HISTOCHEMICAL SIMILARITY BETWEEN INDIVIDUALS FROM DIFFERENT LOCATIONS

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INTRODUCTION

Ocotea odorifera is a woody tree species, perennial, reaching 20 to 25 m high, with 50 to 70 cm in diameter at 1,30m of the soil surface (Reitz *et al.,* 1978). Characteristic of Atlantic Rain Forest, the species is found in shallow soils, well drained, showing crown dense and rounded (Lorenzi 1992) between altitudes of 10 to 1200 m in regions with average annual temperatures ranging from 12 C to 23 C (Carvalho 1994). It has wide geographical distribution, ranging from southern Bahia until the Rio Grande do Sul State, occurring also in Argentina and Paraguay (Kageyama *et al.,* 2003). This species is economically important due to the quality of its wood and the essential oil extracted of its roots, bark and leaves and was intensively explored in the Brazilian Atlantic Forest in the decades of 40 - 70 (Santa - Catarina *et al.,* 2001), with consequent reduction of the original population, which resulted in its inclusion in the list of species threatened with extinction. Nowadays it is recognized as a priority for conservation in germplasm banks (Vieira 1999).

Inorganic and organic extracts of leaves, barks and roots of *O. odorifera* showed to be phytotoxic to some cultivated and non - cultivated plant species (Borges *et al.,* 1993; Carmo, 2001; Carmo *et al.,* 2007; Gatti *at al.* 2008). It is widely know that phytotoxic substances are produced and vary in concentration in the body of the plant, under the influence of biotic and abiotic factors. So, it is expected that individuals of *O.odorifera* living under different climatic conditions must constitute ecotypes, differing each other morphologically and/or physiologically.

OBJECTIVES

The goal of this study was investigate the morphology, anatomy and histochemistry of *O. odorifera* leaves sampled in Minas Gerais State, Southeast Brazil, and compares the results with data of individuals from Parana and Santa Catarina State, South Brazil.

MATERIAL AND METHODS

O. odorifera leaves were collected from adult individuals of the Silvicultura, a forest area of the Viçosa Federal University, in the Municipality of Viçosa, Minas Gerais State, Brazil. This place is located 20045'13.81"S and 42052'55.20"W, between 895 and 610m of altitude. The regional climate is Cwa, mesothermic wet, according to the Köppen classification (Kottek *et al.,* 2006), with rainy season from September to April and dry season from April to September. The average annual precipitation is 1221.4 mm, the average relative humidity is 81%, the mean air temperature is 19.4o C, ranging between 14.8oC and 26.4oC (Assembléia legislative de Minas Gerais, 2009).

Anatomical and histochemical investigations - Samples of the collected leaves were taken of its medium region and fixed in FAA50 (Johansen 1940), dehydrated in an ethanol series, and embedded in methacrylate resin (Historesin, Leica Microsystems, Nussloch Gmbh, Heidelberg, Germany). Using an automatic rotary microtome (Leica RM 2155, Mycrosystems Inc., Leica Mycrosystems, Deerfild, III), equipped with a disposable stainless steel blade, transverse sections (8 μm thick) were cut.

For structural characterization some sections were stained with Toluidine blue at pH 4.0 and for total proteins, other sections were stained with xylidzine pounceau (XP) (O'Brien & McCully 1981) and for neutral polysaccharides, it was used PAS - periodic acid/Schiff reagent (McManus 1948). The slides were mounted in synthetic resin (Permount Fisher, Pittsburgh, Penn.).

To investigate the major classes of the secondary metabolites, fresh samples of the median region of leaves were sectioned transversely using a freezing microtome (Leica CM 1850 Mycrosystems Inc., Leica Mycrosystems, Deerfild, III) and the material were investigated using histochemical tests as described in Table 1. The slides were mounted with glycerine - water. Control sections were performed simultaneously to each histochemical test adopting the standard procedures recommended. The observations and photo-

graphic documentation were taken under light microscope (AX70TRF, Olympus Optical. Tokio, Japan), equipped with U - Photo system of photomicrography.
These results were compared with data published by Toledo *et al.,* (2004).

