Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v38i4.31400

Ecophysiological, anatomical and ultrastructural characteristics of *Vitex polygama* Cham. (Verbenaceae) submitted to different concentrations of fluoride

Zélia Terezinha Teixeira Rossi[1*], Evaristo Mauro de Castro[2], Giuslan Carvalho Pereira[3], João Paulo Rodrigues Alves Delfino Barbosa[2], Graziele Wolff de Almeida Carvalho[3], Marco Aurélio de Lima Silva Rossi[2] and Fabrício José Pereira[2]

*[1]Instituto Federal de Minas Gerais, Cx. Postal 35, 35570-000, Formiga, Minas Gerais, Brazil. [2]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. [3]Instituto Federal de Minas Gerais, São João Evangelista, Minas Gerais, Brazil. *Author for correspondence. E-mail: zelia.rossi@ifmg.edu.br*

ABSTRACT. Fluorides released during the manufacture of aluminum, fertilizers, glass and ceramics are considered to be highly toxic to plants, causing damages in relatively small concentrations. This study aimed to evaluate fluoride (F) accumulation and its effects on *Vitex polygama* Cham. (Verbenaceae) exposed to simulated rain containing potassium fluoride in concentrations of 5, 10, 15 and 20 mg L^{-1}. *Vitex polygama* presented no visual symptoms of foliar injury and accumulated a relatively small amount of F-, significant only for the treatments with higher concentrations of the pollutant. F- promoted an increase in non-photochemical quenching (qN) and in the coefficient due to non-photochemical extinction (NPQ), which indicates higher dissipation of radiant energy in the heat form. Damages in the chloroplast structure, in cellular membranes and in epicuticular waxes, besides cytoplasm granulation and irregularities in the epidermal cell walls were also detected in plants exposed to the pollutant. The results show a considerable resistance of *Vitex polygama* to F and reinforce the prognostic value of physiological, anatomical and ultrastructural analysis in the detection of damages caused by the pollutant in the leaf structure of this species.

Keywords: chlorophyll a fluorescence, potassium fluoride, scanning electron microscopy, transmission electron microscopy, biomonitoring studies.

Características ecofisiológicas, anatômicas e ultraestruturais de *Vitex polygama* Cham. (Verbenaceae) submetida a diferentes concentrações de flúor

RESUMO. Fluoretos, liberados durante a manufatura de alumínio, fertilizantes, vidro e cerâmica, são considerados altamente tóxicos para as plantas, causando danos em concentrações relativamente pequenas. Este estudo teve como finalidade avaliar o acúmulo de flúor (F) e seus efeitos sobre *Vitex polygama* Cham. (Verbenaceae) em plantas expostas a chuva simulada contendo fluoreto de potássio em concentrações de 5, 10, 15 e 20 mg L^{-1}. *Vitex polygama* não apresentou sintomas visíveis de injúria foliar e acumulou uma quantidade relativamente pequena de F-, significativa apenas para os tratamentos com maiores concentrações do poluente. O F- promoveu aumentos no coeficiente devido à extinção não fotoquímica (qN) e no coeficiente devido à extinção não fotoquímica (NPQ), que indicam maior dissipação de energia radiante na forma de calor. Danos na estrutura dos cloroplastos, em membranas celulares e nas ceras epicuticulares, além de granulação do citoplasma e irregularidades na parede celular de células epidérmicas, foram também detectados em plantas expostas ao poluente. Os resultados demonstram uma considerável resistência de *Vitex polygama* ao F e reforçam o valor prognóstico de análises fisiológicas, anatômicas e ultraestruturais na detecção de danos causados pelo poluente na estrutura foliar dessa espécie.

Palavras-chave: fluorescência da clorofila a, fluoreto de potássio, microscopia eletrônica de varredura, microscopia eletrônica de transmissão, estudos de biomonitoramento.

Introduction

Fluorine and fluorides, pollutants which can be released by human activities linked to brickworks, aluminum factories, glassworks, steelworks, ceramic factories, phosphate fertilizer plants and uranium smelters (Gheorghe & Ion, 2011), are mainly absorbed in the form of gases, by the leaves of the vegetation near the polluting industry, accumulating in tips and foliar margins (Miller, 1993) and causing chlorosis and necrosis (Mesquita, Tanaka, Cantarella, & Junior, 2011). However, even in the absence of visible injuries, plants exposed to F can

accumulate those pollutants and present changes in physiological, anatomical and ultrastructural characteristics (Singh-Rawal, Jajoo, & Bharti, 2010; Mesquita et al., 2011). Pollutants can also accumulate in cellular organelles (such as mitochondrias, chloroplasts and vacuoles), causing changes in their structures (Miller, 1993). Moreover, it is known that fluoride can affect the operation of photosystem II (PSII) inhibiting the electron transport rates on it (Singh-Rawal et al., 2010).

The use of bioindicators (biological processes, species or communities that permit to assess the quality of the environment and its changes over time due to anthropogenic disturbances or natural stressors) has proved to be very beneficial because it enables to assess the cumulative impacts of chemical pollutants over time and indicates indirect biotic effects of pollutants that would not be identified by physical or chemical measurements (Holt & Miller, 2010). The study of how a plant found in areas polluted by fluorides respond macroscopically and microscopically to this stressor agent can help indicating it as a bioindicator of this pollutant.

