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Environmental Contamination and Toxicology

Air Pollution Effects on the Structure of *Citrus aurantium* Leaves

G. K. Psaras and N. S. Christodoulakis

Institute of General Botany, University of Athens, Athens 157 84, Greece

Individual air pollutants cause acute and chronic plant injury, act on stomata and affect carbon dioxide exchange as well as plant growth and development. The action of pollutants on intact plants has been reviewed recently by Lentzian and Unsworth (1983). Plant responses to combinationsof pollutants have been reviewed by Reinert et al. (1975). Inhibition of photosynthesis by several air pollutants has been reported repeatedly (White et al. 1974; Capron and Mansfield 1976; Ormrod et al. 1981). Besides, structural modifications of cell organelles have been reported after fumigation by SO_2 (Wellburn et al. 1972). This data provides an explanation for the air pollution originating elimination of primary productivity, which has been reported by environmental scientists (Fischer et al. 1973; Winner 1981; Margaris et al. 1985).

Although chlorosis and subsequent necrosis are common phenomena caused by artificial treatment with pollutants, fine structural leaf characteristics of plants exposed to long-term air pollution in natural conditions are little explored. Light microscope examination of air pollution affected leaves of plants common in natural ecosystems of Athens' metropolitan area revealed chlorosis phenomena (Psaras et al. 1986). Patel and Devi (1984) studied ultrastructural variations in the leaves of *Streblus asper* (Moraceae) growing in a polluted environment resulting from the activities of a fertilizer producing complex. They reported irregular outlines of chloroplasts, accumulation of electron-dense materials in the thylakoids and minor structural deformations of other organelles. Electron microscope examination of the leaves of a common subshrub of greek phryganic formations (*Phlomis fruticosa;* Labiatae) grown in a heavily air polluted natural ecosystem of Athens metropolitan area revealed pronounced ultrastructural anomalies of chloroplasts, mitochondria and microbodies of the mesophyll cells (Psaras and Christodoulakis 1987). This organelle destruction of the photosynthesizing tissue as well as the minimization of the ecosystem

Send reprint requests to G.K.Psaras at the above address.

