Vol.49, n. 2 : pp. 313-321, March 2006
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY

A N I N T E R N A T I O N A L J O U R N A L

Effects of Simulated Acid Rain on Leaf Anatomy and Micromorphology of *Genipa americana* L. (Rubiaceae)

Bruno Francisco Sant'Anna-Santos, Luzimar Campos da Silva, Aristéa Alves Azevedo* **and Rosane Aguiar**

Departamento de Biologia Vegetal; Universidade Federal de Viçosa; Av. P. H. Rolfs, s/n; 36570-000; aazevedo@ufv.br; Viçosa - MG - Brasil

ABSTRACT

Experiments were conducted in order to characterize the injuries on leaf structure and micromorphology of G. americana *and evaluate the degree of susceptibility of this species to simulated acid rain. Plants were exposed to acid rain (pH 3.0) for ten consecutive days. Control plants were submitted only to distilled water (pH 6.0). Leaf tissue was sampled and fixed for light and scanning electron microscopy. Necrotic interveinal spots on the leaf blade occurred. Epidermis and mesophyll cells collapse, hypertrophy of spongy parenchyma cells, accumulation of phenolic compounds and starch grains were observed in leaves exposed to acid rain. The micromorphological analysis showed, in necrotic areas, plasmolized guard cells and cuticle rupture. Epidermal and mesophyll cells alterations occurred before symptoms were visualized in the leaves. These results showed the importance of anatomical data for precocious diagnosis injury and to determine the sensitivity of* G. americana *to acid rain.*

Key words: *Genipa americana,* simulated acid rain, leaf anatomy, leaf micromorphology

INTRODUCTION

The sources of atmospheric deposition can be categorized as either natural or anthropogenic. Unlike the case of fluoride that is emitted by a fewer industries such as the aluminum ones, there are many anthropogenic sources that acidify rain water (Horner and Bell, 1995). Nitrogen and sulphur oxides are the major sources of atmospheric acidity; both are products of combustion, and both are converted in the atmosphere to strong acids, mainly nitric and sulphuric acids that acidify the rain water (Cowling and Linthurst, 1981). Rain that presents a concentration of H^+ ions greater than 2.5 μeq^{-1} and pH values lower than 5.6 is considered acid (Evans, 1984).

Urban air pollution is a major environmental problem, mainly in the developing countries (Mage et al., 1996). In Brazil, cities such as Piracicaba, São Paulo, Cubatão and Rio de Janeiro experience the impact of atmospheric pollution and acid rain (Klumpp et al., 1996, 1999; Lara et al., 2001). Acid precipitation with pH values below 5.0 was registered in different regions in Brazil (Santos and Souza, 1988; Mello, 2001; Mello and Almeida, 2004).

Several experiments have been carried out in the field and in greenhouses to investigate the effects of acid rain on plants (Paparozzi and Tukey, 1983; Percy and Baker, 1987; Turunen and Huttunen, 1991; Nouchi, 1992; Temple et al., 1992; Gabara et al., 2003; Silva et al., 2005a; 2005b). Some species are more sensitive to acid rain than others

* Author for correspondence

(Evans, 1984; Silva et al., 2005a; 2005b). The incidence and severity of leaf injury to acid rain is associated to many variables as plant species, age of tissue and plants, foliar wettability, leaf pubescence and environmental factors (Dickison, 2000).

In the tropical regions there are few studies on pollution using plant species (Klumpp et al., 1996; Klumpp et al., 1999). Forest ecosystems in southeastern Brazil are potentially susceptible to problems related to acid deposition because of increases in the consumption of oil products, natural gas, and coal used to produce energy for the different economic sectors of the region (Mello and Almeida, 2004).

The Rio Doce State Park (RDSP) is considered the largest area of tropical semidecidous forest under legal protection in Minas Gerais State (IEF, 1994). Located in a region known as "Steel Valley", the RDSP has an area of approximately 36,000 hectares and there are charcoal burners in the south and large-sized steel industries in the north.

Preliminary precipitation chemistry analysis from 1985 to 1986 in RDSP detected pH values between 3.47 to 7.62 (Castro et al., 1987). The minimum value observed has large potential to cause damaging effects to the ecosystem (Castro et al., 1987). Jordão et al. (1996) observed that plant populations were under pollution impact occasioned by anthropogenic activities in this region. *Genipa americana* occurs in the RDSP and Silva et al. (2000) showed evidence of air pollution impacts on this species.