Vitex polygama Cham., popularly known as "tarumã", "Maria-preta" and "velame-do-campo" belongs to the family Verbenaceae (Cantino, Harley, & Wagstaff, 1992), can be found as trees or shrubs and present wide distribution. In Brazil, it appears in the States of Minas Gerais, Espírito Santo, Rio de Janeiro and São Paulo (Moldenke & Moldenke, 1957). They have been found in Poços de Caldas, south of Minas Gerais (Van Den Berg, Santos, Castro, & Ferreira, 2007). In this region, there are four companies of crystal manufacture and one aluminum smelter (Cappellin, 2008), activities that can release F in the atmosphere (Fornasiero, 2001).

This study aimed to evaluate fluoride accumulation and the changes caused by this pollutant in visual, ultrastructural and anatomical characteristics of *V. polygama*, as well as its effects on the chlorophyll *a* fluorescence of the PSII. These features can help to elucidate the action mechanism of F in the species, besides being an important subsidy to suggest the use of *V. polygama* in monitoring programs of atmospheric pollution by fluoride.

Material and methods

Saplings of *V. polygama* Cham. (Verbenaceae) were provided in tubes by the nursery of CEMIG-Lavras (*Companhia Energética de Minas Gerais*). The saplings with around 25.0 cm were transferred to plastic bags of 3.0 L, which were filled with a mixture of soil, sand and manure, in a 2:1:1 ratio, respectively. Plants stayed 120 days in a greenhouse of the Plant Physiology Sector in the Biology Department of *Universidade Federal de Lavras* (UFLA), for acclimatization, and, during this period, received nutrient solution of Hoagland and Arnon with 0.5 ionic strength (Hoagland & Arnon, 1950) every 15 days. Plants were also irrigated by deionized water, maintaining the substrate always humid.

Seven days before the beginning of the experiment, the plants were transferred to the laboratory of Ecology of the Biology Department (UFLA), for acclimatization, where they remained until the end of the rainfall simulations. This was performed in order to decrease the interference of environmental stressors on the response of the plants to the treatments. During the experimental period, plants were submitted to a photoperiod of 12 hours, with global radiation around 19.4 ±3.4 W m^{-2}, which corresponded to an average value of 88.2 ± 15.4 μmol photons m^{-2} s^{-1}. The maximum, medium and minimum temperature during the period was 24.2 ±1.0; 22.4 ±1.7; 20.8 ±1.3°C, respectively.

For 10 consecutive days, the plants were submitted to 9.15 mm simulated rain per day, for 15 minutes. Two chambers from the model proposed by Evans, Gmur, and Costa (1977) were used to simulate rainfall, one for the control and other for the fluoride treatments. The control treatment consisted only on deionized water, and the fluoride treatments were prepared dissolving potassium fluoride (KF) in deionized water, in the following concentrations: 5, 10, 15 and 20 mg L^{-1}. The pH values, in all the treatments, were measured using a portable pHmeter Hanna HI 98127, and adjusted to 6.0 using a solution of HCl 2N. The experiment was conducted in a completely randomized design, with eight replications per treatment.

During the experimental period, daily observations were carried out to detect the occurrence of injuries related to the fluoride action.

To determine the fluoride content, samples of leaves (young, in expansion and fully expanded) were collected in all the plants, 24 hours after the last simulation. The samples were previously dried at 70°C until constant mass was obtained, and ground to particles with dimensions inferior to 1.0 mm. Aliquots of 0.5 g were extracted with 0.1 M percloric acid (Garcia-Ciudad, Garcia-Criado, & Pontón-San Emeterio, 1985). For potentiometric determination of fluoride content, it was used an ionic strength adjuster (ORION) proposed by Larsen and Widdowson (1971), with specific electrode. The fluoride quantification analyses were performed at the Laboratory of Plant Anatomy in

Universidade Federal de Viçosa (UFV). The means of fluoride content in the dry mass were compared by Scott-Knott test at 5% probability, using the statistical program Sisvar 5.6 (Ferreira, 2011).

To evaluate the effects of fluoride on the photosystem II (PSII) operation, parameters of chlorophyll *a* fluorescence were measured before the beginning of the simulations (day 1) and 24 hours after the end of the experiment (day 11). The measurements were performed with the miniaturized pulse-amplitude-modulated photosynthesis yield analyzer (Mini-Pam) of H. Walz (Effeltrich, Germany) with a leaf clip holder model 2030-B (Bilger, Schreiber, & Bock, 1995). Measurements of the photochemical (qP) and non-photochemical (qN) quenching, coefficient due to non-photochemical extinction (NPQ), apparent electron transport rate (ETR) and quantum yield of photosystem II were obtained from central fully expanded leaflets located in the first node.

For the statistical analysis, the experimental design was completely randomized with five treatments and three replications. The data were submitted to analysis of variance (ANOVA) and, when the concentration effect proved to be significant, regressions were made according to the model adjustment, using the software Sisvar 5.6 (Ferreira, 2011). A Shapiro-Wilk normality test was used, and the fluorescence data (which proved not normal) were transformed using the equation $(x+1)^{0.5}$, where x represents the real mean.

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were performed for ultrastructural analysis. The sample preparations and the observations in TEM and SEM were conducted in the Laboratory of Electron Microscopy and Ultrastructural Analysis (LME) of the Phytopathology Department (UFLA). Twenty-four hours after the last rain simulation, leaf fragments with approximate 0.5 cm^2 were removed from the central leaflet located in the first node, and fixed in Karnovsky solution (glutaraldehyde (2.5%) and paraformaldehyde (2.5%) in cacodylate buffer, pH 7.0, 0.05 M + $CaCl_2$ 0.001M). After, the fragments were washed (three times of 10 minutes) in cacodylate buffer 0.05 M and post-fixed in osmium tetroxide (1%) in the same buffer.

