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Pollution emissions from a petrochemical complex and other environmental stressors induce structural and ultrastructural damage in leaves of a biosensor tree species from the Atlantic Rain Forest

Andrea Nunes Vaz Pedroso[a,*], Filippo Bussotti[b], Alessio Papini[c], Corrado Tani[c], Marisa Domingos[a]

[a] Instituto de Botânica, Núcleo de Pesquisa em Ecologia, Caixa Postal 68041, CEP 04045-972 São Paulo, SP, Brazil
[b] Università degli Studi di Firenze, Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Piazzale delle Cascine 28, 50144 Firenze, Italy
[c] Università degli Studi di Firenze, Dipartimento di Biologia, Via Micheli 3, 50121 Firenze, Italy

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ABSTRACT

Tibouchina pulchra (Cham.) Cogn., a pioneer tree species commonly found in the Atlantic Rain Forest in southeast Brazil, proved to be a promising biosensor species for indicating the potential effects of air pollution on this type of forest. This study aimed to investigate whether the present levels of nitrogen oxides, sulfur oxides, ozone and particulate matter in industrial and urban areas in the Cubatão region induce structural and ultrastructural changes in this species, to explore the synergy between air pollutants and the effects of other environmental stressors as contributors to microscopic symptoms and to verify whether these changes are seasonally dependent. The field study consisted of exposing potted saplings of *T. pulchra* in three sites around the petrochemical industry (industrial area), in downtown Cubatão (urban area) and in a site far from the industrial emissions (reference area). An experimental study using open top chambers (OTC) with filtered air and non-filtered air was also carried out to confirm the field results. Four exposure periods of 12 weeks were conducted during the field and the OTC experiments, covering wet and dry periods. The effects were more pronounced in plants exposed in the industrial areas during the dry period, which were confirmed by OTC experiments. Crystal density variations were linearly explained by decreases in NO_2, SO_2 and the sum of all hourly concentrations of ozone (SUM00), as well as increases in PM_{10}, relative humidity and rainfall. Variations in lipid peroxidation correlated positively with air temperature and SUM00. Tannins and starch grains decreased. Collapsed and sinuous cell walls, dense cytoplasm, invagination of plasma membranes and vacuome were the main effects observed at the structural level. Occurrence of pectic warts, alterations in chloroplasts and numerous vesicles close to the cell walls were the main ultrastructural effects detected. These results confirmed that pollutants and environmental variables acted synergistically to induce oxidative stress in this species, enhancing our understanding of the bioindicator potential of *T. pulchra* for monitoring complex mixtures of air pollutants in the tropics.

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1. Introduction

Industrial activities produce a large quantity of air pollutants, such as sulfur oxides, nitrogen oxides and particulate matter containing toxic organic and inorganic components. In addition, ozone (O_3) originates from photochemical reactions in the atmosphere between primary pollutants, such as nitrogen oxides and volatile organic compounds (VOC). Atmospheric concentrations of both primary and secondary pollutants in the surroundings of industrial sources as well as damage to natural ecosystems have been shown to be higher than in the countryside (WHO, 2005; Paoletti, 2006).

The industrial complex, started in the city of Cubatão (SE, Brazil) in 1950, is one of the world's most famous examples of environmental pollution, with consequent decline of the Atlantic Rain Forest, known for its high species biodiversity (Domingos et al., 2003a). An oil refinery is among the most polluting industries in the Cubatão region (CETESB, 2015). It is the major emission source within a petrochemical complex, which is also composed of other

* Corresponding author. Tel.: +55 11 5067 6129; fax: +55 11 5073 3678.
E-mail address: andreanvpedroso@gmail.com (A.N.V. Pedroso).

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petrochemical and chemical plants located at the foot of mountains covered by the Atlantic Forest. The refining processes at the oil refinery in particular, in association with the operation of its new thermoelectric power plant fueled by natural gas, still emit considerable amounts of SO_2, NO_2, VOCs and particulate matter (PM) enriched by toxic constituents like trace metals (Nakazato et al., 2015). Increased ozone concentrations have also been registered around this petrochemical complex (CETESB, 2015).

The potential impacts of air pollutants from the industrial pole of Cubatão on the Atlantic Rain Forest were extensively monitored between 1990 and 2000 with standardized and native indicator plant species (Klumpp et al., 2001; Domingos et al., 2003a,b), helping to establish a more refined pollutant emission control policy. *Tibouchina pulchra* (Cham.) Cogn. (Melastomataceae) was shown to be a promising biomonitor tree species growing in the Atlantic Rain Forest (Domingos et al., 2003a). It is a pioneer species and well distributed throughout the Cubatão region (Martínez-Ramos, 1985; Leitão-Filho et al., 1993). *T. pulchra* was successfully used as a fluorine and metal accumulator in the same region (Klumpp et al., 1996a,b; Domingos et al., 2003b). In addition, although it does not display visible foliar injury induced by air pollutants, among them ozone (Moraes et al., 2003, 2004; Furlan et al., 2008; Santos and Furlan, 2013), several studies have identified biochemical and physiological effects of air pollution on this species (Domingos et al., 1998, 2003a,b; Furlan et al., 2004; Klumpp et al., 1996a,b, 1998, 2001; Silva and Moraes, 2013), revealing its biosensor potential for biomonitoring purposes (*sensu* Falla et al., 2000; De Temmerman et al., 2004).

