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Air Pollution Effects on the Guard Cells of the Injury Resistant Leaf of *Laurus nobilis* L.

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The imperative need for cleaner air has led to detailed investigations not only on the sources and types of air pollutants but also on the effect that these compounds have on various life forms of our planet. The plants, being handicaped for they are unable to move, are the first "victims" of the air pollutants. Extensive literature exists on the structural damages (Wellburn et al 1972; Fisher et al. 1973; Reinert et al 1975; Lentzian and Unsworth 1983; Patel and Devi 1984; Psaras and Christodoulakis 1987, 1987a; Smith et al 1990; Hasemann and Wild 1990; Hasemann et al 1990; Trivedi and Singh 1990, Eleftheriou and Tsekos 1991) and functional problems (White et al 1974; Capron and Mansfield 1976; Ormrod et al 1981) that plants suffer after beeing exposed to air pollutants. Many investigators prefer to deal with damages, caused to various organs, in plants growing in non polluted environments, after being fumigated with certain air pollutants. Others investigate the problems in plants growing in polluted areas thus being subject to long-term exposure to air pollutants. Generally it seems that primary producers suffer injuries, most of the times serious, that finally lead to the supression of photosynthesis with all the undesirable consequences that this situation has for the ecosystem (Fisher et al 1973; Winner 1981).

Unfortunately Athens is not only the most polluted city in Greece (Margaris et al 1985) but also an example to be avoided among the most polluted cities in the world. Some measures have been taken but only recently did the authorities start thinking about certain decisions that may have an effect on stoping the unbearable conditions that more than 3 millions of people experience every day. The situation becomes more serious in traffic loaded streets where poorly maintained vehicles fumigate everything in their passage, since catalytic converters were only recently introduced. Therefore the conditions are still far from satisfactory in supporting plant life and

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serious problems have been reported to occur in plants forced to be city dwellers (Psaras and Christodoulakis 1987, Psaras and Christodoulakis 1987a). The exception of L. nobilis was a pleasant surprise. It has recently been introduced as an injury resistant species (Christodoulakis and Fasseas 1990). It presents no signs of external injuries. The cellular organelles of the mesophyll cells remain intact. It is of great interest that chloroplasts in this species not only are they structurally perfect but also accumulate starch which is an indication for their normal function. This plant happens to be a mediterranean evergreen sclerophyllous species. The same is true for another mediterranean species, Olea europaea, which also presents impressive tolerance against air-pollution-induced injuries along with the Australian originating species, Eucalyptus camaldulensis which seems to resist in an equally successful way (Christodoulakis and Koutsogeorgopoulou 1991). All the three species mentioned above have the ability to withstand the unfavorable conditions of the mediterranean climate, particularly those of the summer season. If this fact is considered along with their resistance to air pollution induced injuries, it might lead to the hypothesis that the structural characters of the leaf which help the plant overcome the problems of the "hostile" mediterranean climate also establish the resistance against damages caused from air pollutants. If this is true, we have to accept that these plants are probably escaping the problem by not being actually exposed. Therefore it seems a good approach to study plant cells and the stucture of their organelles, just outside the protective "shield" of the leaf. Such an investigation could be focused on the guard cells of the stomata, the only cells on the leaf epidermis that possess chloroplasts. They occur on the lower surface of the leaf in mediterranean evergreen sclerophylls. These cells are exposed to air pollutants and besides their unequally thickened cell wall, they have no other means of protection. Moreover, whenever stomata are open, the guard cells are primarily and directly affected by the stream of the polluted air entering the leaf. So it seems that a close look at these cells and especially their chloroplast structure, will give an answer to the question of whether the resistance in these plants is based on the protection of the so much speculated characters of the mediterranean leaf or on the protoplasm itself.

For all these reasons Laurus nobilis, was thought to be suitable for the investigation concerning the ability of the guard cells to retain their structure and possess intact chloroplasts.