G. americana, popularly known as 'Jenipapeiro', is a fast growing tree species found throughout Brazil. It has single, subcoriaceous, and glabrous leaves (Lorenzi, 2002). The wood is used for construction and for cabinet-work. The fruits are edible and provide a delicious liqueur that is much consumed. In some regions of Brazil, the juice is used as a blue colorant (Lorenzi, 2002). The aim of this study was to characterize the injuries on leaf structure and micromorphology of *G. americana* and evaluate the degree of susceptibility of this species to simulated acid rain.

MATERIALS AND METHODS

Seedlings and saplings of *Genipa americana* were obtained from germinated seeds and supplied by the RDSP nursery. The seedlings (about two months old with cotyledons and two nodes with leaves) were supplied in 290 cm^3 containers (53 mm internal diameter, 190 mm height, presenting a circular section with 8 vertical grooves) filled with soil. Saplings (about four months old) were transferred from plastic bags to plastic pots (2,000 ml capacity) filled with sand. The plantlets were acclimatized in a covered area for 15 to 20 days. The plants received Hoagland nutritive solution (1/4 strength) every five days throughout the experiment and were daily submitted to simulated acid rain (SAR) event of twenty minutes for 10 consecutive days. The simulated rain was applied using a chamber constructed and adapted by Alves (1988) from the model proposed by Evans et al. (1977). Before and after each simulation, the plants were maintained under a luminous panel, consisting of eight incandescent high pressure mercury lamps (E-27, 220-230 Volts, 250 Watts) for fifteen minutes under radiant flow density of 95 $W.m^{-2}$ and remained the rest of the time under ambient light conditions (28.8/9.2^{0}C average day/night temperatures, relative humidity 79.1%, precipitation 0.0 mm).

The SAR was prepared by mixing 1N sulphuric acid and distilled water to reduce the pH to a value equal to 3.0. The pH value of the SAR used in this study was chosen based on the data of Silva et al. (2005a; 2005b). This value of pH was approximately similar to the minimum value registered in 'Steel Valley' by Castro et al. (1987). Distilled water alone was used in the control treatment (pH 6.0). Treatments were applied in a completely randomized design with two treatments (pH 3.0 and pH 6.0) and ten plants (5 seedlings and 5 saplings) per treatment.

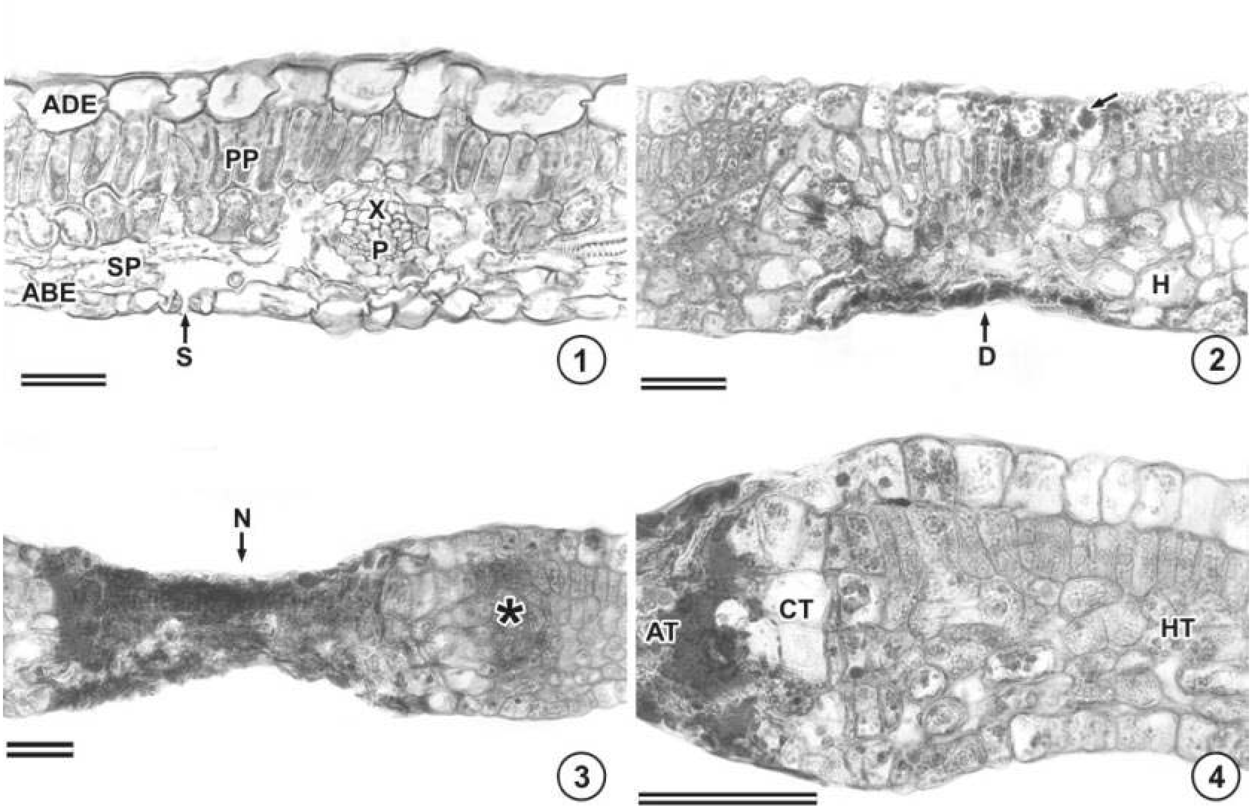
The appearance and location of leaf necroses related to SAR were recorded daily. For the anatomical and micromorphological characterization of injuries, leaf blades samples of five representative seedlings (n = 5) and saplings (n = 5), one leaf of each plant, were collected 24 hours after the last rain application. Leaf material came from the middle region of the expanding leaf at the second node from shoot apex.