For TEM, after the post-fixation in OsO_4 during four hours, the material was contrasted using uranyl acetate, dehydrated in increasing series of acetone (25, 50, 75, 90 and 100% for three times) and embedded in Spurr's resin. Ultrathin sections were cut on a Reichert-Jung Ultracut E ultramicrotome with a diamond knife, contrasted with plumbum citrate, and finally examined and photographed with a Transmission Electron Microscope Zeiss EM 109, with digital camera coupled (Alves, 2004).

For SEM, after the post-fixation in OsO_4 during one hour, the material was dehydrated in increasing series of acetone (25, 50, 75, 90 and 100% for three times) and immediately taken to drying at critical point with CO_2 used as media. The dried samples were sticked on stubs, exposing them to both sides of the leaf surface and taken to metallization with gold plating. The samples were examined under a LEO Evo 40 Scanning Electron Microscope. Obtained images were stored in digital files (Alves, 2004).

Results and discussion

The species *V. polygama* Cham. did not show any symptoms of apparent leaf injury at the end of the experiment, in any of the fluoride treatments (Figure 1).

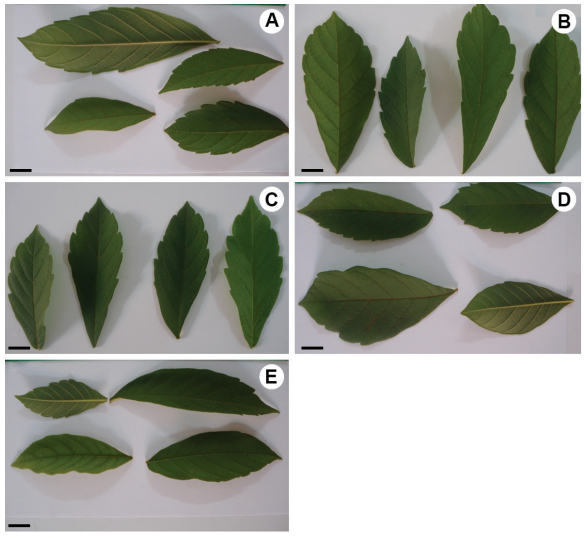

Figure 1. Leaflets of *Vitex polygama* exposed to different concentrations of potassium fluoride in the simulated rain, during 10 days. A: control treatment (0 mg L^{-1}); B: 5 mg L^{-1}; C: 10 mg L^{-1}; D: 15 mg L^{-1}; E: 20 mg L^{-1}. Bars = 2 cm.

The analysis of fluoride concentration in *V. polygama* dry mass show that only the higher concentrations of potassium fluoride (15 and 20 mg L^{-1}) were responsible for significantly higher accumulations of the pollutant ($F=9.946$; $p < 0.005$), being 47.1 and 81.3% higher than in the control treatment, respectively (Figure 2). This indicates that more elevated concentrations of fluoride are required to result in accumulation of the element in *V. polygama* plants, which can be explained by the fact that the exposure to higher concentrations of F promotes a better retention of the pollutant in the leaves (Campos, Azevedo, & Sant'Anna-Santos, 2010).

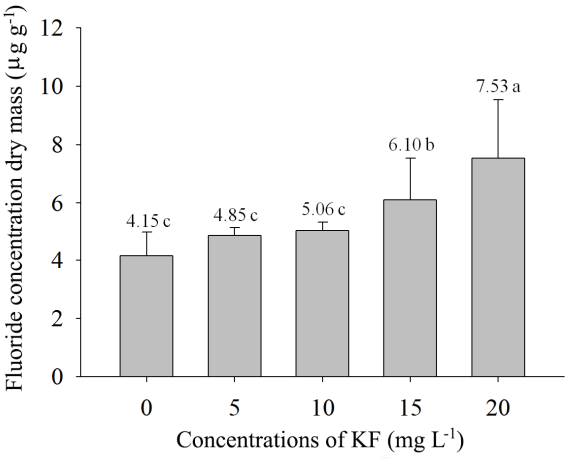

Figure 2. Fluoride concentrations (μg g^{-1} MS) in plants of *Vitex polygama* exposed to different concentrations of potassium fluoride (0, 5, 10, 15 and 20 mg L^{-1}) in the simulated rain, during 10 consecutive days. The vertical bars indicate the standard deviation (n=8). Averages followed by the same letter do not differ among themselves according to Scott-Knott test at 5% of probability.

The obtained data show that the species presents a relatively low accumulation of fluoride. The maximum level of fluoride accumulated in this experiment was about 7.53 μg g^{-1} in the treatment with higher content of potassium fluoride in the simulated rain (20 mg L^{-1}). When other species were exposed to the same concentrations of KF, during the same period of time in the laboratory (Sant'Anna-Santos et al., 2007; Campos et al., 2010) higher accumulation rates of the pollutant were detected, if compared to the ones presented by *V. polygama*.

The low accumulation of F and the absence of visual injuries in *V. polygama* indicate that this species may present some mechanism that is responsible for decreasing the absorption and the accumulation of the pollutant. According to Klumpp, Klumpp, Domingos, and Silva (1996), low accumulation rates associated to a long period of exposure to the pollutant are some of the aspects to be considered when proposing a species as fluoride accumulator.