MATERIALS AND METHODS

Leaves from cultured individuals of L. nobilis, growing on a partition isle of one of the most polluted and traffic-loaded streets down-town Athens, were collected, the same time and day with leaves from practically non-polluted plants growing wild on the western slope of mount Hymmetus, near the Kaesariani monastery, in the basin of Athens. Care was taken in concern to the age of the leaves and their position on the upper meridional side of the plant canopy so that the two samples should be comparable. Thirty pieces from each of these leaves were 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 series of ethanol solutions and embedded in Durcupan ACM (Fluka). Semi- and ultra-thin sections were cut on an LKB Ultrotome III. Sections observed with the light microscope were stained with Toluidene Blue "O". For Electron Microscopy sections were double stained with uranyl acetate and lead citrate and observed with a Philips 300 electron microscope.

RESULTS AND DISCUSSION

Observations on the leaf responce to the long term exposure to air pollutants, were primarily focused on the distribution and frequency of the stomatal complexes on the lower epidermis. They revealed that the stomatal frequency in normal leaves is 204,8 ± 12,5 while in polluted it climbs to 252,9 ± 18,3 using the $x \pm t\sigma_{n-1}$ formula for 32 degrees of freedom and 0,05 probability. The difference between the means (48,1) was tested and found to be significant. This result is rather difficult to evaluate. It is well known that stomatal frequency in evergereen sclerophylls varies according to the vegetative period a leaf was formed, the age or the position of the leaf on the plant and finally the conditions under which the individual grows (availability of water, solar radiation etc). Although care was taken to use leaves of the same age and from the same side of the plants, the exact conditions under which the plants were grown could not be checked. It seems quite possible that microclimatic conditions near the deck of the street are more stressing thus forcing the leaves of the plants to further develop such a xeromorphic feature as the large number of small stomata is. On the other hand it seems that the distiribution of stomata on the lower epidermis of the polluted leaves is somehow peculiar. Stomatal complexes in <u>L. nobilis</u> are accomodated in isles, just between the traces below which the conductive tissue extends. In polluted leaves this distribution can also be observed but strangely many stomata develop on the borders of each isle, crowded, together in contact to the elongated epidermal cells which indicate the existence of the conductive tissue just below them (arrows in Fig 1 C and D).

