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Effects of air pollution on needles of *Cedrus atlantica* (Endl.) Carriere in industrial area of Pančevo (Serbia)

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ABSTRACT Research was done on needles of *Cedrus atlantica* (Endl.) Carriere from polluted and unpolluted environment. Stereological method was used for quantitative examination of changing of different structures of the needles. The obtained results showed that the volume density of mesophyll cells and intercellular spaces were statistically significantly increased, while the volume density of epidermis, central cylinder and resin ducts were decreased in polluted environment. The number of healthy mesophyll cells was decreased, while the number of damaged mesophyll cells was increased in polluted environment. The surface area of damaged mesophyll cells was significantly increased in polluted environment. Histological investigation was used to identify the injured mesophyll cells of types I, II and III of the needles by the appearance of tannin inclusion in central vacuole, and their distribution in the needles. Histological results showed that damaged mesophyll cells of types I, II and III were differently located in the needles. Damaged mesophyll cells of type III were predominantly distributed around stomata, while damaged cells of type II were located in the middle of the needle. Cells of type I were observed near the endodermis.

Key words: *Cedrus atlantica*, needle, air pollution, stereology, histology

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INTRODUCTION

Pollution complexes emitted into the atmosphere as a result of industrial activities are known to affect the physiological and biochemical condition of plants, depending on the chemical composition and concentration of pollutants. Forest health is deteriorating all across Europe and air pollution is found to be the prime cause of tree damage. Coniferous trees are especially sensitive to different pollutants.

Changes of structure of conifer needles caused by air pollutants have been analysed in many papers (Fink 1988; Back & Huttunen 1992; Back *et al.* 1994; Onder & Dursun 2006). The most injurious air pollutants are SO_2, NO_x, HF, HCl and O_3 (Reinikainen & Karenlampi 1987; Jacobson *et al.* 1990a, 1990b; Jordan *et al.*1990; Momen & Helms 1996). Decreased intensity of photosynthesis, disorders in function of some enzymes, damaging of chloroplasts and mitochondria were caused by effects of air pollution (Sutinen 1989; Eguchi *et al.* 2004). The stereological point-counting method for measuring volume density or volumetric proportion of leaf tissues has become widely used (Parkhurst 1982; Albrechtova & Kubinova 1991). Stereology is a precise tool for acquiring

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quantitative three-dimensional information based on two-dimensional data obtained from microscopic structures in tissue sections. There are no stereological and histological investigations about effects of air pollution on the needles of *C. atlantica*. The aim of this study was to examine the effects of air pollution and acid rain on the structure of the needles of *C. atlantica* in the polluted area. The purpose was to identify level degree and extent of the damaged in still green needles at the light microscope.

MATERIAL & METHODS

Sampling of the cedar needles for investigation was done in the vicinity of fertilizer and chemical factories, in the industrial zone of Pančevo. Needles for control were collected from spruce trees located in area about 6 km from the factory, in the opposite direction of dominant wind. Samples were taken from three trees from controlled and three trees from polluted area. From each tree three healthy looking green needles were used for stereological and histological investigation. The samples for microscopic observation were taken about 2 mm from the top of the needle. The needles were always sectioned in a drop of 2% glutaraldehyde and put into a fixative solution of 2% glutaraldehyde in phosphate buffer (0.1 M, pH =7.0), postfixed in 2% OsO_4 and embedded in Araldite. Procedures of prefixation and fixation were done according to (Soikkeli 1978). Ten cross-sections of 1mm thickness from each needle were stained by toluidine blue. Weibel¢s multipurpose test grid (M 42) was used to estimate volume density of different structures in the needles, number of healthy and damaged mesophyll cells and their profile area (Weibel *et al.* 1966). Volume density (V_V) is defined as the percentage of the total volume of a well-defined reference space occupied by any given component within it. The injured cells in cross-section of needles were identified by the appearance of tannins. Air pollutants cause alteration of tannin inclusion, and the comparison with healthy needles can give a result of the damaging level (Soikkeli 1981). In normal healthy cells tannins are of the small granular type. In the first stage of cell injury (type I) tannins are evident as more or less scattered particles in the central vacuole. In the second stage of cell injury (type II) tannins are evident as big clumps with or without granules and with thin ribbon at the margin of the central vacuole.

In the third stage (type III) tannins form a ribbon and occupy whole central region of the cell, pushing back cytoplasm to plasma membrane (Soikkeli 1981). Light microscope GALEN III was used for these investigations. Data were subjected to Student - t test (P) and expressed results as mean ± SEM.

Table 1. Volume density (mm°) of different structures in the needles of *C. atlantica* (mean ± S.E.M.)

Vv (mm°)	Control	air pollution	P
Epidermis	0.21 ± 0.0036	0.17 ± 0.0026	<0.001
Mesophyll	0.48 ± 0.0073	0.55 ± 0.0101	<0.001
Intercellular spaces	0.12 ± 0.0043	0.15 ± 0.0093	<0.01
Resin ducts	0.03 ± 0.0017	0.01 ± 0.0016	<0.001
Central cylinder	0.14 ± 0.0047	0.09 ± 0.0029	<0.001

Table 2. Volume density (mm°) of mesophyll cells in the needles of *C. atlantica* (mean ± S.E.M.)

Vv (mm°)	Control	Air pollution	P
Healthy cells	0.97 ± 0.0049	0.47 ± 0.0171	<0.001
Damaged type I	0.02 ± 0.0049	0.22 ± 0.0112	<0.001
Damaged type II	0	0.3 ± 0.0161	0
Damaged type III	0	0	0

RESULTS AND DISCUSSION

The values obtained for volume density measurements of different structures in the needles from the vicinity of Pančevo are presented in Table 1.