For light microscopy, the samples were fixed in Craft III (Berlyn and Miksche, 1976) dehydrated in ethyl/butyl series, and embedded in histological paraffin. Transversal sections (10 μm thick) were obtained using a rotatory microtome (model Spencer 820, American Optical Corporation, New York, USA). The sections were stained with 1% alcoholic safranin, for sixty minutes, and 1% astra blue, for 3 minutes, and further were mounted in synthetic Canada balsam. A test with lugol was performed to confirm the presence of starch grains. The photographic documentation was made using a photonic microscope (model AX70TRF, Olympus Optical, Tokyo, Japan) with a U-Photo system.

For scanning electron microscopy (SEM), the samples were fixed in glutaraldehyde (2%), followed by thiosemicarbazide (1%) as mordent, and post fixed in 1% osmium tetroxide (Silveira, 1989). The material was dehydrated in ethyl series and submitted to critical point drying using liquid CO_2 in Balzers equipment (model CPD 020, Bal-Tec, Balzers, Liechtenstein). The material was covered in gold using the catodic spraying process in a Sputter Coater (model FDU010, Bal-Tec, Balzers, Liechtenstein). The photographic documentation was performed using a scanning electron microscope (model JSM-T200, Jeol Co., Tokyo, Japan).

RESULTS

G. americana had dorsiventral and hypostomatic leaves with uniseriate epidermis and mesophyll formed by 1-2 layers of palisade parenchyma and 2-3 layers of spongy parenchyma (Fig. 1). Interveinal necroses spots with a blackened appearance were the visual symptoms observed in response to SAR. Seedlings and saplings showed similar foliar symptoms.



Figures 1-4 - Leaf blade structure of *Genipa americana* (Light micrographs; transverse sections of expanded leaf middle region). **(1)** Control treatment. **(2-5)** Simulated acid rain treatment (pH 3.0). **(2)** Necrotic area - adaxial epidermal cells with dark contents (arrow), hypertrophy in spongy parenchyma and collapse of abaxial epidermal cells. **(3)** Total collapse of leaf tissues in interveinal necrosis and alterations in the differentiation pattern of cells near necrosis (*). **(4)** Formation of a cicatrization tissue in region adjacent to necrosis. ADE, adaxial epidermis; ABE, abaxial epidermis; S, stomata; PP, palisade parenchyma; SP, spongy parenchyma; P, phloem; X, xylem; H, hypertrophy; D, collapsed epidermis; N, necrotic area; CT, cicatrization tissue; AT, affected tissue; HT, healthy tissue (Bars = 50 µm).

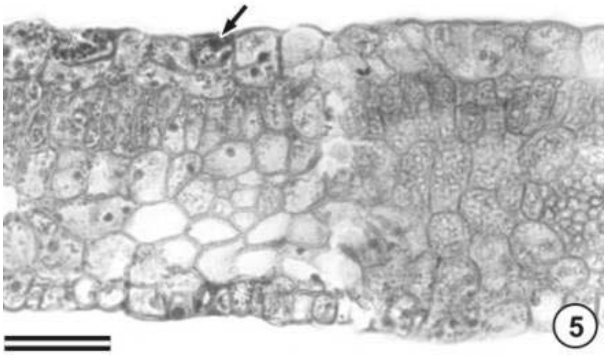


Figure 5 - Injuried leaf blade of *Genipa americana* (Light micrographs; transverse sections of expanded leaf middle region). Region without visible necrosis: alterations in shape of epidermal and mesophyll cells; dark contents in epidermal cells (arrow). (Bar = 50 µm).