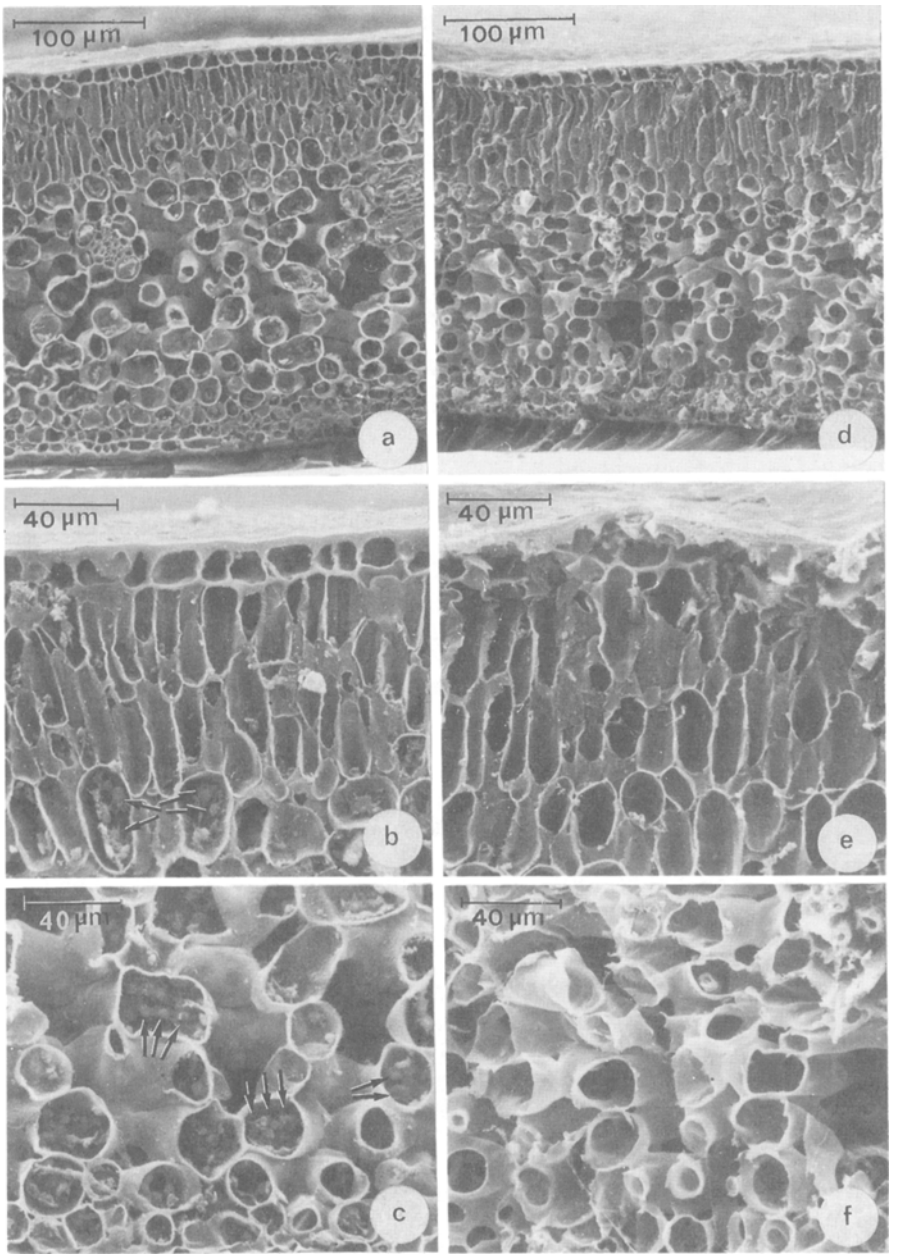

Figure 1. Pairs of scanning electron micrographs of normal (a-c) and polluted (d-f) leaves of *Citrus aurantium*. a,d, cross sections; b,e, upper epidermis and palisade parenchyma; c,f, spongy parenchyma. Note the absence of chloroplasts from polluted leaves.

primary productivity (Margaris et al. 1985) are attributed to the compound action of several toxic air pollutants of the photochemical smog of Athens.

This work describes the long-term air pollution effects on the structural features of the leaves of *Citrus aurantium*, a decorative species planted throughout the heavily air polluted city of Athens.

MATERIALS AND METHODS

Leaves from individuals of *Citrus aurantium* L. grown in a heavily air polluted region of Athens (Margaris et al. 1985) and from others grown in a practically non-polluted area were cut and fixed at the same time in phosphate buffered 3% glutaraldehyde at pH 7 for 2 h at $0^{o}C$ and postfixed in 1% OsO_4 in the same buffer. After dehydration with an ethanol series, plant material for scanning electron microscopy was critical point dried in CO_2, coated with gold-palladium and viewed with a Cambridge S150 Stereoscan scanning electron microscope. Plant tissue for light and transmission electron microscopy was embedded in Durcupan ACM (Fluka). Sections for light microscopy were stained in toluidine blue O and examined with a Carl Zeiss light microscope. Thin sections obtained on an LKB III ultramicrotome, were double stained with uranyl acetate and lead citrate and observed with a Philips EM 300 transmission electron microscope.

RESULTS AND DISCUSSION

A comparative scanning electron microscope examination of leaves from healthy and air pollution affected plants reveals that leaf epidermal tissue of the former seems to be more smooth than the one of the latter (cf. Figs 1a,b to 1d,e). Concerning mesophyll, although we cannot observe any differences in tissue development or cell arrangement, we can distinguish a drastic reduction in the number of chloroplasts in the affected plants of both the palisade (cf. Fig.1e to 1b) and the spongy (cf. Fig. 1f to 1c) parenchyma cells. It seems likely that mesophyll cells in normal leaves bear a large number of chloroplasts whilst in affected leaves they are practically empty.

A confirmation of the scanning electron microscope observations comes from light microscopy (Fig. 2). Both the palisade (Fig. 2b, d) and the spongy mesophyll cells (Fig. 2c,e) of normal leaves possess numerous chloroplasts accumulating several starch grains (Fig. 2d,e); on the contrary both palisade (Fig. 2g,i) and spongy (Fig. 2h,j) mesophyll cells of the affected plants seem to be empty.

Transmission electron microscope examination of the leaves reveals details of the few remaining plastids in affected leaf mesophyll cells. The effects of air pollution on chloroplasts seem to be detrimental. Plastids are more or less spherical (Fig. 3a,c) or lobed (Fig. 3b) and they contain only a few stroma thylakoids either randomly distributed (Fig. 3a,c) or arranged in parallel

