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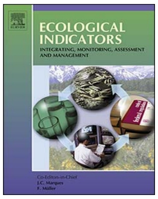

Original Articles

Sensibility of *Spondias purpurea* L. (Anacardiaceae) exposed to fluoride-simulated fog

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ABSTRACT

Fluoride is the atmospheric pollutant with the highest phytotoxicity arising from aluminum smelting fertilizers, glass and, ceramic industry. The most affected plant organ is the leaf, in which the fluoride penetrates through two main ways: absorption by epidermis cuticle or absorption by stomata. Species susceptible to fluoride are potential tools in bioindicator studies because they provide a means for detecting the presence of the pollutant in the environment at a low cost. The aim of this study is to evaluate the sensibility of *Spondias purpurea* L. (Anacardiaceae) to fluoride through the simulated fog. Simulation was performed by applying 500 ml of solution containing 15 mg L^{-1} of potassium fluoride per plant for 20 min daily and for 10 consecutive days. The data of leaflet abscission, visual damage, and climate conditions were recorded daily. At the end of the experiment, data from the cell death analysis, electrolyte leakage determination, and accumulation of the fluoride in the dried matter were collected. Moreover, samples for phenolic compound accumulation, anatomical, and micromorphological analyses were collected. After 24 h, the appearance of visible damage was observed in the leaves with fluoride treatment. This damage consisted, mostly, of necrosis, chlorosis, and leaf apical shriveling. The necroses manifested in gray and brown discoloration. Leaf abscission was intense in the young leaves. Microscopic damage consisted of protoplast retraction, phenolic compound accumulation, collapse, hyperplasia, and cell rupture. Turgidity loss, epicuticular wax erosion, and damage of stomata and trichomes beyond the presence of fungal hyphae were noted in the epidermis. Evans Blue detected dead cell groups in the transition region between the necrosis and the apparent healthy area. Fluoride affected the selective permeability of the membrane, which was observed in this study according to the high rate of electrolyte leakage. Plants exposed to fluoride accumulated 14.48 times more fluoride in their leaves than plants in the control group. It follows that *S. purpurea* is susceptible to fluoride and responds quickly to the presence of this pollutant. Marginal and apical necrosis, presence of phenolic compounds, fluoride accumulation, anatomical alterations, and leaflet abscission in the young leaves are biomarkers of fluoride effects in this species. Therefore, *S. purpurea* is potentially useful in biomonitoring programs.

1. Introduction

Among the most common pollutants in the atmosphere, fluoride stands out owing to its high toxicity, even in low concentrations (Klumpp et al., 1996). Rodriguez et al. (2015) show in their studies conducted in Córdoba, Argentina, that the concentration of atmospheric pollutants can vary throughout the year, being higher in the dry seasons, ultimately contributing to their accumulation in the atmosphere. The main sources of fluoride are the aluminum smelting industry, steel mills, fertilizers, glass, and pottery production (Weinstein and Davison, 2004). Once released into the atmosphere, fluoride can advance throughout expansive regions and combine with water particles reducing the pH of rain. The infiltration of rainwater with high levels of fluoride in the soil can contaminate the groundwater, thereby affecting agricultural activities and its consumption by humans (Naazi et al., 2015).

Owing to its high toxicity, fluoride causes different types of damage in plants, with leaves being the most sensitive organ to this pollutant and the main entry point (Fornasiero, 2001). The entry of fluoride in a leaf depends on the physical state of this pollutant. In gaseous form, fluoride penetrates through the stomata. On the other hand, when dissolved in water, the leaf surface absorbs fluoride through the cuticle and epidermis, facilitated by trichomes and stomata (Azevedo, 1995; Pita-Barbosa et al., 2009). Fluoride transportation inside the cells can occur through ionic canals and/or through the apoplast, which accumulates in the margin and in the apex of the leaves due to the

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evapotranspiration current (Miller, 1993).

