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# Air Pollution Effects on the Ultrastructure of *Phlomis fruticosa* Mesophyll Cells

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Industrial, agricultural and domestic activities of man result in the release of several toxic compounds into the atmosphere. In urban and industrial areas sulphur dioxide and oxidants generated by chemical reactions between oxides of nitrogen and hydrocarbons from vehicle exhausts result in a "photochemical smog" (Lendzian and Unsworth 1983). In Athens metropolitan area(Greece) the photochemical smog became a serious problem recently(Margaris et al.1985).

Plant physiologists and environmental scientists suggest that a basic effect of air pollution on plants leads towards the minimization of their productivity (Winner 1981; Margaris et al. 1985). On the other hand the action of individual pollutants on intact plants has been studied from biochemical as well as structural viewpoint. Thus the study of plant responses to $SO_2$ exposure revealed that this agent causes acute and chronic injury. The first symptom of acute injury is the appearance of bleached areas in the leaves. Chronic injury results in chlorosis and subsequent necrosis due to destruction of chlorophylls and final chloroplast lysis (Lendzian and Unsworth 1983). It has been documented that ultrastructural characteristics of leaves are affected prior to any visible injury. Electron microscope examination of $SO_2$ fumigated plant-attached leaves of *Vicia faba* revealed chloroplast thylakoids starting to swell whilst photosynthesis rate was drastically reduced (Fischer et al. 1973). Swelling of chloroplast thylakoids has also been observed by Wellburn et al. (1972) in *Vicia faba* after $SO_2$ and $NO_2$ fumigation.

The first light microscope-detected effects of air pollution on the leaf structure of plants common in natural ecosystems of Athens metropolitan area, have been reported by Psaras et al. (1984). A chlorosis phenomenon in *Urginea maritima* leaves as well as an indication of detrimental effects of *Phlomis fruticosa* mesophyll chloroplasts were documented.

In this work further investigation has been undertaken in order to elucidate the precise effects of air pollution on the ultrastructure of the photosynthesizing mesophyll cells.

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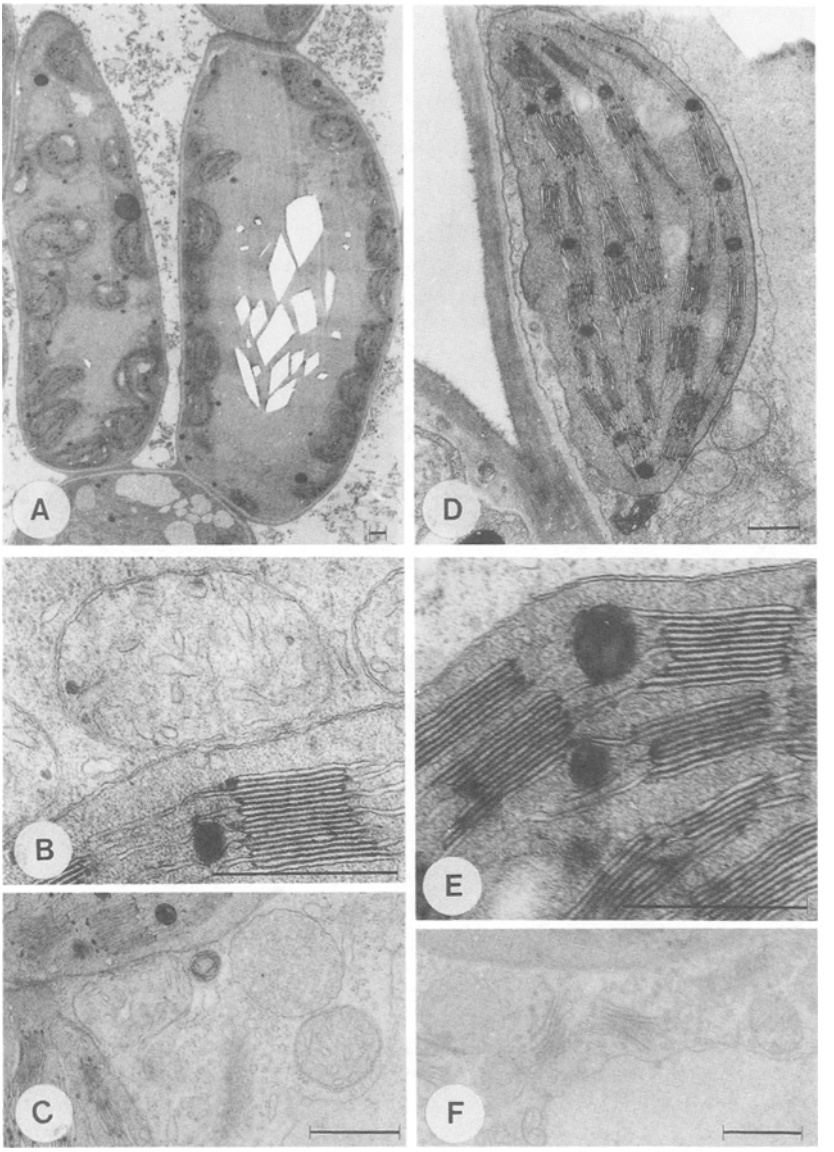