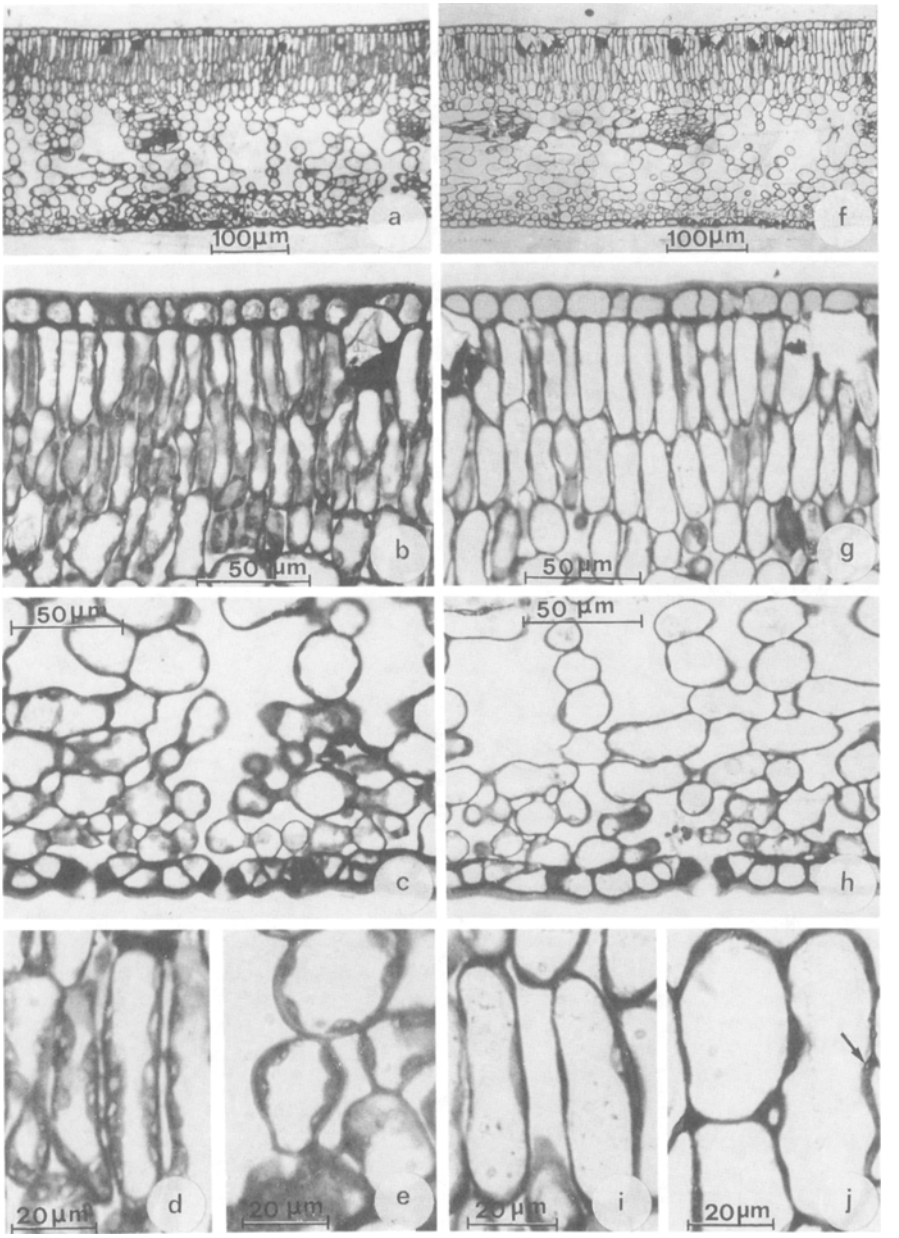

Figure 2. Light micrographs from non polluted (a-e) and polluted (f-j) leaves. a,f, cross sections; b,g, details of upper epidermis and palisade tissue; c,h, details of spongy cells; d,i, high magnification of palisade cells and e,j, spongy cells; arrow indicates a position with a few plastids. Starch accumulation and chloroplast distribution in non polluted leaves in evident.