The onset of these symptoms occurred after the first rain application preferentially on the adaxial surface of expanding leaves; in sequence cellular damage develops internally. In young leaves, some necroses began from the abaxial epidermal cells.

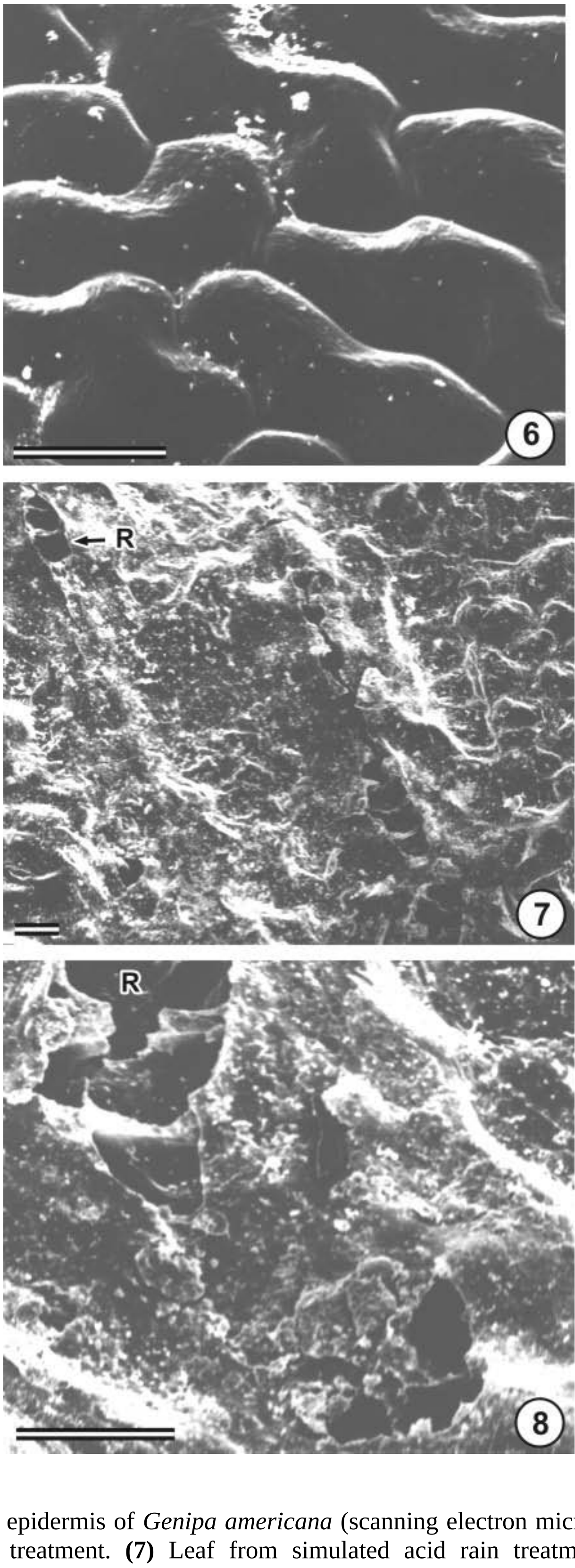
The structural analysis of the injuries caused by SAR showed the occurrence of epidermal cells presenting dark contents and hypertrophy in spongy parenchyma cells that had a more compact arrangement (Fig. 2). The lesion development resulted in total collapse of leaf tissues in affected areas (Fig. 3). The cells near necrotic areas showed alterations in the differentiation pattern (Fig. 3) without a typical palisade and spongy mesophyll. Rounded chloroplasts with large starch grains were observed in region between necrotic and healthy tissues. In this region, a cicatrization tissue consisting of large cells with suberized walls was formed as a result of the mesophyll cell division (Fig. 4), in some necrosed leaves. Epidermal and mesophyll cells with alterations in shape and contents were identified in leaves submitted to acid rain but without visible necrosis (Fig. 5).

In the control treatment, the micromorphological analyses showed the presence of turgid epidermal cells with distinct contours (Fig. 6). In the leaves submitted to SAR, adaxial epidermis presented altered contours, plasmolised aspect and rupture of several cell groups in necrotic areas (Fig. 7). The rupture of the epidermis cells exposed the parenchyma cells to acid rain treatment (Fig. 8). The abaxial epidermis with stomata is shown in Figure 9. The plants submitted to SAR presented stomata with deformed aperture (Fig. 10) as a consequence of rupture of overlying cuticle and plasmolysis of guard cells, leading to the formation of depressions.

