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Air Pollution Effects on the Leaf Structure of *Laurus nobilis*, an Injury Resistant Species

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The emission of air pollutants from the various industrial, domestic and social activities of man create a serious problem by turning the atmosphere in to a mixture of toxic and suffocating gases wich are disastrous to both plants and animals. The action of air pollutants to plants has long ago been the matter of several investigations. Structural modifications of plant cell organelles (Wellburn et al. 1972; Fisher et al. 1973; Reinert et al. 1975; Lentzian and Unsworth 1983; Patel and Devi 1984), inhibition of photosynthesis (White et al 1974; Capron and Mansfield 1976; Ormrod et al 1981) and finally, elimination of primary productivity (Fisher et al 1973; Winner 1981; Margaris et al 1985) have been reported by environmental scientists.

Concerning plant responses at the ultrastructural level it is well documented that chronic injury is displayed as chlorosis and subsequent necrosis due to chlorophyll destruction which is also followed by thylakoid swelling and chloroplast lysis (Lendzian and Unsworth 1983, Psaras and Christodoulakis 1987; 1987a). Moreover it was also observed that ultrastructural injuries appear prior to any other visible modification on the leaf (Lendzian and Unsworth 1983).

Although the problems caused by the long-term exposure to air pollution are serious for the existence of plants, the support of plant life in heavily polluted areas is essential for the improvement of man's life in a hard-to-breath atmosphere. In Athens the problem of photochemical smog is so serious that life in the metropolitan area becomes dangerous (Margaris et al.1985). That is why the Laurus nobilis (laurel) plants growing on the partition isle of a continuously traffic-loaded and heavily polluted main street in Athens, attracted our attention and became the subject of our investigation

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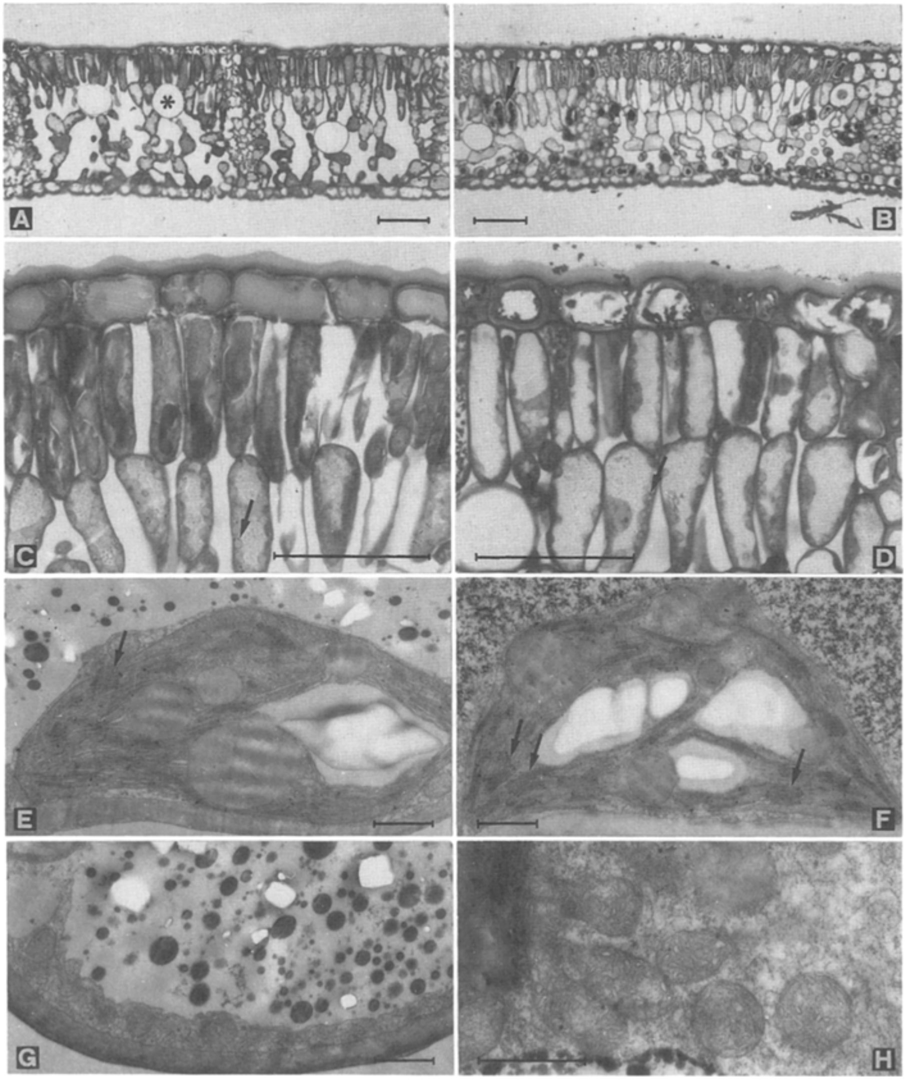