Figure 1. Electron micrographs of photosynthesizing mesophyll cells of non-polluted *Phlomis fruticosa* plants. A, Palisade cells in longitudinal section. A single layer of chloroplasts lines inner surfaces of the cell walls. In the central vacuole the sites of several crystals appear. B, Well-developed mitochrondria attached to a chloroplast. C, Portion of palisade cell showing a microbody and mitochondria in an intimate association with chloroplasts. Microbody matrix exhibits a fine granular appearance. D, A chloroplast in a part of a palisade cell. E, A portion of a palisade chloroplast showing the thylakoid system, E, Dictyosome of a palisade cell. Bars represent 0.5 µm.

## MATERIALS AND METHODS

*Phlomis fruticosa* L. (Labiatae) is a subligneous plant common in phryganic (drought deciduous) plant communities of Mediterranean-climate regions in Greece (Margaris 1976). Fully developed leaves of *P.fruticosa* were collected from a heavily air polluted and from a practically non polluted region inside Athens metropolitan area (Margaris et al. 1985). The polluted site is a smog-covered one, facing the industrial area and the city of Pireaus, therefore vehicular and industrial emissions are superabundant. On the contrary the non polluted area is located on the west slope of mountain Hymettus, east of Athens. Great care was taken in collecting the samples from uniform terrain conditions in order to minimize variations resulting from microhabitat factors.

Suitable pieces of leaves were fixed in phosphate buffered 5% glutaraldehyde at ph 7 for 3 h at 4°C. Buffer-washed material was post fixed in 1% $OsO_4$ at the same temperature. Washing and dehydration of the tissue in graded ethanol series followed. Finally the tissue was embedded in Durcupan ACM (Fluka). Ultrathin sections were double stained in uranyl acetate and lead citrate (Reynolds 1963) and examined in a Philips 300 electron microscope.

## RESULTS AND DISCUSSION

Electron microscope examination of palisade parenchyma, the most specialized type of photosynthetic tissue (Esau 1965), of non polluted *P.fruticosa* plants revealed that the elongated cells of this tissue accomodate numerous chloroplasts lining the inner surfaces of the cell wall in a single layer (Fig. 1A). As in most higher plants (Mühlethaler 1977), the thylakoid system of these organelles in *P.fruticosa* photosynthetically active cells, consists of grana stacks connected to a number of stroma thylakoids (frets, Fig. 1D) which finally give a membrane continuum (Jacobi 1977). The number and size of the thylakoids varies in each grana stack in such a way that precise distinction between grana and stroma thylakoids is not always possible (Fig. 1E). The same cells possess well developed mitochondria with pronounced cristae (Figs 1B,C) and several microbodies, especially in the proximity of chloroplasts, with homogeneous matrix lacking crystalline inclusions (Fig. 1C). Small vesicle producing dictyosomes were found as well (Fig. 1F).

The examination of palisade cells in plant leaves collected from polluted sites revealed several structural anomalies both in cytoplasm organization and cell organelles (Fig. 2A). The cytoplasm of these cells is occupied by large number of vesicles of various diameter with translucent contents, while the rest of it seems to be granular (Fig. 2B). The major damaging effect of air pollution was confirmed on the membrane system of chloroplasts. Dilated peripheral grana thylakoids give the first signs of destruction (Fig. 2C). In sections perpendicular to the surface of a thylakoid cisterna displacement of adjacent thylakoids cannot be observed at