Once in contact with the leaf surface and inside this organ, fluoride can cause visual damage such as leaf shriveling, necrosis, and chlorosis (Rodrigues et al., 2017; Silva et al., 2000). Anatomic and micromorphological studies have proven to be important in the assessment of damage by fluoride, as they revealed that microscopic injuries precede visual symptoms (Sant'Anna-Santos et al., 2012). The physiological analysis shows that contamination by fluoride reduces the photosynthetic rate (Divan Junior et al., 2007; Elloumi et al., 2017). It is also associated with chloroplast disruption, dysfunction in the enzymes of the photosystem II and enzymes involved in the antioxidation process (Peixoto et al., 2005; Mondal, 2017; Zouari et al., 2017). In association with micromorphological damage, studies show that fluoride promotes cuticle erosion, stomata obliteration, protuberances, and depressions in the epidermis (Campos et al., 2010; Louback et al., 2016; Sant'Anna-Santos et al., 2012). In the mesophyll, studies have found disorganization of the tissues, accumulation of phenolic compounds, and plasmolysis of parenchymatous cells (Azevedo, 1995; Louback et al., 2016; Sant'Anna-Santos et al., 2012, 2014).

Plants are used in atmospheric biomonitoring, for the presence of fluoride in the air, because of the inherent sensibility in some species (Klumpp et al., 2001). This sensibility is associated with genetic, edaphic, and climatic factors (Weinstein and Davison, 2004). The characterization of the effects of fluoride on physiology and morphoanatomy of the sensitive species allows the identification and selection of biomarkers for fluoride pollution, which can be used in biomonitoring programs (Klumpp et al., 2001). It is worth mentioning that biomonitoring cannot replace physical–chemical methods, but it offers a low-cost alternative to obtain relevant information (Klumpp et al., 2001). Many companies have used plants as bioindicators. Eurobionet is an example of a network air quality monitoring system implemented in Europe to collect systematic data in certain cities throughout Europe. Eurobionet's goal is to create a standardized response inventory with the selected bioindicator species for the different pollutants (Klumpp et al., 2001). Installing a similar biomonitoring system in Brazil would require the use of tropical species because the species must fit the regional climate (Oliva and Figueiredo, 2005). In the Serra do Mar, Cubatão-SP, the Environmental Company of the São Paulo State (CETESB) has developed a significant biomonitoring system using plants to detect the presence of fluoride and other pollutants. Their work has the intention to evaluate the temporal and spatial distribution of the pollutant and to inform the public about their opinion on the air quality through scientific data (CETESB, 2015).

Spondias dulcis, a tropical species sensible to fluoride (Silva et al., 2000), is used for active monitoring to detect the presence of fluoride in air in the region of Ouro Preto, Minas Gerais – Brazil (Louback et al., 2016; Sant'Anna-Santos et al., 2014). Sant'Anna-Santos et al. (2012) selected among visual, morphoanatomical, and physiological symptoms the most appropriate characters to be used as biomarkers of fluoride effects.

Species of genus *Spondias* show a narrow genetic interrelation based on the AFLP (Amplified Fragment Length Polymorphisms) marks (Santos et al., 2002). Furthermore, studies reveal a high biologic compatibility so that it is possible to propagate some species from this genus by grafting (Santos and Oliveira, 2008). From the same genus, *Spondias purpurea* is a subtropical species native to forests in Central America, whose fruit, known as "seriguela," is appreciated as a food source as well a natural medicine used to treat diseases such as diarrhea, ulcer, dysentery, and swelling (Engels et al., 2012). The "seriguela" fruit is gaining economic importance owing to the juice and drink industries in addition to its use as a stimulus in biological studies and development of the technologies that assist postharvest management (Maldonado-Astudillo et al., 2014). "Seriguela" seeds were also studied for bioabsorption. The seed biomass can accumulate metals that pollute hydric resources (Arshadi et al., 2015).