The analysis of the chlorophyll *a* fluorescence showed that before the beginning of the simulations (day 1), there was no effect of potassium fluoride concentration on fluoresce parameters (qN: F=0.358, p=0.8382; NPQ: F=1.26, p=0.2898; qP: F=0.526, p=0.7169; ETR: F=0.925, p=0.4518; Yield: F=0.337, p=0.8525). This indicates that the plants of all treatments were submitted to the same initial conditions, and that subsequent changes in fluorescence parameters, if detected, would possibly be due to the exposure to fluoride.

Twenty-four hours after the end of the simulations (day 11), it was possible to verify that the KF concentrations did not affect significantly the photochemical quenching (F=0.775, p=0.5436), the electron transport rate (F=0.327, p=0.8591) and the yield of PSII (F=0.132, p=0.9704). However, it was detected a significant effect of KF concentrations on the non-photochemical quenching (F=8.508, $p < 0.05$) (Figure 3) and on the coefficient of non-photochemical extinction (F=10.465, $p < 0.05$) (Figure 4). The linear regression model show that the non-photochemical quenching increases with increasing concentrations of KF in the simulated rain (y=0.0127x+0.2036, R^2=0.6203), which is also verified for the coefficient of non-photochemical extinction (y=0.0169x+0.1791; R^2=0.6296).

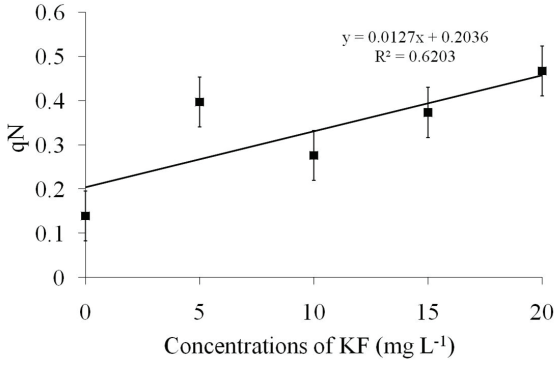

Figure 3. Non-photochemical quenching (qN) in *Vitex polygama* plants, in relation to the concentrations of KF in the simulated rain (in mg L^{-1}), after 10 days of simulation. The vertical bars represent the mean standard deviation.

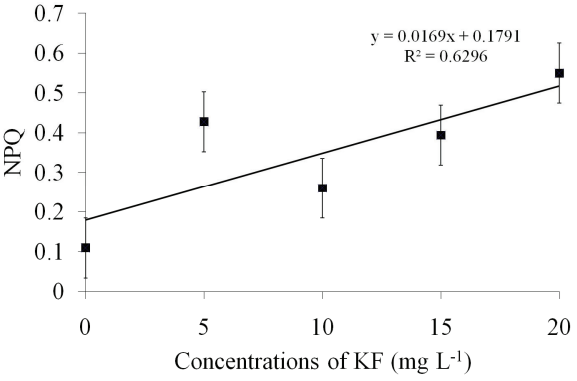

Figure 4. Coefficient due to non-photochemical extinction (NPQ) in *Vitex polygama* plants, in relation to the concentrations of KF in the simulated rain (in mg L^{-1}), after 10 days of simulation. The vertical bars represent the mean standard deviation.

The increases in qN and NPQ in *V. polygama* show that, even in the absence of visual injuries, fluoride constitutes a stress factor for the plant, and this stress manifests in the form of excess energy

dissipation, considering that the increase in qN is related to how much of the electromagnetic energy was not destined to the conversion of photochemical products ATP and NAD(P)H^+-H^+ and consists a reversible regulatory and protective mechanism that involves changes in the thylakoid membranes triggered by transthylakoidal pH gradient, transition states and photoinactivation of PSII (Roháček, Soukupová, & Barták, 2008). The NPQ also characterizes a protective mechanism of the plant to dissipate the excessive radiant energy not used in the photochemical reactions (Silva et al., 2011), but, in this case, acting by decreasing the excitation pressure of PSII, which provides protection against damages in the photosynthetic apparatus, such as electron transport dependent and/or triplet chlorophyll dependent photo damage (Vass, 2012).

The chloroplasts corresponded to the organelle with more ultrastructural alterations in the presence of F, compared to the control treatment, such as larger size (Figures 5B, C, D, F), undulated membranes (Figure 5F) and irregular shape (5B, C, D).

The higher incidence of damages in the chloroplasts may be linked to the higher fluoride accumulation in this organelle, as determined by Chang and Thompson (1966).

