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Air Pollution Effects on the Leaf Structure of Two Injury Resistant Species: *Eucalyptus camaldulensis* and *Olea europaea* L.

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The release of suffocating and toxic gases as well as of particulate pollutants into the atmosphere is a major side effect of the human industrial, agricultural and domestic activities. The impact of these compounds on the various life forms of our planet seems to be very serious. Wild-life is threatened while man-life is on the way of experiencing difficult-to-overcome environmental challenges. A major problem arises through the destruction of the natural "air purifiers", the plants, which are among the first victims of the air pollutants. The effect of the air pollutants on plant structure and function has been in the focus of interest for many investigators. Structural modifications of the organelles, especially the chloroplasts, followed most of the times by membrane swelling and disruption have been reported in detail (Wellburn et al 1972, Fisher et al 1973, Patel and Devi 1984, Psaras and Christodoulakis 1987a, 1987b, Dixit 1988, Krishnayya and Bedi 1989). Besides that and prior to chloroplast disintegration, inhibition of photosynthesis is among the most serious effects resulting from the action of the various components of air pollution to the biochemical pathways of photosynthesis. (White et al 1974, Capron and Mansfield 1976, Ormrod et al 1981, Winner 1981, Margaris et al 1985, Darrall 1989). Other essential pathways of the primary or secondary metabolism in plants can also be affected by air pollutans (Katoh et al 1989).

All these studies mentioned above lead to the conclusion that life is threatened not only by the various chemical compounds, released into the atmosphere, themselves but also by the elimination of the improvement of air quality and the retardation of primary productivity through plant injuries. Therefore investigations for plant species resistant to pollution-induced injuries, do have a meaning. The introduction of these species will improve air quality and establish a moderate rate of primary productivity in the handicaped regions. That is why data concerning an evergreen sclerophyllous species which does not present structural modifications and organelle destruction although forced to be a dweller of a partition isle in a heavily polluted, traffic-loaded main street

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of the smog-suffering city of Athens, Greece, seemed very interesting (Christodoulakis and Fasseas 1990).

In this paper, further investigation is presented. Two common, species were studied. The first, Eucalyptus camaldulensis, a huge tree once imported to Europe from Australia as a marsh-drier in an effort to control malaria, is a drought enduring species mostly known for the essential oils accumulated in its leaves. The second Olea europaea L. var oleaster Brot, is a sclerophyllous tree growing wild in chaparall formations in Greece. Among these two species, the former was chosen as an easily adapting to mediterranean conditions, sized species, which can bring back the green color to a vegetation deficient, heavily polluted area and the latter for both its remarkable ability to grow under the stressful conditions of the mediterranean climate and its connection to the city of Athens from floristc and historical point of view.

MATERIALS AND METHODS

Mature leaves of the same age (Diamantoglou and Mitrakos 1981) were taken from three control and three experimental plants of both species, the same day and time, in November 1989. These leaves had already been marked on the upper meridional area of the tree canopy (sun leaves) while beeing leaf primordia, during the vegetative period, in spring. Exposed plants were found growing on the side of a traffic loaded street in a heavily polluted region (Margaris et al 1985), downtown Athens while the unaffected individuals used for reference, were found in a practically non polluted site on the west slope of Mt Hymmetus, which closes the basin of Athens metropolitan area from the east side. At this site O. europaea grows wild.

Thirty leaves were used for sampling. Ten small pieces from each of these leaves were fixed in phosphate buffered 3% glutaraldehyde at ph 7 for 2 h at 0 °C, rinsed in the same buffer, postfixed in 1% OsO_4 at the same temperature and dehydrated in a graded ethanol series. Fixed tissue was embedded in Durcupan ACM (Fluka). Series of semi-thin sections for LM were stained with toluidene blue "O". Glass slides observed originated from at least ten different leaves for each species and leaf type. Ultra-thin sections were cut on an LKB Ultrotome III, double stained in uranyl acetate and lead citrate and observed in a Philips EM 300.

RESULTS AND DISCUSSION

The rather mesomorphic leaves of E. camaldulensis seem to be similar in both normal and polluted plants when examined under the Light Microscope (Fig.1,A and B). Their outstanding characteristic is the large intercellular spaces with the essential oil which occupy the upper part of the otherwise bilateral leaf. Stomata occur on both surfaces. Palisade cells, at both sides, possess numerous chloroplasts while the vacuole seems to accumulate osmophillic granules of phenolic nature. Leaves from polluted plants present these compounds in the vacuole of their palisade cells but in a more condensed form.

