Restinga showed damage when exposed to emissions from an iron ore pelletizing factory. *C. hilariana* presented higher sensitivity to SO_2 and greater alterations in the analyzed parameters in comparison to *E. uniflora*, thus being considered the most sensitive species.

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