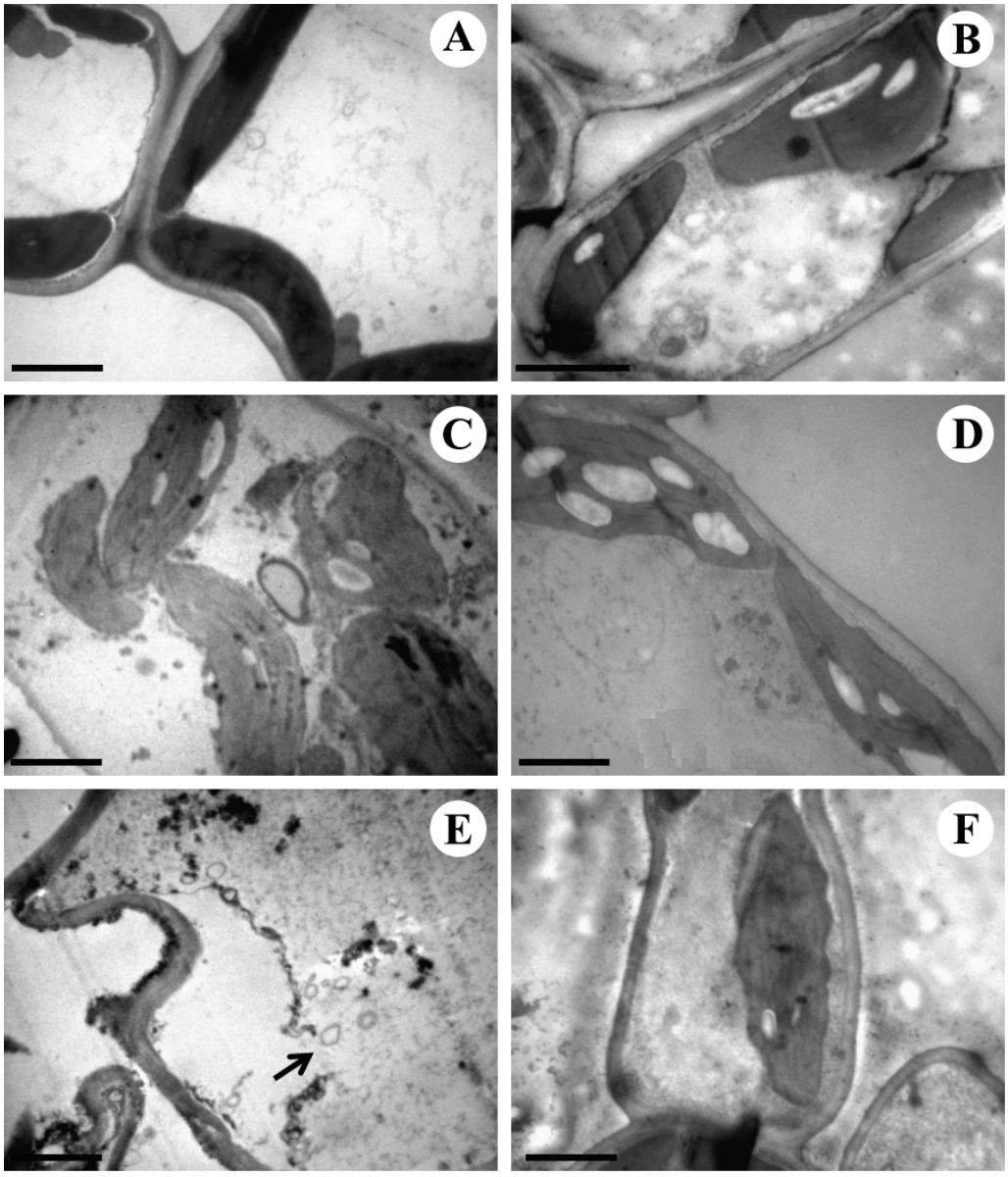

Figure 5. Chlorenchyma photomicrographs of *Vitex polygama* Cham. leaflets (cross-section in transmission electron microscopy) in the control treatment (A) and when exposed to 5 (B), 10 (C), 15 (D and E) and 20 mg L^{-1} potassium fluoride (F) during 10 days. B, C, D, F: deformed chloroplasts, with larger size and undulations in the wall; B, C, D: large starch grains within the chloroplasts; E: plasma membrane detached and broke (arrow); B, E, F: cytoplasm with granular appearance. Bar=2 μm

Larger starch grain were also observed inside the chloroplasts of plants treated with fluoride (Figure 5B, C, D), which was also reported by other authors (Eleftheriou & Tsekos, 1991; Wei & Miller, 1972). In stressful situations like the presence of pollutants, the carbohydrate translocation from the leaves to the roots can be inhibited, which can lead to the accumulation of starch grains in these organelles (Rennenberg, Herschbach, & Polle, 1996).

Another alteration detected in electron micrographs was the plasma membrane detachment from the cell wall and its break (Figure 5E), as also verified by Fornasiero (2001). F can affect cell membranes through two main mechanisms: low availability of energy for the synthesis of these cellular structure components or hydrolysis of the membrane lipids to be used as alternative sources of energy (Bhatnagar & Bhatnagar, 2000).

It was also reported the formation of many small vacuoles in the cytoplasm, leading to a granular appearance of this part of the cell (Figure 5B, E, F), and, in some cells, rupture of vesicles, releasing electron dense compounds (Figure 5E), another possible symptom caused by F inside the cells (Eleftheriou & Tsekos, 1991). The vacuole formation can be related to the tonoplast disruption, forming vesicles and multivesicular bodies (Wei & Miller, 1972). According to Miller (1993), the tonoplast is the most sensitive membrane to fluoride, and its disruption leads to the release of phenolic compounds and other organic substances, which can accelerate the degeneration of other organelles, since they induce osmotic changes in the cytoplasm and exert toxic effects on the cell.

In abaxial and adaxial surfaces of *V. polygama* leaves of the control treatment, we observed epidermal cells with sinuous outlines and elevated central region, smooth cuticle and many tector and glandular peltate trichomes. The peltate trichomes present short stalk and ovoid head. The tector trichomes are multiseriated (Figures 6A and 7A). *V. polygama* leaf is hipostomatic and stomata have random distribution in the epidermis and projected position (Figure 6A).