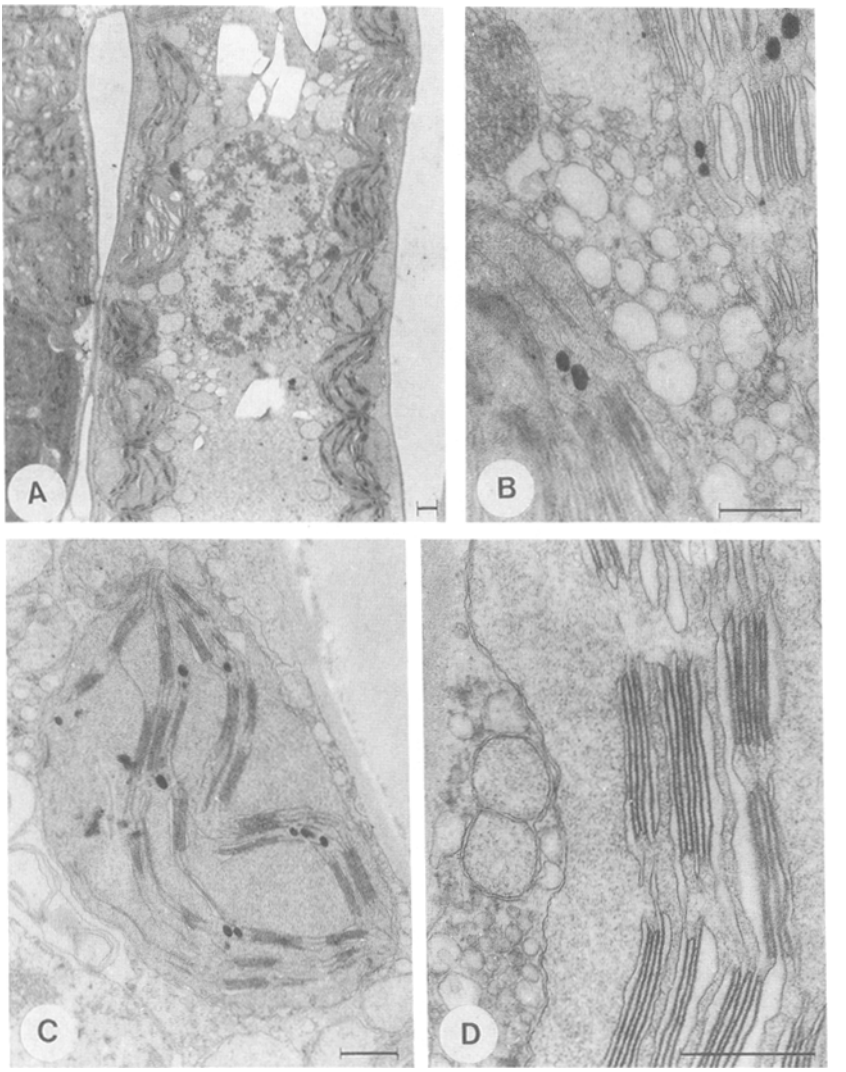


Figure 2. A, A palisade mesophyll cell in longitudinal section. The arrangement of chloroplasts seems to be unaffected. B, A portion of cytoplasm between two chloroplasts. Various diameter vasicles with translucent contents are superabundant. C,D, A slightly affected chloroplast (C). Peripheral thylakoids of grana begin to swell; adjacent thylakoids retain their normal profile (D). Bars represent 0.5 μm.