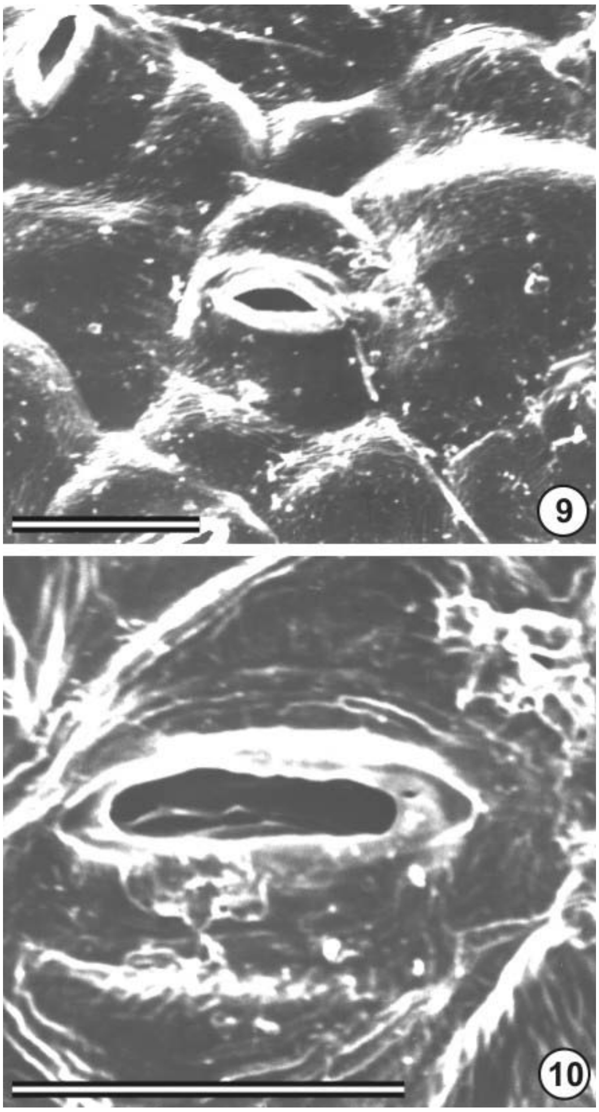
DISCUSSION

Sensitive plants to pollutants can present changes in their morphology, anatomy, physiology and biochemistry (Neufeld et al., 1985; Azevedo, 1995; Hara, 2000; Moraes et al., 2000; Chaves et al., 2002; Gabara et al., 2003; Reig-Armiñana et al., 2004; Silva et al., 2005b). The presence of necrotic spots, observed on *G. americana* leaves exposed to low-pH rain were also related by Silva et al. (2005a) who classified this species (based on morphological changes), in comparation with other four tree species, as moderately injuried when exposed to SAR. The structural and micromorphological analyses of the injuries caused by acid rain clearly showed that *G. americana* was seriously injuried when exposed to SAR (pH 3.0).

The anatomical analysis of injuries caused by pollutants on plant species has been used in various studies to assess the real damage caused by pollutants (Azevedo, 1995; Hara, 2000; Chaves et al., 2002; Reig-Armiñana et al., 2004; Silva et al., 2005a; 2005b). Several authors have related the deleterious effects of acid rain on the anatomy and ultrastructural leaf characteristics (Paparozzi and Tukey, 1983; Percy and Baker, 1987; Gabara et al., 2003; Silva et al., 2005a; 2005b), however, there are relatively few studies in tropical species as *G. americana*.



Figures 6-8 - Adaxial epidermis of *Genipa americana* (scanning electron micrographs). **(6)** Leaf from control treatment. **(7)** Leaf from simulated acid rain treatment: epidermis cells of necrotic area. **(8)** Detail of epidermis rupture (R) showed in Figure 7 (Bars = 5 µm).



Figures 9 and 10 - Abaxial epidermis of *Genipa americana* (scanning electron micrographs). **(9)** Leaf from control treatment. **(10)** Stomata with deformed aspect in leaves exposed to acid rain (Bars = 20 µm).

Leaf orientation appears to be a critical factor in determining lesion development in response to acid rain (Knittel and Pell, 1991). Most of the necroses caused by SAR started from the adaxial leaf surface because they were directly exposed to the pollutant (Silva et al., 2000). However, in *G. americana*, young leaves were vertically oriented and both leaf surfaces were exposed to the acid rain water and manifested the onset of necroses formations.

In *G. americana* the foliar epidermis were the first tissue to be injuried like observed in other plants species treated with acid rain (Evans and Curry, 1979; Rathier and Frink, 1984). The rupture of the epidermis intensified the effects of SAR on leaf internal tissues of *G. americana*.