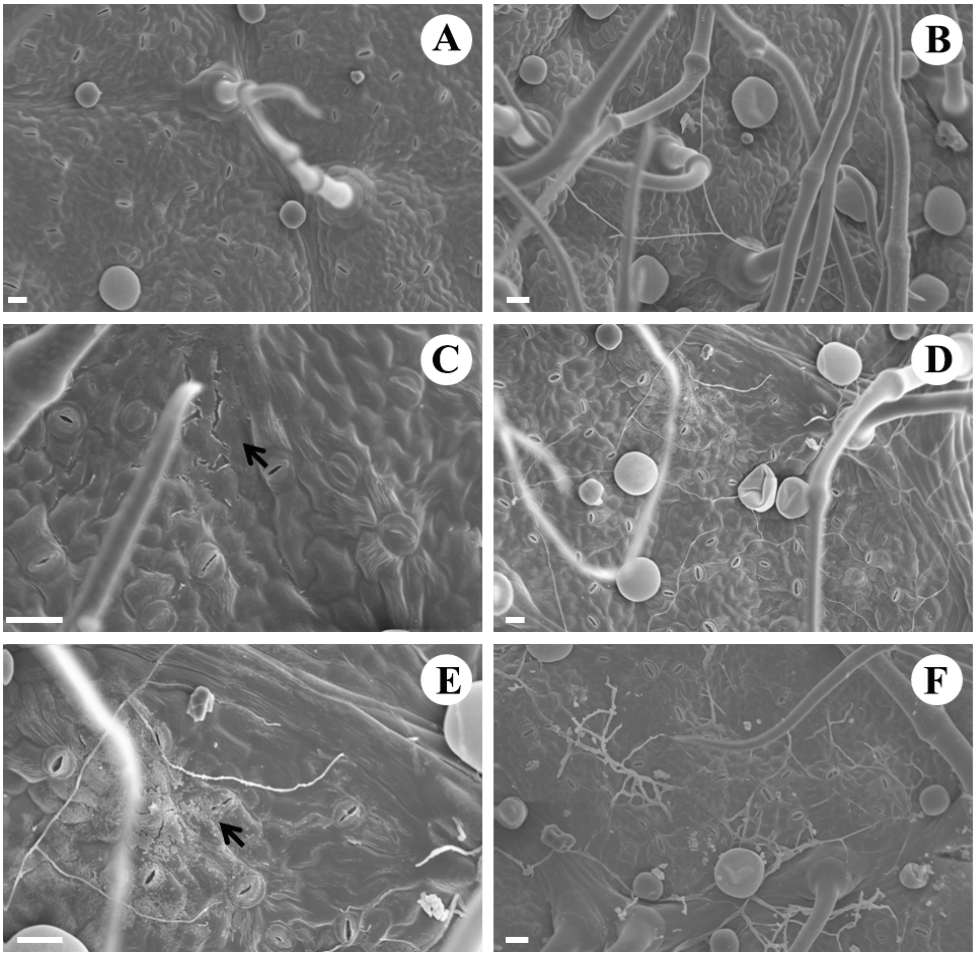

Figure 6 Photomicrographs of abaxial surface in *Vitex polygama* Cham. leaflets in the control treatment (A) and when exposed to 5 (B), 10 (C), 15 (D and E) and 20 (F) mg L^{-1} KF during 10 days. B, D, E, F: fungal hiphae; C: epicuticular wax erosion (arrow); E: epicuticular wax accumulation (arrow). Bars=10 μm (C) and 20 μm (A, B, D, E, F).