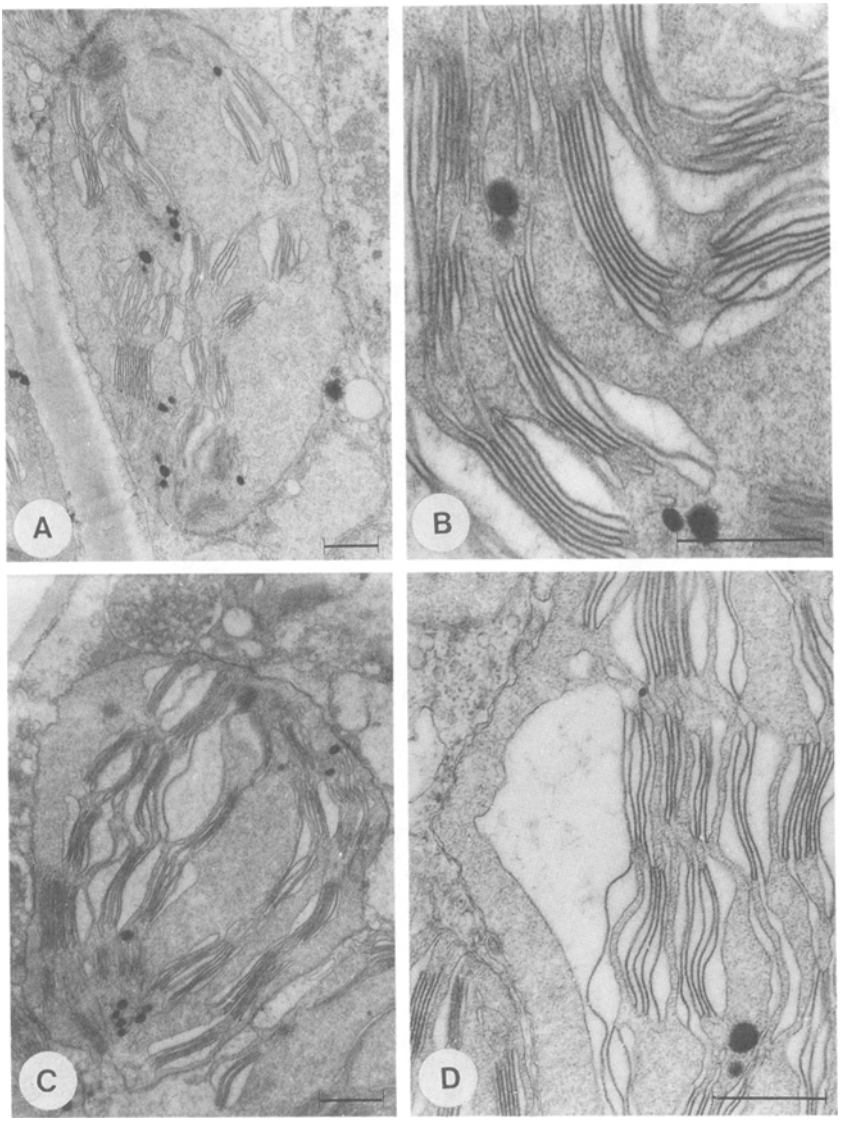


Figure 3. A,B, A moderately affected chlroplast (A). Swelling of peripheral trylakoids of grana is pronounced; some of the grana thylakoids retain their normal profile (B). C,D, A strongly affected chloroplast (C). Swelling and uneven enlargement of thylakoids cause displacement of all grana thylakoid cisternae (D). Bars represent 0.5 µm.

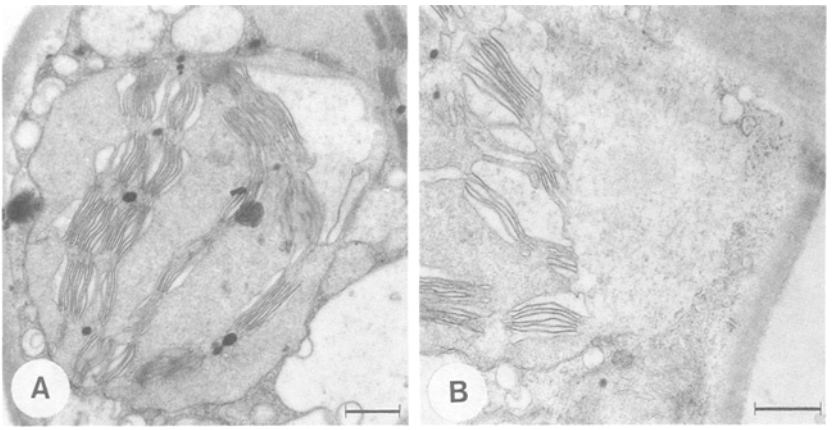


Figure 4. Uneven enlargement of some thylakoids (A) seems to result in breakage of chloroplast envelope (B). Bars represent 0.5 μm.

this stage (Fig. 2D). As chloroplast destruction processes further grana thylakoids start to swell (Fig. 3A). Swelling can be more intense at one end of an individual thylakoid whilst a part of it may retain its normal profile (Fig. 3B). Finally and before chloroplast is completely ruined, grana thylakoid swelling is prominent (Fig. 3C). Thylakoid dilation and swelling affects not only peripheral but also the internal thylakoids of a granum (Figs 3C,D). Displacement of adjacent thylakoids is evident. In a chloroplast condition like this, undilated thylakoids with normal profile in sections can hardly be observed. Moreover swelling and uneven enlargement of thylakoids (Figs 3D,4A) forces the chloroplast to burst resulting in the final lysis of the organelle (Fig. 4B).

These observations on the detrimental effects of air pollution in *P.fruticosa* chloroplasts resemble the structural alterations of these organelles observed in *Vicia faba* after $SO_2$ fumigation (Fischer et al. 1973). Taking in account the fact that swelling of thylakoids is also caused by $SO_2$ and $NO_2$ polluted air (Wellburn et al. 1972), we can assume that these pollutants must be super-abudant in the area where the plants were collected. According to Lendzian and Unsworth (1983), fumigation with sulphur compounds results in alteration of regulatory properties and/or inhibition of Calvin cycle enzymes. Therefore elimination of the primary productivity of a heavily air polluted plant community must be expected (Fischer et al. 1973; Winner et al. 1981; Margaris et al. 1984).