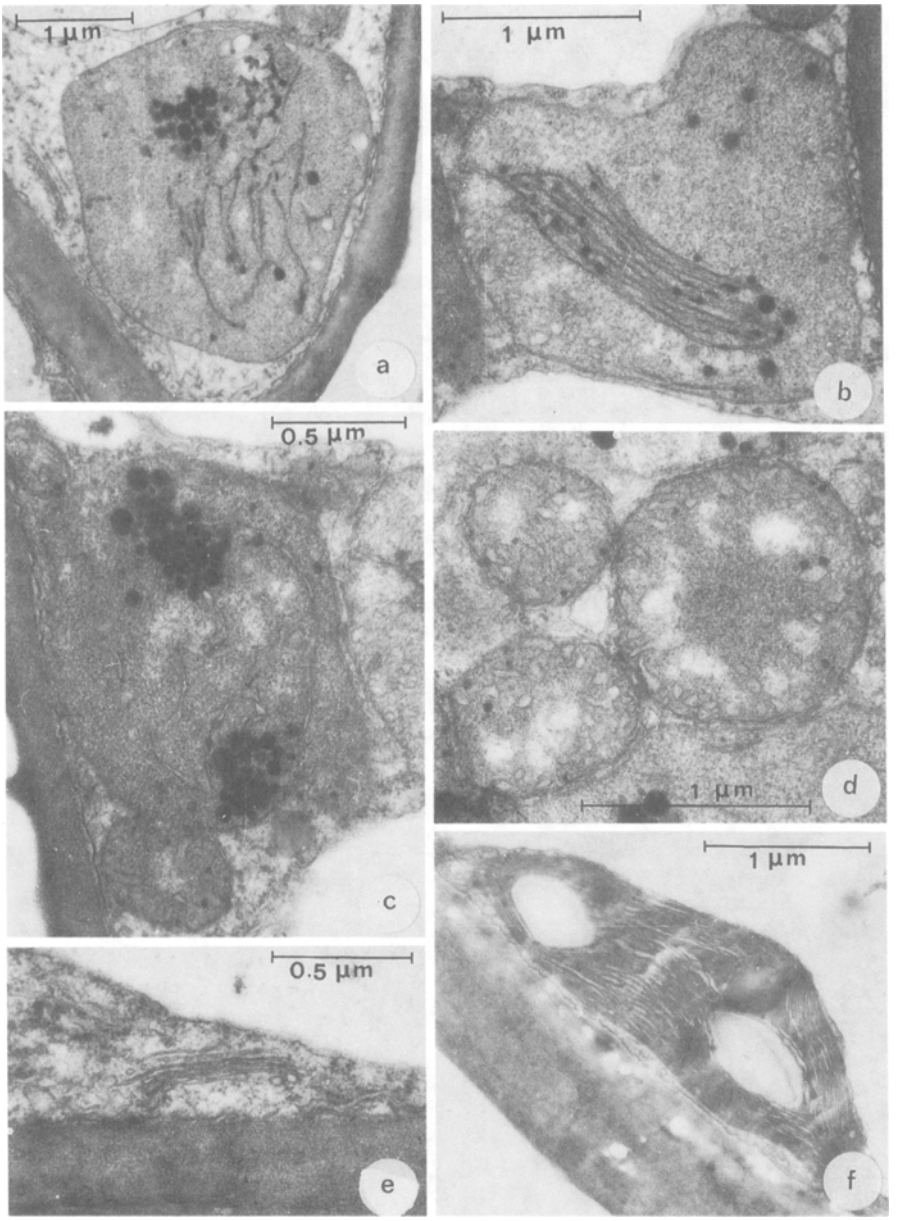

Figure 3. Transmission electron micrographs. a to e, from mesophyll cells of polluted plants; a, a chloroplast with few thylakoids randomly distributed and several grouped plastoglobuli; b, a chloroplast with thylakoids arranged in parallel and several plastoglobuli scattered throughout the stroma; c, a chloroplast with signs of thylakoid dilation; d, mitochondria; e, dictyosome; f, a chloroplast from a non polluted leaf; note the presence of starch grains and thylakoid arrangement.

(Fig. 3b), and several plastoglobuli (Fig. 3a,b,c). Thylakoids in some cases seem to be slightly dilated (Fig. 3c). On the contrary, chloroplasts of normal leaf mesophyll cells are lens-shaped, they contain several starch grains and present a well developed system of stroma and grana thylakoids (Fig. 3f). Mitochondria (Fig. 3d) and dictyosomes (Fig. 3e) do not seem to present any pronounced damage.

Ultrastructural effects of the long-term air pollution on *C. aurantium* leaves refer mainly to the plastids of mesophyll cells. *C.aurantium* affected plastids do not exhibit structural amonalies like the ones reported previously for *Phlomis fruticosa* (Psaras and Christodoulakis 1987). On the contrary their spherical shape, the formation of lobed contour and the existence of abundant plastoglobuli in their stroma are the same deformations as the ones reported for *Streblus asper* by Patel and Devi (1984). We can assume that the final effects of long-term air pollution on leaves of various species are not exactly the same. According to our data the outstanding feature of affected *C.aurantium* mesophyll plastids, always in comparison to normal ones, is their starch deficiency. This observation provides additional information directly confirming the strong reduction of photosynthetic activity caused by air pollution, a proposal mostly supported by environmental scientists (for references, see Lendzian and Unsworth 1983).

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