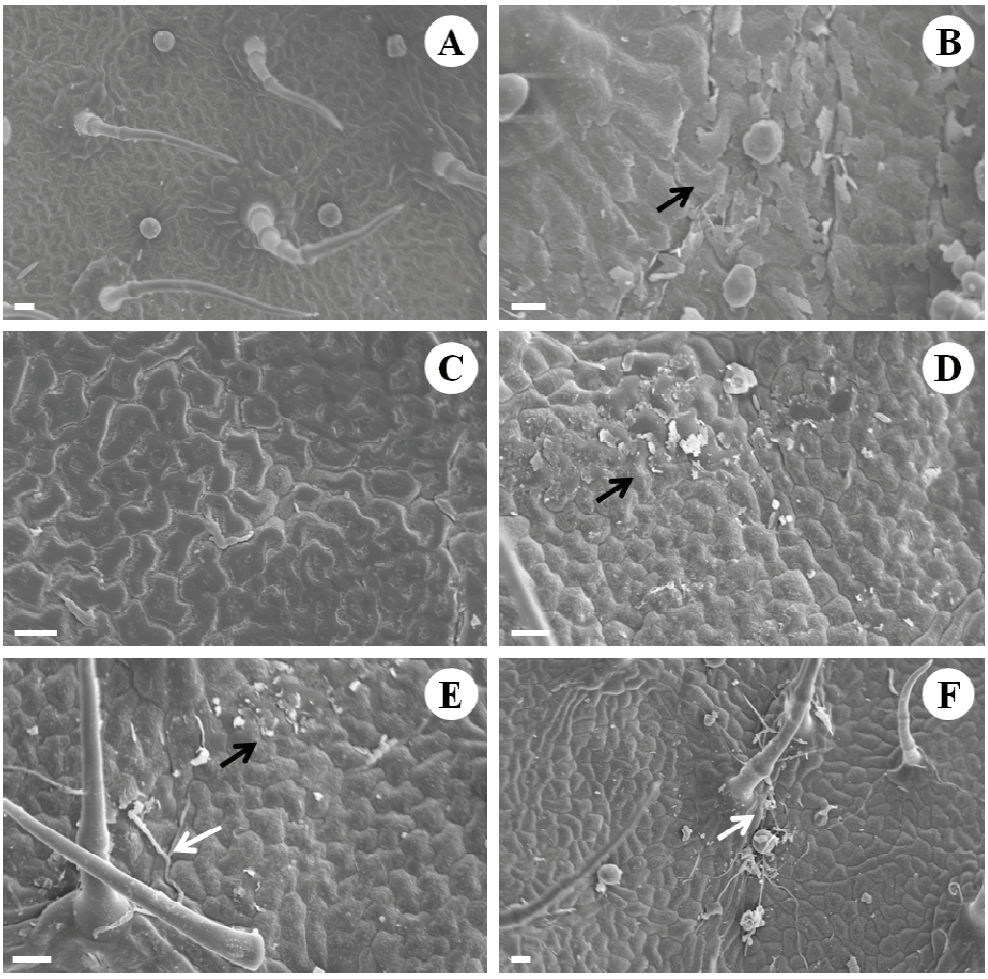

Figure 7 Photomicrographs of adaxial surface in *Vitex polygama* Cham. leaflets in the control treatment (A) and when exposed to 5 (B), 10 (C), 15 (D) and 20 (E and F) mg L^{-1} KF during 10 days. E, F: fungal hiphae (white arrows); C and E: epicuticular wax more prominent; B, D, E: epicuticular wax erosion due to its accumulation (black arrows). Bars=10 μm (B) and 20 μm (A, C, D, E, F).

All the concentrations of KF in the simulated rain (5, 10, 15 and 20 mg L^{-1}) caused changes in the leaf surface of *V. polygama*, which can be related to phytotoxicity. On both sides of the epidermis, the accumulation of epicuticular wax (Figures 6E and 7B, C, D and E) and its erosion (Figures 6C and 7B, D, E) were detected.

In the plants treated with fluoride, the epicuticular wax found to be more pronounced, cracked in some spots (Figure 6C) and losing in others (Figure 7B, D). The epicuticular wax erosion was also detected by other authors in leaflets or leaves exposed to F (Sant'Anna-Santos et al., 2007; Campos et al., 2010), and can result from the loss of integrity of the epidermis (Mesquita et al., 2011). Once the epicuticular wax serves as a barrier against the entry of pollutants through leaf cuticle (Singh & Verma, 2007), the erosion of the wax in the presence of fluoride may have facilitated its entry through the leaf surface.

It was also detected a colonization of *V. polygama* leaf surface by fungal hyphae (Figures 6B, D, E, F and 7F). Once the cuticle of the leaves constitute a barrier that, among other functions, protects the plant against pathogens by consisting of a physical barrier and by providing a dynamic defense based on signaling circuits and effector molecules (Reina-Pinto & Yephremov, 2009), the epicuticular wax disruption in *V. polygama* may have led to the colonization of the leaves by these pathogens.

We suggest that the large amount of trichomes (Figures 6 and 7), may have contributed to the relative resistance of *V. polygama* to the pollutant, given that no external damages were detected in the plants and that the accumulation of fluoride was relatively low, as already discussed. According to Garg and Varshney (1980), greater resistance to pollution often occurs in plants with higher density of trichomes, and plants exposed to greater levels of air pollution may present higher length and density of foliar trichomes.

Conclusion

Vitex polygama Cham. has potential to be used as a biosensor of air pollution by fluoride, since it presented no visual symptoms when exposed to potassium fluoride and the pollutant accumulation was relatively low and just occurred for the higher concentrations of KF.

The detected changes in microscopy, ultrastructure and in chlorophyll *a* fluorescence in *V. polygama* leaves with healthy visual aspect have also proven to be important, since they have prognostic value in the detection of injuries caused by fluoride and can be used as biomarkers in monitoring programs of air pollution by F.

Acknowledgements

To *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), for providing the scolarship to the first author. To CEMIG, for donating the seedlings. To the *Universidade Federal de Viçosa* (UFV), professors Aristea Alves Azevedo and Luzimar Campos da Silva and to Sarah Diniz for the physical and technical support on fluoride quantification. To professor Eduardo Alves, Claudia Labory and Eloisa Leite for the physical support for Transmission and Scanning Electron Microscopy. To *Fundação de Amparo à Pesquisa do Estado de Minas Gerais*, for the financial support in the analysis of Transmission and Scanning Electron Microscopy and to *Conselho Nacional de Desenvolvimento Científico e Tecnológico* for the financial support in the project.

References

Alves, E. (2004). *Apostila do curso introdutório de microscopia eletrônica de transmissão*. Lavras, MG: Faepe.

Bhatnagar, M., & Bhatnagar, A. (2000). Algal and cyanobacterial responses to fluoride. *Fluoride, 33*(2), 55-65.

Bilger, W., Schreiber, U., & Bock, M. (1995). Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia, 102*(4), 425-432.

Campos, N. V., Azevedo, A. A., & Sant'anna-Santos, B. F. (2010). Acúmulo e efeitos fitotóxicos do flúor em folhas de boldo-gambá e capim-cidreira utilizadas para chás. *Pesquisa Agropecuária Brasileira, 45*(7), 646-653.

Cantino, P. D., Harley, R. M., & Wagstaff, S. J. (1992). Genera of *Labiatae*: status and classification. In Harley, R. M., Reynolds, T. (Ed.), *Advances in Labiatae science*. (p. 511-522). Kew, UK: Royal Botanic Gardens.