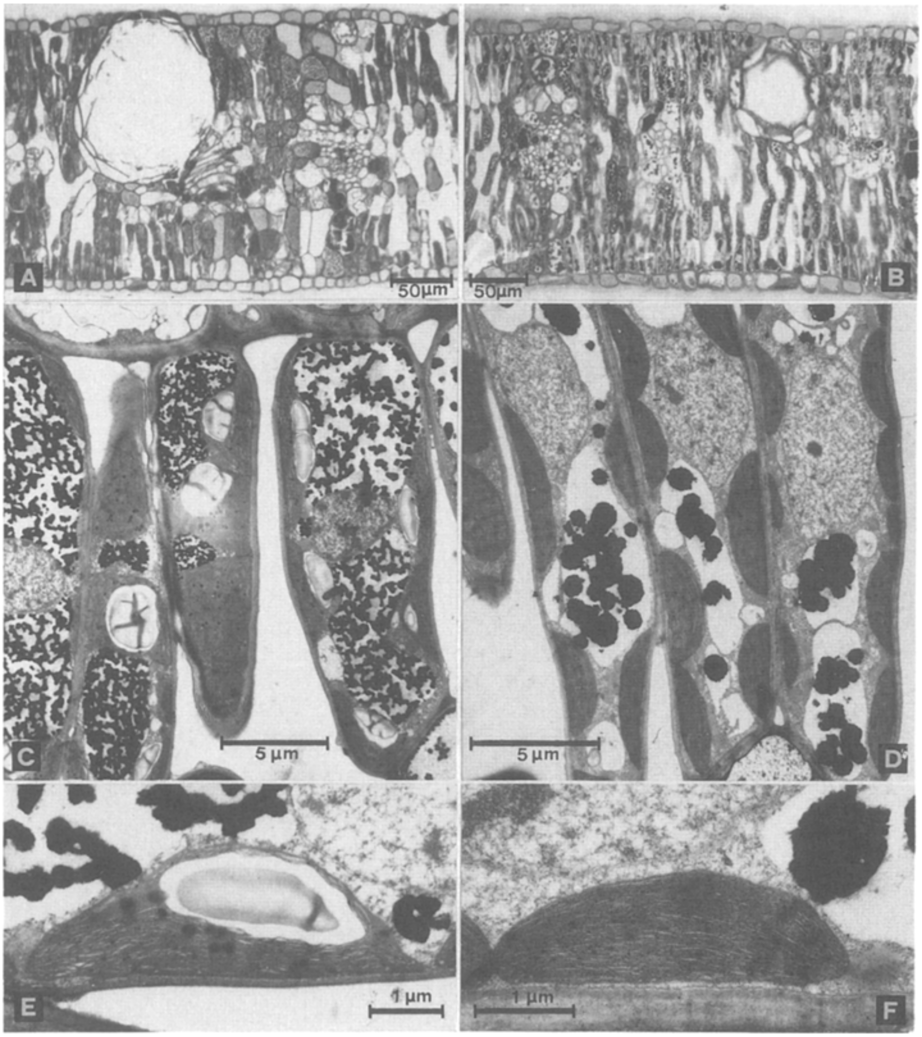

Figure 1. E. camaldulensis. A/ and B/ light micrographs from normal and polluted leaves, C/ and D/ electron micrographs of palisade cells from normal and polluted leaves respectively, E/ chloroplast from a normal leaf and F/ chloroplast from a polluted leaf

Electron micrographs from the same species revealed that mesophyll cells in the leaves of polluted plants remain intact and their organelles retain their structure (Fig.1,C and D). Chloroplasts appear with well preserved fretwork but do not seem to accumulate even traces of starch (Fig.1,F) as this happens in the same organelles of the cells in non polluted leaves (Fig.1,E). The large starch granules in the latter indicate a positive balance of photosynthesis. Mitochondria also seem to retain their shape and internal membrane structure while a little problem for the cell structure arises from the abudant phenolic compounds that leach from the vacuole during fixation. These compounds are accumulated in the cells of leaves living under stressful conditions thus their superior quantity in polluted leaf cells is an indication that polluted leaves experience microclimatic conditions far more unfavorable than those of the normal ones. It is also very interesting that the plastids in epidermal cells of polluted leaves do not present membrane swelling or the deformations so far described for the pollution sensitive leaves of some species.

