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# Sclerophyllous leaf characteristics in the detection of plant abiotic stress

**Abstract**

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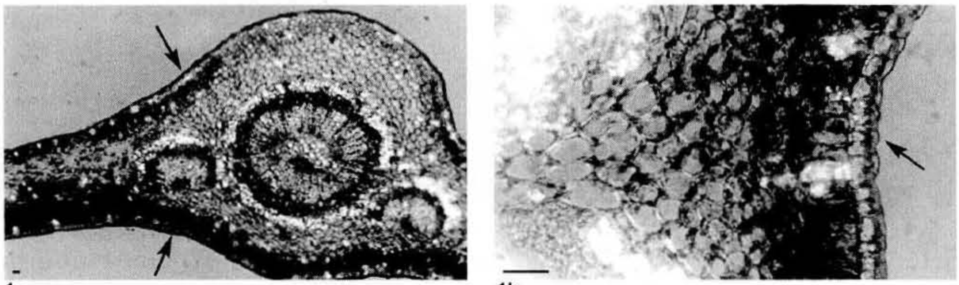
Pollution urban and suburban stress provoke a different tendence towards leaf plant sclerophylly. Sclerophylly and phenol compound synthesis appeared to be active respomnses in leaves of *Citrus aurantium* L., *Cupressus sempervirens* L., *Ficus magnolioides* Borzì, *Myrtus communis* L., *Nerium oleander* L., *Parietaria judaica* L, *Pinus pinea* L, *Platanus hybrida* Brot., *Quercus ilex* L. and cotyledon of *Sinapis alba* L., from sites located along polluted urban areas or sea-spray polluted periurban areas or, in the end, subjected to magnetic treatment as *Sinapis alba* L. Our investigations have shown that some plants are better suited to a city environment, some have displastic cells (*Pinus pinea* L., *Nerium oleander* L.), other are able to accumulate air pollution or sea aereosol and their cells are not displastic, but are full of secondary metabolites (*Cupressus sempervirens* L., *Ficus magnolioides* Borzì, *Parietaria judaica* L., *Platanus hybrida* Brot.), others still show no sign of alteration, and almost all are prone to a higher (*Myrtus communis* L., *Quercus ilex* L.) or lower degree (*Sinapis alba* L.) of sclerophylly. Biostructural and spectrophotometric tests are carried out, paying particular attention to urban vehicolar lead concentrations.

## Introduction

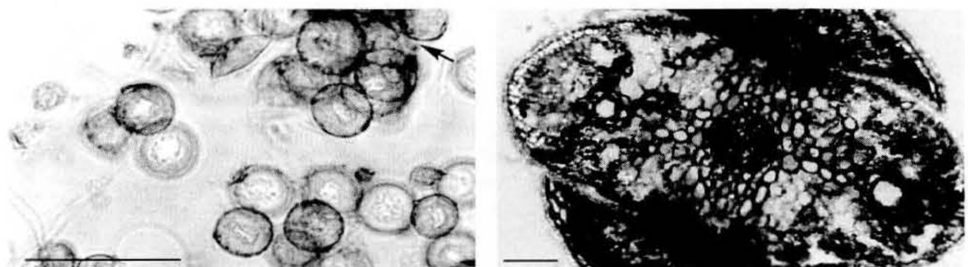
Sclerophylly and phenol compound synthesis appeared to be active responses to environmental stress in leaves of urban and sububan areas (Palermo, Sicily). These adaptations have been described by Bussotti & al. (1995, 1998); Kainulainen & al. (1996); Karolewski & Gietrych (1995); Schnitriler & al. (1996); Zobel & Nighswander (1990) for drought, low nutrient supply, ozone and other pollutants, intense irradiation and salt spray respectively.

Here we describe the morphological and histochemical alterations occurring in field-grown leaves of *Citrus aurantium* L., *Cupressus sempervirens* L., *Ficus magnolioides* Borzì, *Myrtus communis* L., *Nerium oleander* L., *Parietaria judaica* L., *Pinus pinea* L., *Platanus hybrida* Brot., *Quercus ilex* L. and cotyledon of *Sinapis alba* L., from sites located along polluted urban areas or seasprays polluted periurban areas or, in the end, subjected to magnetic treatment (selected because they are the most typical examples of indigenous plants or of plants which are specifically cultivated for ornamental purposes). In biological biodiversity studies, light-microscopy, and electron microscopy, is a good method for



1a 1b

Figs 1a, 1b. *Citrus aurantium* L.: the cells in samples from polluted vehicle stations are not deformed and are identical to the cells of leaves from the control stations; only the cuticles (heavily stained due to the presence of secondary metabolites) appear to react to the polluted environment (1a, 1b) respect to control.



2a 2b

Figs 2a, 2b. *Cupressus sempervirens* L.: phenol content is high in the pollens (2a) and in the section of a leaf (2b) from a polluted vehicle station; however the structure of the leaf is merely more coarcted than that of the control, with only slight cell deformation and wall thickening.

3a 3b 3c

Figs 3a, 3b, 3c. *Ficus magnolioides* Borzì: secondary metabolites are more profuse in leaves from the polluted vehicle stations (3b, 3c) than in the controls (3a) and the leaf veins are more sclerosed. The mesophyll cells are only slightly coarcted. In Fig. 3c: entire lamina leaf of polluted station.