Cappellin, P. (2008). Entre a memória e o mercado: o desenvolvimento da empresa de porte médio no Brasil. *Revista de Sociologia e Política, 16*(31), 49-70.

Chang, C. W., & Thompson, C. R. (1966). Site of fluoride accumulation in navel orange leaves. *Plant Physiology, 41*(2), 211-213.

Eleftheriou, E. P., & Tsekos, I. (1991). Fluoride effects of leaf cell ultrastructure of olive trees growing in the vicinity of the Aluminum Factory of Greece. *Trees, 5*(1), 83-89.

Evans, L. S., Gmur, N. F., & Costa, F. (1977). Leaf surface and histological perturbations of leaves of *Phaseolus vulgaris* and *Helianthus annuus* after exposure to simulated acid rain. *American Journal of Botany, 64*(7), 903-913.

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia, 35*(6), 1039-1042.

Fornasiero, R. B. (2001). Phytotoxic effects of fluorides. *Plant Science, 161*(5), 979-985.

Garcia-Ciudad, A., Garcia-Criado, B., & Pontón-San Emeterio, C. (1985). Determination of fluoride in plant samples by a potentiometric method and near-infrared reflectance spectroscopy. *Communications in Soil Science and Plant Analysis, 16*(10), 1107-1122.

Garg, K. K., & Varshney, C. K. (1980). Effect of air pollution on the leaf epidermis at the submicroscopic level. *Experientia Basel, 36*(12), 1364-1366.

Gheorge, I. F., & Ion, B. (2011). The effects of air pollutants on vegetation and the role of vegetation in reducing atmospheric pollution. In Khallaf, M. K (Ed.). *The impact of air pollution on health, economy, environment and agricultural sources.* doi: 10.5772/17660.

Hoagland, D. R., & Arnon, D. I. (1950). *The water-culture method for growing plants without soil.* Berkeley, CA California Agricultural Experiment Station.

Holt, E. A. & Miller, S. W. (2010). Bioindicators: using organisms to measure environmental impacts. *Nature Education Knowledge, 3*(10), 8.

Klumpp. A., Klumpp, G., Domingos, M., & Silva, M. D. (1996). Fluoride impact on native tree species of the Atlantic Forest near Cubatão, Brazil. *Water, Air and Soil Pollution, 87*(1), 57-71.

Larsen, S., & Widdowson, A. E. (1971). Soil fluorine. *Journal of Soil Science, 22*(2), 210-221.

Mesquita, G. L., Tanaka, F. A. O., Cantarella, H., & Junior, D. M. (2011). Atmospheric absorption of fluoride by cultivated species: leaf structural changes and plant growth. *Water, Air and Soil Pollution, 219*(1), 143-156.

Miller, G. W. (1993). The effect of fluoride on higher plants: with special emphasis on early physiological and biochemical disorders. *Fluoride, 26*(1), 3-22.

Moldenke, H. N., & Moldenke, A. L. (1957). Materials toward a monograph of the genus *Vitex*. *Phytologia, 6*(1), 80-89.

Reina-Pinto, J. J., & Yephremov, A. (2009). Surface lipids and plant defenses. *Plant Physiology and Biochemistry, 47*(6), 540-549.

Rennenberg, H., Herschbach, C., & Polle, A. (1996). Consequences of air pollution on shoot-root interactions. *Journal of Plant Physiology, 148*(3/4), 296-301.

Rohácek, K., Soukupová, J., & Barták, M. (2008). Chlorophyll fluorescence: a wonderful tool to study plant physiology and plant stress. *Research Signpost, 2*(1), 41-104.

Sant'anna-Santos, B. F., Duque-Brasil, R., Azevedo, A. A., Silveira, A. S., Araújo, J, M. & Aguiar, R. (2007). Utilização de parâmetros morfoanatômicos na análise da fitotoxidez do flúor em folhas de *Magnolia ovata* (A. St.-Hil.) Spreng. (Magnoliaceae). *Revista Árvore, 31*(4), 761-771.

Silva, E. N., Ribeiro, R. V., Ferreira-Silva, S. L., Viégas, R. A., Silveira, J. A. G., & Silveira, J. A. G. (2011). Stress induced damages on the photosynthesis of physic nut young plants. *Scientia Agricola, 68*(1), 62-68.

Singh, S. N., & Verna, A. (2007). Phytoremediation of air pollutants: a review. In S. N. Singh & R. D. Tripathy, (Eds.). *Environmental Bioremediation Technologies* (p. 293-314). Berlin, BE: Springer.

Singh-Rawal, P., Jajoo, A., & Bharti, S. (2010). Fluoride affects distribution of absorbed excitation energy more in favour of photosystem 1. *Biologia Plantarum, 54*(3), 556-560.

Van Den Berg, E., Santos, M., Castro, G. C., & Ferreira, C. A. (2007). Estrutura do componente arbóreo de uma floresta de galeria aluvial em Poços de Caldas, MG. *Revista Brasileira de Biociências, 5*(1), 150-152.

Vass, I. (2012). Molecular mechanisms of photodamage in Photosystem II complex. *Biochimica et Biophysica Acta - Bioenergetics, 1817*(1), 209-217.

Wei, L., & Miller, G. W. (1972). Effect of HF on the fine structure of mesophyll cells from *Glycine max*, Merr. *Fluoride, 5*(2), 67-72.

Received on March 22, 2016.
Accepted on September 1, 2016.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.