RESULTS AND DISCUSSION

Structural characterization: The *Ocotea odorifera* leaves are simple, petiolate, margins entire, leather - like consistency, with shiny appearance. The epidermises of leaves are uniseriate in both side, glabra and its cells contain prismatic crystals, with thicker cuticle in adaxial face. The leaves present paracytic stomata, formed by guard cells covered by the projections of the subsidiary cells, and are hypostomatic. The mesophyll has two layers of palisade parenchyma, and the subepidermal shows more compact arrangement than the spongy parenchyma, which is formed by several layers of loose arrangement. Between the parenchyma cells are found idioblasts randomly distributed.
The edge is slightly bent toward the abaxial region and is coated internally by sclereids. The vascular bundles are surrounded by trichosclereid that extend toward the epidermis in the adaxial and abaxial sides. In the midrib region, the vascular system is formed by a continuous line, where the xylem is oriented towards the adaxial face and phloem to the abaxial face, and both tissues are accompanied by perivascular fibers. Underlying the epidermis there are the collenchyma and just beneath there are the parenchyma cells. Inside the cells of parenchyma and collenchyma there are small crystals and the collenchyma sometimes shows cells with lignified walls.
When observed in vivo the O. odorifera leaves shows yellowish in the vascular bundle of the midrib and a greenish aspect in the mesophyll due to the presence of chlorenchyma, and drops of translucent material can be observed inside the idioblasts.
Histochemical tests: The histochemical testes with sudan red showed the presence of lipophilic substances in the mesophyll (mainly in the palisade parenchyma chloroplasts) and in the idioblasts and also highlighted the lipophilic nature of the cuticle. The tests with PAS pointed out mucilages. Neutral lipids were evidenced in idioblasts by the Blue Nile test and show the cellular content pink. Fatty acids were evidenced in mesophyll chloroplasts and in the phloem by copper acetate/rubeanic acid test that make this cells the dark - green. Nadi reagent showed positive result to specific terpenoids in idioblasts wich secretion became purple - violet, thereby determining its oil - resin composition. It were detect total polysaccharides, pectins and mucilages in the cell wall, mainly in the phloem, and in idioblasts secretion. Ruthenium red evidenced the pectic nature of chlorenchyma cells, but this reaction was not too pronounced because these cells walls are sclerified. Starch was evidenced in mesophyll, chlorenchyma, parenchyma and perivascular fibers cells by lugol. Tests with PAS detected phenolic compounds in phloem and in mesophyll, especially in the palisade parenchyma cells. The test with vanillin hydrochloric confirmed the tanic nature of these phenols. The presence of lignin was detected by phloroglucinol in chlorenchyma, xylem, perivascular fibers and trichosclereid. No alkaloids or total protein were detected.
Discussion
The structural features shown in this work for *O. odorifera* leaves is typical of Lauraceae botanical family and are supported by works of Metcalfe (1987), Christophel *et al.,* (1996), Toledo *et al.,* (2004), Marques (2001) and Boeger *et al.,* (2004).
The histochemical tests showed terpenoids like oil - resins founded at idioblasts, which content also may be mucilages, as this work evidenced in accordance with Santos & Oliveira, (1988) and Toledo (2004), although oleiferous cells are more frequent than the mucilagenous cells (Metcalfe 1987). Also, it was detected phenolic compounds like tannins in the palisade parenchyma cells compounding the O. odorifera essencial oil. These results are in accordance with those presented by Toledo (2004).
To Gottlieb & Borin (1999) the coexistence of different metabolic categories, as it is observed in Lauraceae botanical family, may lead to alternate predominance of ecotypes of a species accompanying changes in local ecological environments. It is made possible by an evolutionary status characterized by the simultaneous presence of at least two micromolecular categories of chemical compounds, as shikimate and acetate derivatives.
The presence of essential oils in plant species is widely attested (Silva *et al.,* 2001, Kageyama *et al.,* 2003), and, in some cases, these compounds are involved in the inhibition of germination in plant species (Machado & Craveiro 1986; Harborne 1993). One common constituent of essential oils are monoterpenes that are in turn correlated with the inhibitory effects observed in seeds germination, development of plants and micro - organisms exposed to its presence (Rice 1984).
Terpenoids constitute the largest class of secondary products. In spite of diverse substances of this class are generally insoluble in water (Gershenzon, 2004) the release of terpenes and water - soluble phenolics by members Asteraceae, Lamiaceae, Myrtaceae, Rutaceae, and Rosaceae families may inhibit the stablishiment of other plant species in some communities (Cseke, 2006). Also Indejit & Duke (2003) reported that more classes of secondary compounds associated with inhibitory effects on plants are phenols and terpenes.
Phenolic compounds has significant physiological roles and implications in plant growth and metabolism, reducing auxin and giberelin activities, altering mitosis, cellular respiration, photosynthesis and enzymatic activities (Rice, 1984; Chou, 1999) resulting in physiological and ecological consequences as inhibition of seed germination and plant establishment in plant communities, and thus influencing the local diversity (Harborne 1980). The phenolics importance as allelochemicals arrives, in part, of its solubility in water and inhibitory effects are frequently related to phenolic acids, hydrolysable tannins, flavonoids, quinines, etc. It has been reported phenolic compounds, like pinene, cineole, camphor, safrole, methyleugenol and eugenol as constituent of *O. odorifera* essential.
The morphological and anatomical similarity of results between Boerger *et al.,* (2004) and Toledo (*et al.,* 2004)