Figure 1. A: Cross section of a non polluted leaf. Asterisk marks an oil gland. B: Cross section of a polluted leaf. Arrow indicates a cell with condensed tannins. C: Palisade tissue from a non-polluted and D: from a polluted leaf. Arrows indicate chloroplasts. Bars in all OM micrographs represent 100μm. E: Electron micrograph of the chloroplast in an non-polluted and F: in a polluted leaf. G: Oil droplets and crystals in the vacuole from the cell of a polluted leaf and H: mitochondria from the cells of a polluted leaf. Bars in EM micrographs represent 1μm.

on the purpose to find out how far could the effects of the long-term exposure go and how a heavily fumigated, suffocating plant could react.

MATERIALS AND METHODS

Leaves from cultured individuals of Laurus nobilis were collected the same day and time from a heavily polluted street in Athens and from a practically non-polluted area inside the basin created by the mountains round Athens, on the western slope of mount Hymettus. The leaves were cut into small pieces and fixed immediately in phosphate buffered 3% glutaraldehyde at pH 7 for 2 hours at 0 °C. The tissue was post fixed in 1% OsO_4 in the same buffer for 2 hours at 0 °C, dehydrated in graded ethanol series and embedded in Durcupan ACM (Fluka). Sections were cut on an LKB Ultrotome III. Semithin sections were glass mounted and stained in toluidene blue "O". Ultrathin sections were double stained in uranyl acetate and lead citrate and observed with a Philips 300 transmission electron microscope. Leaves of both types were also prepared for scanning electron microscopy. Dehydration in these leaves was followed by critical point drying in CO_2, coating with gold - palladium and viewing with a Cambridge S150 Stereoscan.

RESULTS AND DISCUSSION

A comparative examination of leaf sections under the light microscope revealed that both types of leaves are equally developed (Figs 1 A and B). A very thick cuticle on a unilayered epidermis is a common structural characteristic of a mediterranean evergreen sclerophyllous plant as Laurus nobilis happens to be. The cuticle and the tannin impregnated epidermal cells offer effective shielding against high light intensities and desiccation (Christodoulakis and Mitrakos 1987). The thicker wall of the epidermal cells in polluted leaves is an indication that exposed plants turn to be more xerophytic. Stomata, as in all evergreen sclerophylls, appear only on the lower surface. A close look in the palisade tissue (Figs 1 A and B) revealed that the cells in both types of leaves possess numerous chloroplasts (indicated by arrows) which accumulate starch granules. Starch was also observed in the cells of the bundle seath extensions. Mesophyll cells accumulating phenolics are common in both leaf types but the existence of condensed tannins, an outstanding characteristic of xeromorphic leaves, was mostly observed in polluted leaves (arrows in Fig.1A). It also seems that the intercellular spaces where the essential oils of the laurel leaf are accumulated (asterisc in Fig 1A) are superior in number in the non-polluted leaves but this variation is rather difficult to be evaluated since the factors controling the oil production are numerous and not well understood.

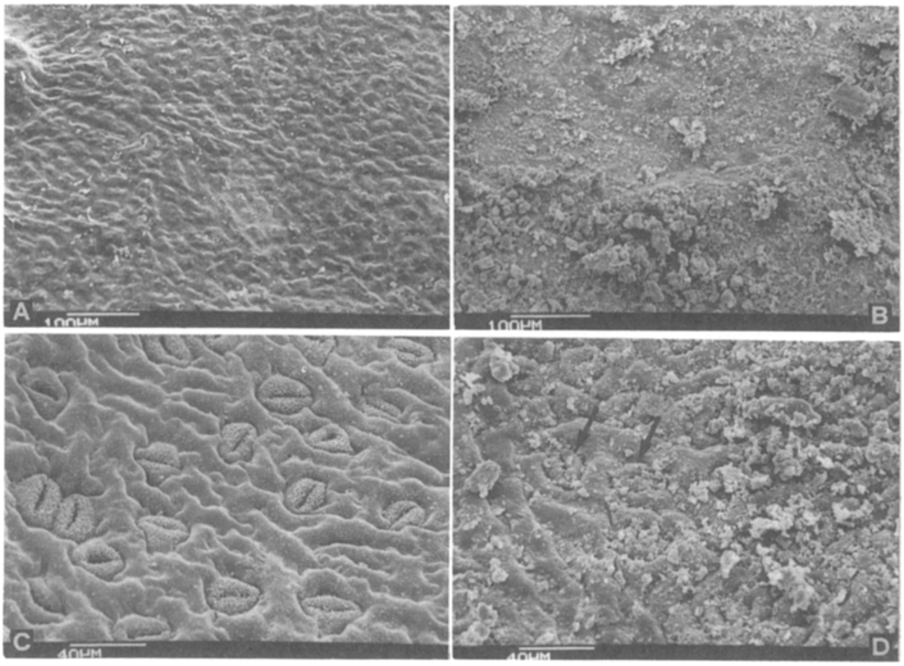

Figure 2. A and B: Upper surface from a non polluted and a polluted leaf. C and D: Lower surface from a non polluted and a polluted leaf respectively. Arrows indicate covered stomata.