The results of examined cedar needles showed that the volume density of mesophyll and intercellular spaces were significantly increased, while the volume density of epidermis, central cylinder and resin ducts were significantly decreased in polluted area.

Volume density of mesophyll cells are presented in Table 2.

Volume density of damaged mesophyll cells was statistically significantly increased, while the volume density of healthy mesophyll cells was decreased in polluted environments. The values obtained for the number of healthy and damaged mesophyll cells are presented in Table 3. The number of healthy mesophyll cells was decreased in polluted area. Damaged mesophyll cells (type I, II and III) were differently presented in the needles of *C. atlantica* (Fig.1). Damaged mesophyll cells of type II were more numerous than damaged mesophyll cells of types I and III in polluted area. The results of surface area of healthy and damaged mesophyll cells are showed in Table 4. The area of healthy mesophyll cells decreased in polluted region, while the area of damaged mesophyll cells was significantly increased in polluted environment.

Table 3. Number of healthy and damaged mesophyll cells (types I, II and III)

April	Total No of cells	Healthy	Damaged	Type I	Type II	Type III
control	182 ± 6.9	142 ± 6.8	40 ± 0.6	40 ± 0.6	0	0
air pollution	114 ± 2.8	63 ± 4	51 ± 2.7	24 ± 2.7	26 ± 2.4	1 ± 0.6

Table 4. Surface area of healthy and damaged mesophyll cells (μm²)

Surface area of mesophyll cells	Control	Air pollution	P
Healthy cells	1762 ± 48	1486 ± 54	<0.001
Damaged type I	2028 ± 341	2551 ± 79	<0.05
Damaged type II	1842 ± 56	2971 ± 207	<0.001
Damaged type III	0	3003 ± 864	ns.

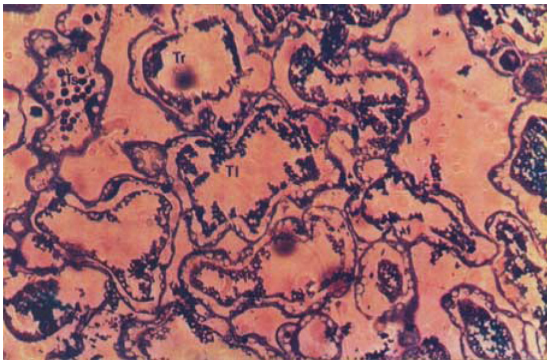

Fig.1. Micrograph of mesophyll cells (type I, II and III) on the cross-section of *C. atlantica* needle, 40X.

The results of histological research showed that the polluted environment caused damage of mesophyll cells, which was manifested by alteration of tannin inclusion and decrease in number of starch grains. Histological results showed that the damaged mesophyll cells of types I, II and III were differently located in the needles. Air pollutants penetrate the needle through the stomata during respiration and attack directly the mesophyll cells around the stomata.

This is the reason why the most damaged mesophyll cells (type III) were predominantly distributed around the stomata. Injury extended straight to the central cylinder and damaged mesophyll cells of type II were observed in the middle region of the needle, while mesophyll cells of type I were located near the endodermis.

Recent studies show that relationships between leaf anatomy parameters and photosynthesis are important in leaf acclimation to high or low irradiances (Pandey & Kushwaha 2005.).

The detailed and unbiased estimators of mesophyll parameters are becoming crucial for modeling physiological processes that underlie photosynthesis, such as gas transport or radiative transfer. (Aalto & Juurola 2002). It is believed that the present stereological analysis is a promising new tool for plant physiological and modeling research. (Albrechtova *et al.* 2007). Results obtained in this morphometric and stereological investigation of damaging level of different needle structures (epidermis, mesophyll, resin ducts, central cylinder and intercellulars) showed that needles of *C. atlantica* is more resistant to air pollutants comparing to structures in the needles of *Picea omorika* which was growing in polluted environment of Pančevo (Ilijin-Jug *et al.*1995).

CONCLUSION

Needles of *C. atlantica* which were exposed to air pollutants showed moderate accumulation of toxicants, decreased resistance and increased extent of injury especially in mesophyll cells. Results of morphometric and stereological examination of different needle structures showed higher level of resistance to air pollutants than the corresponding structures in the needles of other species which were growing in polluted environment. Because of its relative resistance to air pollutants *Cedrus atlantica* can be planted in the industrial environments.

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REZIME

Uticaj aeropolutanata na četine *Cedrus atlantica* (Endl.) Carriere u industrijski zagađenoj sedini Pančeva (Srbija).

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Istraživanja su obavljena na četinama *Cedrus atlantica* (Endl.) Carriere u zagađenoj i nezagađenoj sredini. Rezultati stereoloških istraživanja su pokazali statistički značajno povećanje volumenske gustine ćelija mezofila i intercelulara, dok su rezultati dobijeni merenjem volumenske gustine epidermisa, centralnog cilindra i smonih kanala ukazali na njihovo smanjenje u zagađenoj sredini. Broj zdravih mezofilnih ćelija je bio smanjen, dok je broj oštećenih mezofilnih ćelija bio uvećan u zagađenoj sredini. Površina oštećenih ćelija mezofila bila je takođe značajno uvećana u zagađenoj sredini. Rezultati histoloških ispitivanja su pokazali da su oštećene mezofilne ćelije tipa I, II i III različito raspoređene u četini. Oštećene mezofilne ćelije tipa III bile su uglavnom raspoređene oko stoma, dok su oštećene ćelije tipa II bile raspoređene u sredini četine. U blizini endodermisa nalazile su se oštećene ćelije tipa I.

Ključne reči: *Cedrus atlantica*, četina, aerozagađenje, stereologija, histologija