The reduction in turgidity of the subsidiary cells observed in *G. americana* could induce alterations in the guard cells permeability (Kozlowski, 1980) and interfere with the gas exchange rates (Evans, 1984). Generally, the damage caused to the stomata impaired the plant growth and yield, because it reduced the photosynthetic rates. In some species of *Liriodendron*, there were reductions in biomass due to acid rain decreases in photosynthetic capacity (Neufeld et al., 1985).

It has been shown that biotic and abiotic stresses such as atmospheric pollutants can induce an increase in the amount of phenolic compounds in plants (Zobel, 1996). In *G. americana* the dark contents observed in leaf tissues, especially in epidermal cells probably were phenolic compounds. Zobel and Nighswander (1991) reported that the accumulation of these compounds was generally followed by cytoplasm degradation and vacuolar content release that led to cell death.

Leaves exposed to low-pH showed hypertrophy and hyperplasia of the mesophyll cells (Dickison, 2000; Silva et al., 2005b). In *G. americana* only hypertrophy occurred. Leaf wrinkling and curling usually associated to hypertrophy and hyperplasia (Evans and Curry, 1979) were not registered.

The occurrence of cells with abnormal quantities of starch observed near necrotic areas in *G. americana* was probably related with the inhibiting effect of pollutants on the translocation of carbohydrates (Rennenberg et al., 1996). The accumulation of large starch grains in the chloroplasts was also observed in mesophyll cells of *Clusia hilariana* and *Lycopersicon esculentum* mesophyll cells after exposure to acid rain (Gabara et al., 2003; Silva et al., 2005b).

The structures of the epidermal and mesophyll cells within a lesion were indistinguishable but healthy cells occurred immediately adjacent to collapsed necrotic region (Knittel and Pell, 1991; Silva et al., 2000; Silva et al., 2005a; 2005b).

The differentiation of a cicatrization tissue (from the parenchyma cells adjacent to the necrosis) on the *G. americana* leaves was similar to cicatrization observed on *C. hilariana* leaves attacked by fungi (Schneider, 1985) and submitted to SAR (Silva et al., 2005b). This cicatrization tissue functions as a barrier, preventing the progress of the necrosis to other regions of the leaf and results of the ability of plants to form new tissues from the proliferative capabilities of parenchyma cells (Dickison, 2000).

On the analysis of damage caused by SAR at structural and micromorphological levels, the present study led to conclude that visual assessments alone were not sufficient to determine the real effects of SAR. The occurrence of cellular injury in the absence of visual macroscopic symptoms in *G. americana* showed that anatomical investigations allowed a more precise injury diagnosis caused by pollutants as observed in soybean (Azevedo, 1995). It would be important to emphasize that a more detailed physiological assessment should be made to evaluate the potential of this species as bioindicator of polluted environments, since *G. americana* presented considerable structural and micromorphological alterations in response to SAR.

ACKNOWLEDGEMENTS

We thank the CNPq (Brazil) for a scholarship and Professor Wagner C. Otoni (UFV) for suggestions in writing this paper.

RESUMO

Experimentos foram conduzidos para avaliar o grau de susceptibilidade e determinar as injúrias causadas pela chuva ácida simulada na anatomia e micromorfologia foliar de *Genipa americana*. Plantas foram expostas à chuva com pH 3,0 durante 10 dias consecutivos. No tratamento controle utilizou-se apenas água destilada (pH 6,0). Amostras foliares foram coletadas e fixadas para microscopia de luz e eletrônica de varredura. Foram observados nas folhas expostas à chuva ácida: necroses pontuais intervenais, colapso das células do mesofilo e da epiderme; hipertrofia do parênquima lacunoso e acúmulo de compostos fenólicos e grãos de amido. A análise micromorfológica evidenciou, nas áreas necrosadas, plasmólise das células-guarda e ruptura da cutícula e da crista estomática. Alterações anatômicas ocorreram antes que sintomas visuais fossem observados nas folhas. Estes resultados comprovam a importância de dados anatômicos na diagnose precoce da injúria e na determinação da sensibilidade de *G. americana* à chuva ácida.

REFERENCES

Alves, P. L. C. A. (1988), *Efeitos nutricionais da chuva ácida simulada e do latossolo Vermelho-Amarelo de Cubatão-SP*, *em soja (*Glycine max L.*) Merril.* M.Sc. Thesis, Universidade Federal de Viçosa, Viçosa, Brasil.

Azevedo, A. A. (1995), *Ação do flúor*, *em chuva simulada*, *sobre a estrutura foliar de* Glycine max *(L.) Merril.* D.Sc. Thesis, Universidade de São Paulo, São Paulo, Brasil.

Berlyn, G. P. and Miksche, J. P. (1976), *Botanical microtechnique and cytochemistry*. Ames: The Iowa State University Press.