Transmission electron microscope examination of the mesophyll cells revealed that chloroplast ultrastructure in the polluted leaf cells was surprisingly retained (cf Fig 1E to 1F). Well developed fretwork and grana with no signs of swelling appear in both types of leaves. Sized oil droplets and starch granules are present in the chloroplasts. In the large vacuole either phenolics or numerous oil droplets and raphide crystals are present (Fig 1G). The later are more common in the polluted leaf cells. Mitochondria retain their structure especially that of the inner membrane (Fig 1H). Microsomes also seem unaffected. Some membrane deformations observed in the cells of both leaf types are common in phenolic accumulating cells and result from the leaching of tannins through the tonoplast during fixation.

SEM micrographs revealed a continuous crust of pollutants over the upper surface of the polluted leaves (cf Fig 2A to 2B). The lower surface is also affected and stomata can hardly be distinguished through the various

particles deposited on the epidermis (Figs 2C and D).
Our observations indicate that although polluted leaves seem to be somehow more xeromorphic than the clean ones the usual deformations of the organelles common in other plants investigated, were not found. It also seems that productivity is not affected since starch granules are accumulated in the chloroplasts of both leaf types. Taking into account the fact that higher temperatures and desiccated air can routinely be monitored at the ground level of a traffic loaded and heavily polluted road, esspecially in a country where vehicle maintenance is depended on the owners good will, we might say that Laurus nobilis is an extremely tolerant species. The leaf characteristics which enable the plant to withstand the stressing conditions of the mediterranenan summer may count for its ability to tolerate air pollution.

REFERENCES

Capron TM, Mansfield TA (1976). Inhibition of net photosynthesis in tomato in air polluted with NO and NO_2. J Exp Bot 27: 1181-1186.
Christodoulakis NS, Mitrakos K (1987). Sstructural analysis of sclerophylly in eleven evergreen phanerophytes in Greece. In: NATO ASI Series, Vol. G15, Plant response to stress. Eds: Tenhunen JD et al. Springer-Verlag, Berlin-Heidelberg.
Fisher K, Kramer D, Ziegler H (1973). Electronmikroskopische Untersuchungen SO_2 - begaster Blater von Vicia faba. I. Beobachtungen aus Chloroplasten mit akuter schadigung. Protoplasma 76: 89-96.
Lentzian KJ, Unsworth MH (1983). Ecophysological effects of atmospheric pollutants. In: Lange OL, Nobel PS, Osmond CB, Ziegler H. (eds) Encyclopedia of Plant Physiology Vol 12D. Springer Verlag, Berlin. pp 465-502.
Margaris NS, Arianoutsou M, Tselas S, Loukas L (1985). Air pollution effects on Attica's natural ecosystems. Bull Environ Contam Toxicol 34: 280-284.
Ormrod DL, Black VJ, Unsworth MH (1981). Depression of net photosynthesis in Vicia faba L. exposed to sulphur dioxide and ozone. Nature 291: 585-586.
Patel JD, Devi GS (1984). Ultrastructural variations in leaves of Streblus asper growing near a fertilizer complex. Phytomorphology 34: 140-146.
Psaras GK, Christodoulakis NS (1987). Air pollution effects on the ultrastructure of Phlomis fruticosa mesophyll cells. Bull Environ Contam Toxicol 38: 610-617.
Psaras GK, Christodoulakis NS (1987a). Air pollution effects on the structure of Citrus aurantium leaves. Bull Environ Contam Toxicol 39: 474-480.

Reinert RA, Heagle AS, Heck WW (1975). Plant responses to pollutant combinations. In: Mudd JB, Kozlowski TT (eds). Responses of plants to air pollution. Academic Press. London, New York.
Wellburn AR, Majernik O, Wellburn FAM (1972). Effects of SO_2 and NO_2 polluted air upon the ultrastructure of chloroplasts. Environ Pollution 3: 37-49.
White KL, Hill AC, Bennett JH (1974). Synergistic inhibition of apparent photosynthesis rate of alfalfa by combinations of sulphur dioxide and nitrogen dioxide. Environ Sci Technol 8: 574-576.
Winner WE (1981). The effect of SO_2 on photosynthesis and stomatal behavior of mediterranean-climate shrubs and trees. In: Components of productivity of mediterranean-climate regions - Basic and applied aspects. Margaris NS, Mooney HA (eds) Dr W Junk Publ. pp 91-103.

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