LM studies indicate that stomata in polluted leaves possess denser

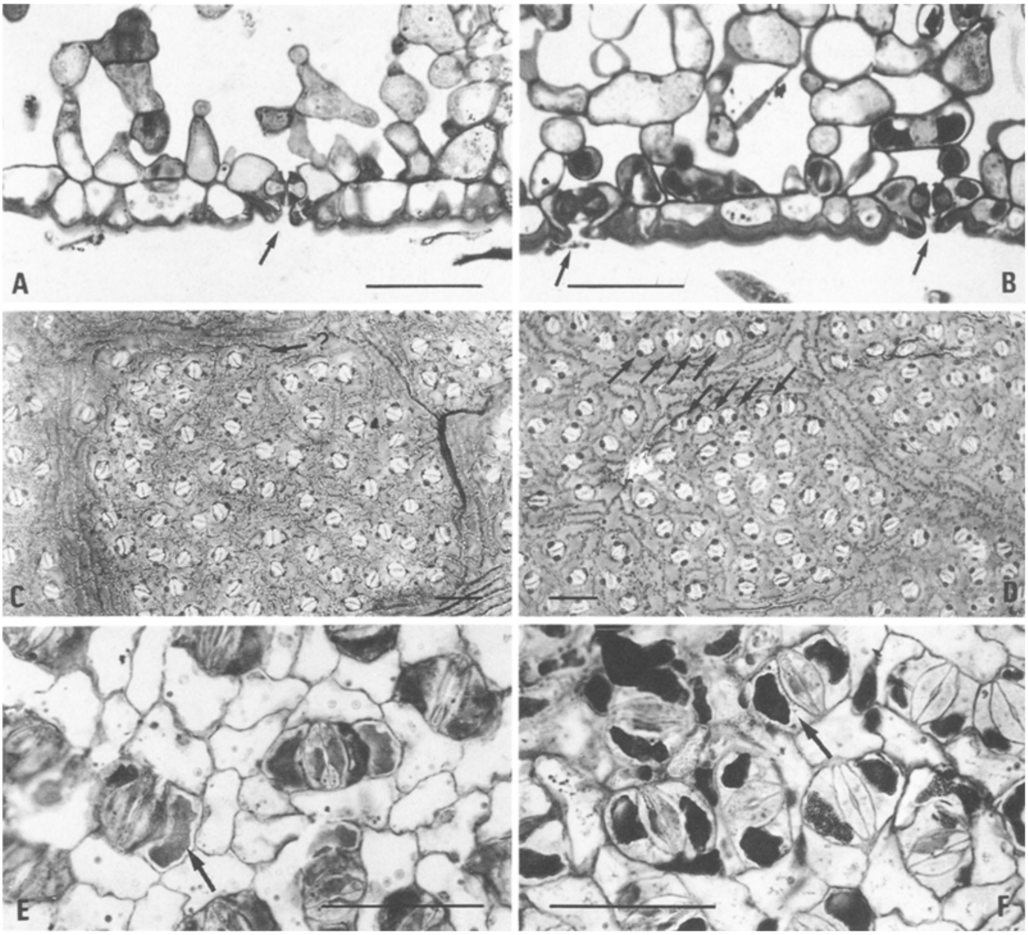

Figure 1. Light micrographs from normal (left) and polluted (right) leaves of L. nobilis. A/ and B/ cross sections of lower epidermis with stomata, C/ and D/ peeled pieces of lower epidermis, E/ and F/ paradermal sections from the lower surface. All bars 50μm.

cytoplasm (Fig 1 A and B) while the vacuolar contents of the parastomatic cells are strongly condensed as we can easily observe in paradermal leaf sections (Fig 1 E and F).

Numerous stomatal complexes were carefully examined under the EM but only a few were properly oriented and sectioned and therefore could be photographed (Fig 2 A). The guard cells of the stomatal complexes (Fig 2 B) seem to be unaffected. The structural characteristics of the chloroplasts remain intact (Fig 2C). Their internal membranes do present the special arrangment common in the guard cell chloroplasts and only rarely do they seem to have undergone some swelling. Chloroplast envelope also appears intact while plastoglobuli are present in numbers far inferior than those in the chloroplasts of the mesophyll cells. Starch granules are an indication for the normal activity of the chloroplasts which, generally, in the guard cells, is somehow peculiar.