The hairy leaves of O. europaea have been investigated in detail along with other ten evergreen sclerophyllous species comprising the chaparall formations growing wild in the areas characterised by mediterranean climatic conditions in nearby Athens metropolitan area (Christodoulakis 1984). Among the outstanding features of these hypostomatic leaves (Fig.2,A) is the unilayered epidermis, the multilayered palisade tissue, the scale-like hair on both sides and the numerous inosclereids distributed not only between epidermal and palisade cells but also throughout the mesophyll. It seems that the evaluation of the structural differences in the leaves of various individuals is rather risky because this species, having adopted the hard leaf strategy as well as the evergreen habit, is very flexible in using various options from the vast genetic potential which has evolved under the constant pressure of the unfavorable conditions prevailing in the mediterranean regions. Therefore leaf size and thickness, palisade development, hair cover, stomatal frequency and secondary metabolite accumulation are characteristics that vary dramaticaly even in the leaves of the same individual, according to their age or their position on the plant (sun or shade) etc. (Christodoulakis 1984). These variations can be exagerated in trees of cultured varieties, growing in different places or beyond the limits of the natural distribution of the species, shaded near tall buildings in urban areas or watered by municipal services. Ultrastructural changes and damage of certain organelles seem to be more safe to use as a pollution indicator as well as more important for the evaluation of the contribution of the leaves to the air quality, something that is of great interest for polluted areas. Therefore we consider that efforts to correlate the structural characteristics of the hard leaves to the presence of air pollutants (Eleftheriou 1987) may lead to a weak point in such investigations. On the contrary we suggest that the development and ultrastructure of the anyway sensitive chloroplast, can be used as an unequivocal criterion for the leaf resistance to pollution, especially when a plant grows in a very affected area of one of the most polluted cities in the world, as it happens in our case.

Having all these in mind we can say that polluted leaves (Fif.2,B) are characterized by the accumulation of raphide crystals in the vacuole of all cells but those of the conductive tissue and the highly granular appearance of the nucleus in the mesophyll cells (Fig.2,B insert).