Mitochondria of polluted plants seem to be less developed than those of non polluted plants. They possess a few, weakly defined cristae, particularly if the organelle lies in the proximity of an

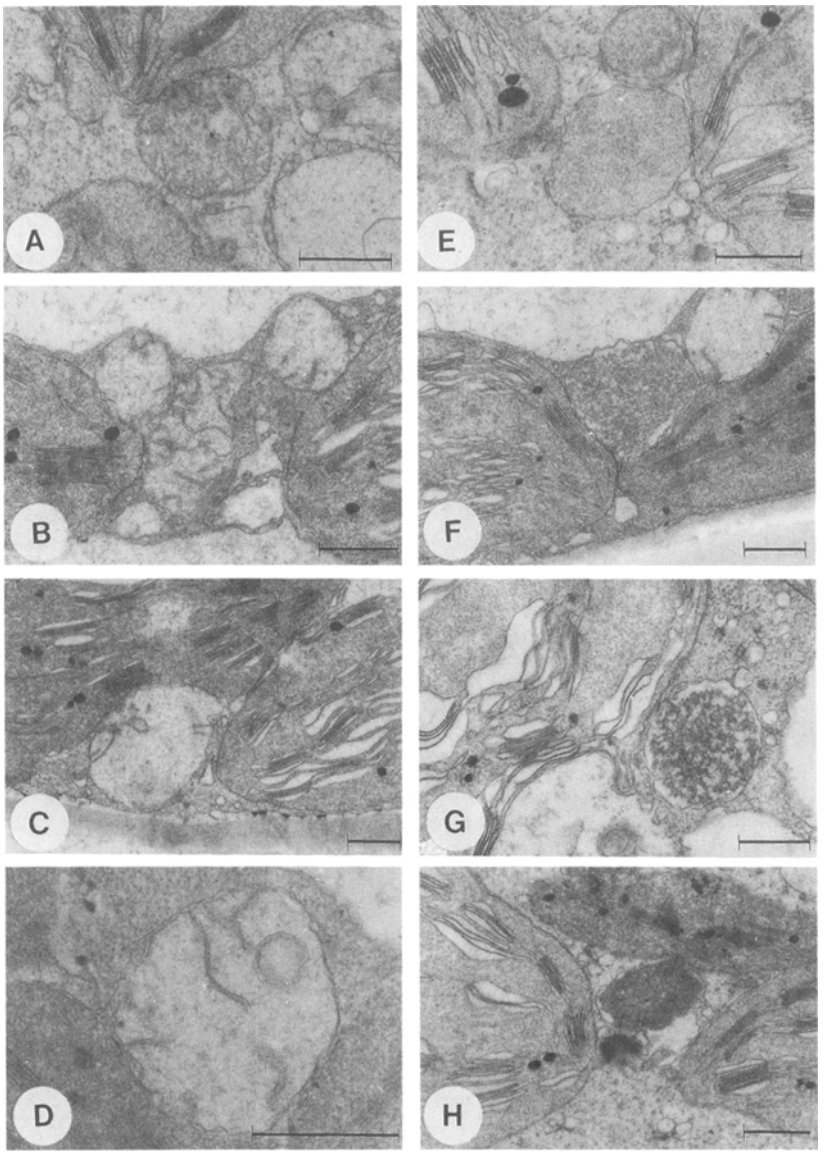


Figure 5. A-D, Series of electron micrographs of mitochondria from air pollution affected palisade cells. Internal cristae seem to be reduced according to the degree of damage of neighbouring chloroplasts (slight damage in upper, strong damage in lower micrographs). E-H, Series of microbodies in the proximity of chloroplasts presenting a probable process of gradual granulation of the matrix (E-G) and finally a separation of the condensed matrix from the microbody membrane (H). Bars represent 0.5 µm.

affected chloroplast (Figs 5A-D). Microbodies are also among the affected organelles. Whenever attached to healthy chloroplasts their structure seems to be normal (cf. Fig. 5E to Fig. 1C) but if they are promixal to affected chloroplasts their matrix gradually becomes granulated (Figs 5F,G) and condenses until final separation from the boundary membrane (Fig. 5H).

Considering the role of plastids and mitochondria and the close functional relations of microbodies to these all (Chollet and Ogren 1975) we can assume that structural anomalies, prior to final destruction of the organelles, undoubtedly lead to severe suppression of cellular metabolism in plants of heavily polluted ecosystems.

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