Castro, P. S.; Ribeiro, J. C.; Ribeiro, G. A.; Soares, V. P.; Barros, N. F.; Gomes, L. C. L.; Neves, J. C. L.; Souza, A. L.; Galvão, D. M. and Valente, O. F. (1987), *Estudos preliminares sobre a química da precipitação na região industrial do Vale do Rio Doce – MG*. Viçosa, MG: SIF/IBDF (Relatório Final).

Chaves, A. L. F.; Silva, E. A. M.; Azevedo, A. A.; Oliva, M. A. and Matsuoka, K. (2002), Ação do flúor dissolvido em chuva simulada sobre a estrutura foliar de *Panicum maximum* Jacq. (colonião) e *Chloris gayana* Kunth. (capim-Rhodes) – Poaceae. *Acta Botanica Brasilica*, **16**, 395-406.

Cowling, E. B. and Linthurst, R. A. (1981), The acid precipitation phenomenon and its ecological consequences. *Bio-Science*, **31**, 649-653.

Dickison, W. C. (2000), *Integrative Plant Anatomy*. Massachusetts: Harcourt/Academic Press.

Evans, L. S., Gmur, N. F. and Kelsch, J. J. (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**, 903-913.

Evans, L. S. (1984), Botanical aspects of acidic precipitation. *The Botanical Review*, **50**, 449-490.

Evans, L. S. and Curry, T. M. (1979), Differential responses of plant foliage to simulated acid rain. *American Journal of Botany*, **66**, 953-962.

Gabara, B.; Sklodowska, M.; Wyrwicka, A.; Glinska, S. and Gapinska, M. (2003), Changes in the ultrastructure of chloroplasts and mitochondria and antioxidant enzime activity in *Lycopersicon esculentum* Mill. leaves sprayed with acid rain. *Plant Science*, **164**, 507-516.

Hara, S. (2000), *Alterações estruturais em folhas de* Panicum maximum *Jacq. submetidas à chuva simulada com flúor*. M.Sc. Thesis, Universidade Federal de Viçosa, Viçosa, Brasil.

Horner, J. M. and Bell, J. N. B. (1995), Effects of fluoride and acidity on early plant growth. *Agriculture, Ecosystems and Environment*, **52**, 205-211.

IEF - Instituto Estadual de Florestas. (1994), *Pesquisas Prioritárias para o Parque Estadual do Rio Doce*, Brasil, Belo Horizonte.

Jordão, C.P.; Pereira, J.C.; Brune, W.; Pereira, J.L. and Braathen, P.C. (1996), Heavy metal dispersion from industrial wastes in the Vale do Aço, Minas Gerais, Brazil. *Environmental Technology*, **17**, 489-500.

Klumpp, A.; Klumpp, G. and Domingos, M. (1996), Bioindicator of Air Pollution in the Tropics. *Gefarstoffe – Reinhaltung der Luft*, **56**, 27-31.

Klumpp, A.; Domingos, M.; and Pignata, M. L. (1999), Air pollution and vegetation damage in South America – State of knowledge and perspectives. In: Agrawal & Agrawal (eds), *Environmental Pollution and Plant Responses*, Boca Raton, FL, USA: Lewis Publishers, pp.111-136.

Knittel, R. and Pell, E. J. (1991), Effects of simulated acidic rain on upper leaf surface of *Zea mays* foliage. *Canadian Journal of Botany*, **69**, 2637-2642.

Kozlowski, T. T. (1980), Impacts of Air Pollution on Forest Ecosystems. *BioScience*, **30**, 88-93.

Lara, L.B.L.S.; Artaxo, P.; Martinelli, L.A.; Victoria, R.L.; Camargo, P.B.; Krusche, A.; Ayers, G.P.; Ferraz, E.S.B. and Ballester, M.V. (2001), Chemical composition of rainwater and anthropogenic influences in the Piracicaba River Basin, Southeast Brazil. *Atmospheric Environment*, **35**, 4937-4945.

Lorenzi, H. (2002), *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. Nova Odessa: Instituto Plantarum de Estudos da Flora Ltda. v. 1.

Mage, D.; Ozolins, G.; Peterson, P.; Webster, A.; Orthofer, R.; Wandeweerd, V. and Gwynne, M. (1996), Urban air pollution in megacities of the world. *Atmospheric Environment*, **30**, 681-686.

Mello, W.Z. (2001), Precipitation chemistry in the coast of the Metropolitan Region of Rio de Janeiro, Brazil. *Environmental Pollution*, **114**, 235-242.

Mello, W.Z. and Almeida, M.D. (2004), Rainwater chemistry at the summit and southern flank of the Itatiaia massif, Southeastern Brazil. *Environmental Pollution*, **129**, 63-68.