Microscopic leaf injuries caused by air pollutants in woody plants from temperate regions have been indicated as biomarkers of phytotoxic levels of ozone and other environmental stressors (Günthardt-Goerg and Vollenweider, 2007; Vollenweider et al., 2003, 2013, 2015). Inclusively, these investigations have helped to establish the bioindicator potential of such biosensor plant. Although little is known about the microscopic responses of tropical tree species (Tresmondi and Alves, 2011; Moura et al., 2014), we may assume that the combination of air pollutants around the aforementioned petrochemical complex in Cubatão acts synergistically with other environmental stressors on the native biosensor species of the Atlantic Rain Forest. This synergy may induce leaf injuries at the structural and ultrastructural levels (Falla et al., 2000; De Temmerman et al., 2004). This study aimed to investigate whether the present level of industrial air pollution in Cubatão is high enough to induce structural and ultrastructural changes in the leaves of *T. pulchra*, to explore the possible synergy between air pollutants and other environmental stressors in inducing microscopic symptoms and to verify whether the severity of these microscopic symptoms are seasonally dependent.

2. Materials and methods

2.1. Description of the study area and plant exposure

The industrial area chosen for conducting this field study is located in the city of Cubatão, southeast Brazil (23°53′42″S; 46°25′31″W). The area is composed of 19 industries, totaling 230 pollutant emission sources (CETESB, 2015).

The field study was performed in the petrochemical complex of that industrial area, located at the foot of mountain slopes covered by the Atlantic Rain Forest. This area is mainly under the influence of inorganic compounds, like nitrogen and sulfur oxides, and secondary pollutants, among them ozone. The pollution dispersion is hindered by the rugged local topography. It is also affected by local meteorological conditions (CETESB, 2015). The predominant climate in Cubatão is Af (tropical, without dry season), according to Koeppen's classification (Alvares et al., 2013), which is characterized by high relative humidity during the whole year due to the high annual rainfall (2600 mm on average). The average annual temperature is 23 °C. The winds blow in opposite directions in a 24-h period. They are stronger during the daytime and blow from S and SW to NE. Weaker winds blow from NE to SW at night. This wind pattern determines the pollution dispersion from the industrial and urban areas to the mountain slopes during the day (Fig. 1).

Three exposure sites were selected at different altitudes of Caminho do Mar road built on the slopes of the Serra do Mar and close to the petrochemical area: CM1 (23°51′46″S; 46°26′16″W); CM2 (23°51′26″S; 46°26′50″W) and CM3 (23°51′51″S; 46°27′44″W). The fourth site was located in downtown Cubatão (CET – 23º52′45″S; 46°26′16″W), which is affected by both petrochemical and vehicular pollutant emissions and where a monitoring station of air pollutants and meteorological conditions is managed by the Environmental Company of São Paulo State (CETESB) (Fig. 1). The fifth exposure site was located in a low pollution area, to the southwest of the industrial complex, in the Pilões River Valley (PRV – 23º54′19″S; 46º29′30″W). This last site was used as the reference because it is located out of the predominant wind direction (Fig. 1) and is thought to have been less affected by the industrial and vehicular emissions than the other sites.

The results from the field study were compared to those from an experimental study carried out in parallel in four open top chambers (OTC) assembled next to the CET site (Fig. 1), in an area belonging to the Polytechnic School of the University of São Paulo (23°53′10″S–46°26′16″W; 15 m a.s.l.). Two chambers received ambient and non-filtered air (NF), and the other two chambers received filtered air (FA). Thick and fine particles were removed from the air by mechanical filters and inorganic and organic gases by a series of chemical beds prepared with activated carbon and aluminum oxide pellets impregnated with potassium permanganate, all of them from Purafil Inc. The same airflow was set in the chambers using manometers. More details about the OTC design are described in Esposito and Domingos (2014).

All the saplings of *T. pulchra* for both field and OTC experiments were obtained from the plant nursery managed by the São Paulo State Energy Company (CESP). They all had similar developmental characteristics (approximately six-months old with a height of 10–20 cm). All the saplings were transplanted into 1.5 L pots containing standardized substrate (a mixture of a commercial substrate primarily composed of *Pinus* bark and fine vermiculite in a 3:1 ratio) and fertilized with Hoagland solution. They were kept inside a glasshouse under filtered air and at an ambient temperature at the Institute of Botany in São Paulo until exposure under field or OTC conditions.

The saplings were exposed during four consecutive periods of 12 weeks each (from May/2011 to October/2012) in both field and OTC experiments (Table 1). Three of the field experiments were conducted concurrently with OTC experiments, with the aim to better establish the associations between microscopic injuries in saplings and environmental stressors. In all cases, the saplings were exposed under shading nets (reduction of 50%) in order to protect against excessive radiation and were continuously irrigated by means of strings, in accordance with the guidelines from the German Association of Engineers (VDI, 2003).

Each exposure experiment in both conditions was started with 12 plants per field site or OTC. Sub-lots of six plants were randomly collected after six and 12 weeks for microscopic analysis of leaves, summing up a total of 392 plants sampled and analyzed.

2.2. Description of microscopic analyses

Structural and ultrastructural symptoms at both tissue and cell levels were searched for in leaf blade fragments taken from the

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