There are strong pieces of evidence establishing the sensibility of *S. purpurea* to fluoride; however, studies on this species involving fluoride or other pollutants are absent from the scientific literature. Considering that *S. purpurea* belongs to the same genus of *S. dulcis*, already established as a bioindicator of the presence of fluoride, the hypothesis of the present work is that *S. purpurea* is also sensitive to fluoride. Therefore, this study has the goal of verifying the sensitivity of the *S. purpurea* L. (Anacardiaceae) to fluoride by utilizing characters of visual symptomatology, anatomical and micromorphological analyses, fluoride accumulation, dead cell evaluation, and electrolyte leakage.

2. Materials and methods

2.1. Botanic material

Hardwood cuttings (with 45 cm length and 4 cm diameter) were taken from *S. purpurea* L. (Anacardiaceae) from one healthy adult plant, located in Viçosa, MG, Brazil (20°42′52″ **S** latitude – 42°51′02″ **W** longitude, and altitude of 649 m). They were subjected to 600 mg of AIB and planted in pots containing sand and low liberation fertilizer, Osmocote®, and were regularly drizzled with water. After 2 months, 1–4 side shoots of about 15 cm developed in each hardwood. The total seedling heights were approximately 60 cm (45 cm hardwood plus 15 cm side shoots).

After 3 months, the plants were transferred to the greenhouse located in the UFV Growth Plants Unit, 649 m of altitude, Lat 20°45′20″ e and Long 42°52′40″. The plants were transplanted in 3.8-L pots containing an organic substrate, Tropstrato HT®, based on pine bark, peat, and expanded vermiculite normally used to cultivate hardwood vegetables. One week after the transplant, the plants received Hoagland nutritive solution, 0.5 ionic power, and pH = 6.5 (Hoagland and Arnon, 1950). The hydroponic solution was composed of 0.5 mM $NH_4H_2PO_4$, 3 mM KNO_3, 2 mM$Ca(NO_3)_2.4H_2O$, 1 mM $MgSO_4.7H_2O$, 23.13 µM H_3BO_3, 4.57 µM $MnCl_2.4H_2O$, 0.382 µM $ZnSO_4.7H_2O$, 0.16 µM $CuSO_4.5H_2O$, 0.0695 µM MoO_3, and 9 µM Fe-EDTA. Hoagland solution (100 ml per plant) was applied weekly, during all acclimation period and until the end of the experiment, totaling 6 weeks (Silva et al., 2000).

Environmental conditions such as temperature [°C], relative humidity of air [%], and wind speed [$m.s^{-1}$] were recorded for each day using the manual register device Kestrel® (model 4300; Nielsen-Kellerman, EUA). Light radiation [$\mu mol.s^{-1}.m^{-2}$] was measured with a radiometer (model LI-185B; LI-COR, Lincoln Nebraska, EUA). In the course of the experiment, the average temperature was 27.49 °C, relative humidity of air was 51%, wind speed was 0.31 $m.s^{-1}$, and light radiation was 346.8 $\mu mol.s^{-1}.m^{-2}$. The experiment was also carried out on cloudy days.

2.2. Fluoride application

The application technique was based on the study by Pita-Barbosa et al. (2009). The plants were exposed to the spray containing fluoride (15 $mg\,L^{-1}$) daily, at 9 am, for 10 days. The volume applied in each plant was 500 ml of solution stipulated as sufficient to wet the entire surface of the plant. For this, a precompression hand sprayer Guarany® was used in the thinnest regulation forming water droplets on the leaf surface.

Fluoride was added as potassium fluoride (fluoride 15 ppm) to deionized water at pH 6.0 (Silva et al., 2000). For the control treatment, only deionized water, pH 6.0, was used. The fog simulation by spray occurred outside of the greenhouse. Four properly standardized experimental units (n = 4) were used for each treatment (fluoride and control).

2.3. Symptomatology

S. purpurea has alternate pinnate leaves with 7–23 leaflets. These