Moraes, R. M.; Delitti, W. B. C.; Moraes, J. A. P. V. (2000), Respostas de indivíduos jovens de *Tibouchina pulchra* Cogn. à poluição aérea de Cubatão, SP: fotossíntese líquida, crescimento e química foliar. *Revista Brasileira de Botânica*, **23**, 441-447.

Neufeld, H. S.; Jernstedt, J. A. and Haines, B. L. (1985), Direct foliar effects of simulated acid rain. I. Damage, growth and gas exchange. *The New Phytologist*, **99**, 389-405.

Nouchi, I. (1992), Acid precipitation in Japan and its impacts on plants – 1. Acid precipitation and foliar injury. *JARQ*, **26**, 171-177.

Paparozzi, E. T. and Tukey, H. B. J. (1983), Developmental and anatomical changes in leaves of Yellow Birch and Red Kidney Bean exposed to simulated acid precipitation. *Journal of the American Society of Horticultural Science*, **108**, 890-898.

Percy, K. E. and Baker, E. A. (1987), Effects of simulated acid rain on production, morphology and composition of epicuticular wax and on cuticular membrane development. *The New Phytologist*, **107**, 577-589.

Rathier, T. M. and Frink, C. R. (1984), Simulated acid rain: Effects on leaf quality and yield of broadleaf tobacco. *Water, Air and Soil Pollution*, **22**, 389-394.

Reig-Armiñana, J., Calatayud, V., Cerveró, J., García-Breijo, F. J., Ibars, A. and Sanz, M. J. (2004), Effects of ozone on the foliar histology of the mastic plant (*Pistacia lentiscus* L.). *Environmental Pollution*, **132**, 321-331.

Rennenberg, H.; Herschbach, C. and Polle, A. (1996), Consequences of air pollution on shoot-root interactions. *Journal of Plant Physiology*, **148**, 269-301.

Santos, O. C. O. and Souza, J. R. S. (1988), Estudo preliminar da acidez das águas de chuvas em Belém, Pará. *Boletim do Museu Paraense Emílio Goeldi*, **32**, 1-11.

Schneider, S. Z. (1985). *Anatomia foliar de* Clusia hilariana *Schlechtendal e* Clusia spiritu-sanctensis *G. Mariz et Weinberg (Guttiferae) ocorrentes no estado do Espírito Santo*. M.Sc. Thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

Silva, L. C.; Azevedo, A. A.; Silva, E. A. M. and Oliva, M. A. (2000), Flúor em chuva simulada: sintomatologia e efeitos sobre a estrutura foliar e o crescimento de plantas arbóreas. *Revista Brasileira de Botânica*, **23**, 383-391.

Silva, L. C.; Azevedo, A. A.; Silva, E. A. M. and Oliva, M. A. (2005a), Effects of simulated acid rain on the growth and anatomy of five Brazilian tree species. *Australian Journal of Botany*, **53**, 1-8.

Silva, L. C.; Oliva, M. A.; Azevedo, A. A.; Araújo, J. M. and Aguiar, R. (2005b), Micromorphological and anatomical alterations caused by simulated acid rain in Restinga plants: *Eugenia uniflora* and *Clusia hilariana*. *Water, Air and Soil Pollution*, **168**, 129-143.

Silveira, M. (1989), Preparo de amostras biológicas para microscopia eletrônica de varredura. In: Souza, W. (Ed.). *Manual sobre técnicas básicas em microscopia eletrônica.* Técnicas básicas. Rio de Janeiro: Sociedade Brasileira de Microscopia Eletrônica. v.1. pp. 71-90.

Temple, P. J.; Riechers, G. H. and Miller, P. R. (1992), Foliar injury responses of Ponderosa pine seedlings to ozone wet and dry acidic deposition, and drought. *Environmental and Experimental Botany*, **32**, 101-113.

Turunen, M. and Huttunen, S. (1991), Effect of simulated acid rain on the epicuticular wax of Scots pine needles under northerly conditions. *Canadian Journal of Botany*, **69**, 412-419.

Zobel, A. and Nighswander, J. E. (1991), Accumulation of phenolic compounds in the necrotic areas of austrian and Red Pine needles after spraying with sulphuric acid: a possible bioindicator of air pollution. *The New Phytologist*, **117**, 565-574.

Zobel, A. M. (1996). Phenolic compounds against in defence air pollution. In: Yunus, M. and Iqbal, M. (Eds.). *Plant response to air pollution*. Chincester: John Wiley. pp. 241-266.

Received: September 17, 2004;
Revised: April 18, 2005;
Accepted: January 04, 2006.