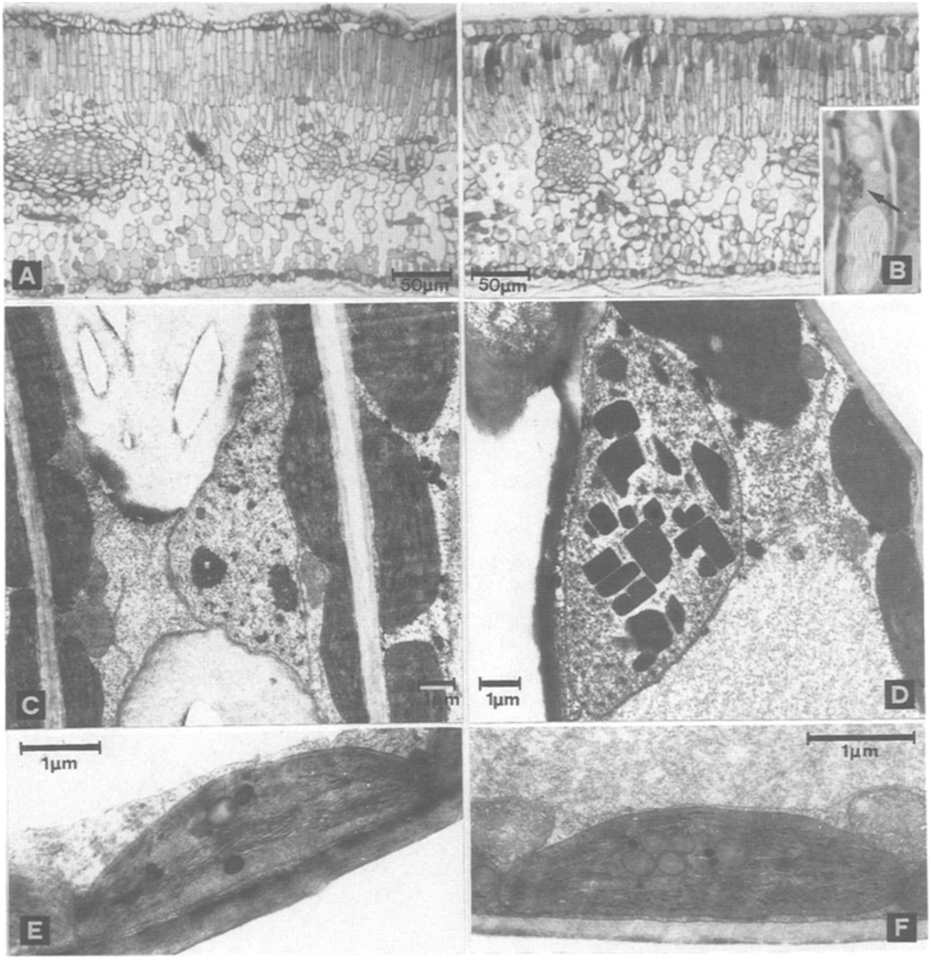

Figure 2. O. europaea. A/ and B/ light micrographs from normal and polluted leaves. Raphide crystals and granular nucleus (arrow) are shown in the insert, C/ and D/ electron micrographs of the nucleus in mesophyll cells of normal and polluted leaves, E/ chloroplast from a normal leaf, F/ injury resistant chloroplast from a polluted leaf; intact mitochondria can also be observed.

Epidermal and mesophyll cells in normal and polluted leaves look similar in electron micrographs. Chloroplasts in the cells of both the palisade and spongy parenchyma seem to remain intact in polluted leaves (cf Fig.2E to Fig. 2F). Most of the times they are extraordinarily long and present a dence fretwork, with large grana stalks which do not seem to undergo dilation. Plastoglobuli do not differ in the chloroplasts of the two leaf types. Starch granules were sometimes observed only in the chloroplasts of the normal leaves while the existence of electron dense, crystal like inclusions in the nuclei of the mesophyll cells in the polluted leaves, was something difficult to explain (cf Fig.2C to Fig.2D). Mesophyll cells in normal leaves of O. europaea, observed during a two year cycle and throughout their life story, rarely present such inclusions in the nucleus, at any time of the year (Christodoulakis 1984). The nature of these crystals was not identified. Mitochondria and microsomes also seem to remain intact. The electron dense appearance of the mesophyll cells in both leaf types is due to lipid leaching from the vacuole, during fixation. At this point we have to make clear that the leaves from both species studied are characterized by the large quantities of secondary metabolites (phenolics, lipids etc) accumulated in the cells. This becomes a serious problem during fixation and sectioning while the fine structural details are also affected to some extend.

Our observations indicate that polluted leaves of these two species as well as those of Laurus nobilis appear more xeromorphic. Among the characteristics common in water stressed leaves experiencing high light intensisties, is the accumulation of phenolic compounds. These compounds are, in L. nobilis and E. camaldulensis (O. europaea accumulates secondary metabolites of lipid nature), more abundant, most of the times in their condensed form, in polluted leaves. Although this may indicate that microclimatic conditions in polluted areas are more stressing, it seems contradictory to the reduction of the phenolics in the leaves of polluted Japanese cedars, probably due to the repressing effect that sulfur dioxide has to the sicimic acid pathway, reported by Katoh et al (1989). Although tannins are considered to be multi-protective agents, their presence in the leaf cells depends on the environmental conditions which induce their biosynthesis. The balance between climatic conditions iducing phenolic accumulation and the inverse effect that sulfur dioxide probably has, may be negative under the climatic conditions prevailing in Japan but it is strongly positive in our hot and arid climate. Besides that, the character of this climate is supposed to be responsible for the structure of the hard leaf which in turn probably establishes the resistance of these species to pollution damage.

Intact fretwork in the chloroplasts of the polluted leaves, although starch is missing, is an indication that the organelle structure keeps on supporting photosynthesis under the unfavorable conditions of a polluted atmosphere and stresses the need for a detailed physilogical approach which will integrate our knowledge on these injury resistant species. The use of these species in heavily polluted areas could then be a serious step towards the improvement of the air quality.

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