works and the results presented here evidenced that *O. odorifera* leaves morphology and anatomy does not respond to the range of variations of environmental conditions between Minas Gerais and Paraná State.

Toledo *et al.,* (2004) sampled the specimens for his work at Colombo and Araucaria, Parana State. Colombo is located at 25º17'33.42"S and 49º13'23.05"W, elevated 1018m above the sea and Araucária is at 25º35'36.51"S and 49º24'31.69"W coordinates, at 892m of altitude. Boerger *et al.*, 2004), in turn, collected his data at Itapoã, Santa Catarina State, at coordinates $26^0$07'01"S and $48^0$36'58"W, at 18m above the sea. The climate of this south Brazilian region is subtropical mesotermic wet, Cfc and Cfb according Köpen classification, with temperatures varying between - 3ºC to 18ºC in winter and 10ºC to 22ºC in summer. In this region, soils are predominantly Cambisol, Red /Yellow Alic Latosol, Eutrophic Red Yellow Podzolic and also Hydromorphic gley

Morpho - anatomical characteristics of leaves are greatly influenced by environmental factors that vary in space and time (Givnish, 1984; Pyykko, 1979) and leaves morphology has been used to compare responses of individual of the same taxa to different environment conditions (Bongers & Popma, 1990; Geeske *et al.*, 994, Turner *et al.*, 995). Our results suggest that these parameters are conservatives to this specie.

In addition our results confirm the hypothesis of Gottlieb (1972) that individuals of *O. odorifera* from Minas Gerais are morphologically and anatomically indistinctive of those from Santa Catarina State although they are physiologically different, constituting ecotypes (Turesson, 1922) or specifically chemo types. Gottlieb (1972) pointed out that populations of *O. odorifera* from São Paulo, Rio de Janeiro, Espirito Santo and Minas Gerais States contains methyleugenol, eugenol and 1 - nitro - 2 - phenylethane, as main constitutes of its essential oil instead of safrol (Gottlieb & Magalhães, 1959) in response of different environmental conditions.

CONCLUSION

The morpho - anatomical and histochemistry investigation of *O. odorifera* leaves from Minas Gerais State do not revealed diferences from those individuals from Paraná State leading us to conclude that individuals from that populations do not constitute morphological ecotypes.

To Capes and CNPq

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