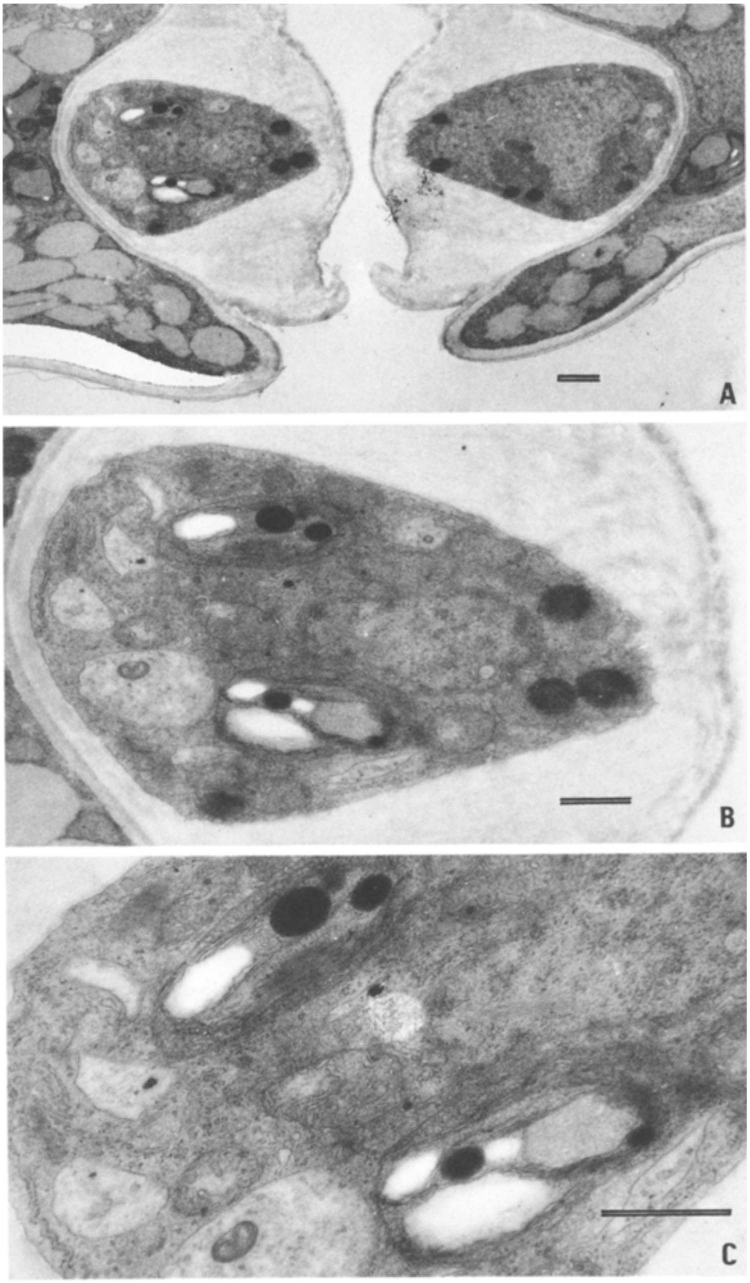

Figure 2. Electron micrographs of stomata from affected leaves. A/ cross section of the guard cells, B/ a guard cell. The nucleus and various organelles can be observed, C/ chloroplasts of the guard cell. Intact mitochondria and membranes of the rough ER can be observed. All bars 1μm.

Mitochondria appear numerous in the guard cells, with well preserved membranes. The nucleus is not granular as in the mesophyll cells and the nuclear envelope does not indicate any signs of swelling. Finally the cytoplasm appears more even than that of the mesophyll cells. Vacuoles are small with some phenolic inclusions. Oil droplets can rarely be observed while crystals are absent. Membranes of rough ER can also be observed. The whole guard cell gives the impression of an intact, organized and functioning system, totaly unaffected by air pollution while the slight dilation sometimes observed in the fretwork can also be commonly observed in the chloroplasts of the mesophyll cells in plants growing under clean-air conditions. This is probably due to the secondary metabolites, especially phenolics, usually accumulated in the leaf cells of the mediterranean evergreen sclerophyllous species. These metabolites, mostly protein coagulators, leach from the vacuole during fixation and react with the membranes dilating or disintegrating some of them.

It seems that we have two critical points to discuss. The first has to do with the uptake of the air pollutants by the leaf and the second with the damage process that takes place after the flux of the air pollutants to the leaf. Pollutant flow into the leaf is affected by many factors. The morphology of the leaf, it's orientation, the epidermal characteristics as well as the air movement across the leaf (Guderian et al 1985, Tingey and Taylor 1982, Hill 1971). Pubescence and cuticular waxes are important factors that influence uptake. Stomatal resistance, also a significant factor, is affected by the morphology of the stomatal complexes, i.e. size of the stomatal apperture, "sunken" stomata etc, as well as by the frequency they appear on the leaf surface. Concernig the structural characteristics of the leaves of <u>L. nobilis</u> we have a detailed description (Christodoulakis 1992). We know that the structure of these leaves contributes to the drought resistance of the species and therefore may also control the concentration of air pollutants in the mesophyll. Keeping it significantly lower than the ambient concentration, by elliminating air flow into the leaf will, unquestionably, affect photosynthetic rates. But the existence of assimilatory starch in the well developed chloroplasts of the mesophyll cells does not indicate supression of photosynthesis (Christodoulakis and Fasseas 1990). Therefore pollutans positively flux into leaf along with air essential for photosynthesis. The higher stomatal frequency as well as the peculiar accomodation of stomata, their dense cellular contents and the thicker cuticle observed in polluted leaves are indications of a more xeromorphic reaction probably due to microclimatic conditions in the polluted area.

What remains to be discussed is the second critical point, that has to do with the damage process in the leaf. It seems that the cells within the leaf of L. nobilis can resist damage and keep all the organelle membranes intact (Christodoulakis and Fasseas 1990). This means that genetically controlled sensitivity which is the overriding determinant of injury (Treshow and Anderson 1991), is very low or non existing in this species. A final argument for that is now available. It comes from the guard cells which altough remaining outside the protective structures of the leaf, continuously in contact with air pollutants, do not present any signs of injury.

Finally we may conclude that Laurus nobilis is a plant species with genetically based resistance against air-pollution-induced injuries. Having a relatively wide disribution in nature, can also be cultivated under a variety of climatic conditions. It can be introduced as a natural air purifier in cities or near polluting industries.

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