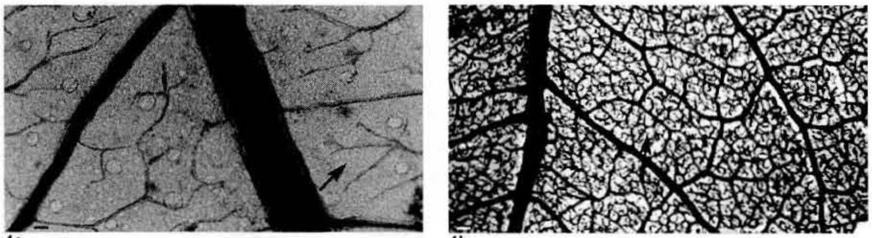
evaluating the extent of damage as well as in resolving dose-response questions. The microscopes can reveal injuries before any are apparent to the naked eye (Melati & al. 2001). Enzymes involved in phenol metabolism such as phenylalanine ammonia lyase, polyphenol oxidase and peroxidase are stimulated by stresses therefore the result is greater lignification and on the whole more conspicuous sclerophylly (Bussotti & al. 1998). Activation of these enzymes would stimulate oxidation of phenols to quinones and cause accumulation of their polymerization products. The foliar surface, and particulary the cuticle, is the first zone of impact of abiotic stress on leaves. During recent years, with modifications in the nature and level of environmental stress (decrease of primary pollutants, increase of nitrogen deposits and of organic micropollutants, increase of $CO_2$ and of climatic change etc.), physiomorphological impact on plants and in particular on the leaf is different (Schonherr & Riederer 1989). It is observed that the leaf surface characteristics are modified by stress. The use of more sophisticated leaf and cuticular appropriate and more sensitive method of detecting new plant stress and understanding at what level these recent stress act: have they a direct, chemical effect on cuticle and on biosynthesis (wax biosynthesis?) or a more general impact on the plant metabolism (aging?). The objective of our project is to finalize bioindication and biomonitoring environmental methodologies by means of using herbaceous and arboreal plant species in relation to environmental troubles in urbanized and in periurban areas, to assess the environmental quality of these sites.

## Materials and methods

All the live material was fixed in FAA and stored in 70% alcohol. Entire leaves (*Citrus aurantium* L., *Cupressus sempervirens* L., *Ficus magnolioides* Borzì sin., *Ficus macrophylla* Desf. ex Pers., *Myrtus communis* L., *Nerium oleander* L., *Parietaria judaica* L., *Pinus pinea* L., *Platanus hybrida* Brot., *Quercus ilex* L., *Sinapis alba* L., (see figs from 1a to 10c) were boiled for several minutes with 10% NaOH and 1% fuxine (Fuchs, 1963).Some leaves were cryosectioned, and stained with fast blue BB 0,08 % in acetate buffer (pH 6,5) at room temperature for 30 minutes and mounted in gelatine-glicerinate: phenols were red or brown in colour (Gahan 1984). Leaf surface, margins and venation, stomata (size and density per $mm^2$), thickness, epidermal cells (and anticlinal walls ,adaxial and abaxial) and cuticle, mesophyll, laticifers, crystals and trichomes were examined and revealed with the photos. Fully developed, mature leaves were used for this study. The sites chosen for sampling (Table 2) are situated in urban and suburban areas of Palermo, and are characterised by different motor traffic flows. Biostructural and spectrophotometric tests were carried out, paying particular attention to concentrations of toxic (Pb) metals with atomic absorption spectrophotometer (Cenci & al. 1998). Lead concentration were measured and expressed in µg/g of dry weight of material. Magnetic treatment of *Sinapis alba* L., cotyledons was conducted in the laboratory of Dep. of Physics into E 104 B/EPR(Varian). The exposure time required to induce the stimulus is 15 and 30 seconds with 30mT (inductive treatment) after magnetic treatment the larger cotyledons were grown in water in the dark and incubated at 21 °C in Petri dishes, containing 2 disks of filter paper wetted with distilled water (non-inductive medium).

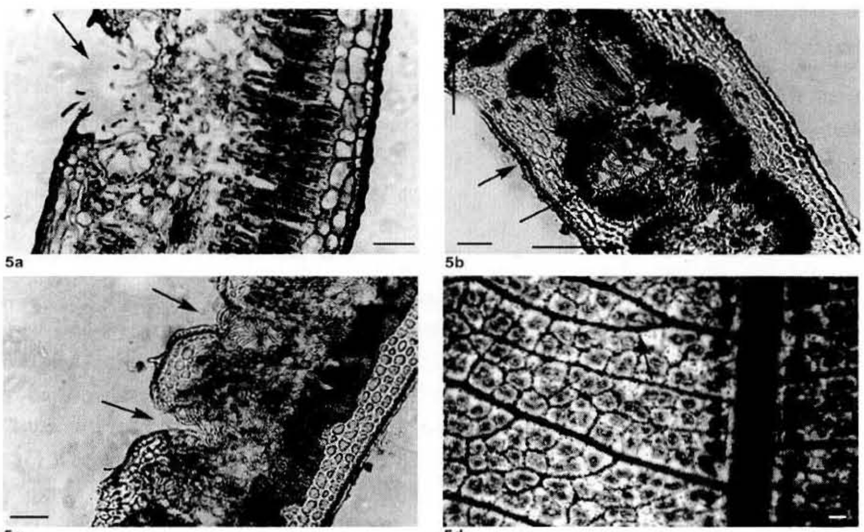
## Results

Our investigations have shown that some plants are better suited to a city environment, thanks to their special leaf structures: some have displasic cells (see *Pinus pinea* L., *Nerium oleander* L., Figs 7a, 7b, 7c, 7d; 5a, 5b, 5c), others are able to accumulate air pollution or sea aerosols and their cells are not displastic but are full of secondary metabolites (see *Cupressus sempervirens* L., *Ficus magnolioides* Borzì, *Parietaria judaica* L., *Platanus hybrida* Brot., Figs 2a, 2b; 3a, 3b, 3c; 6a, 6b; 8a, 8b); others still show no signs



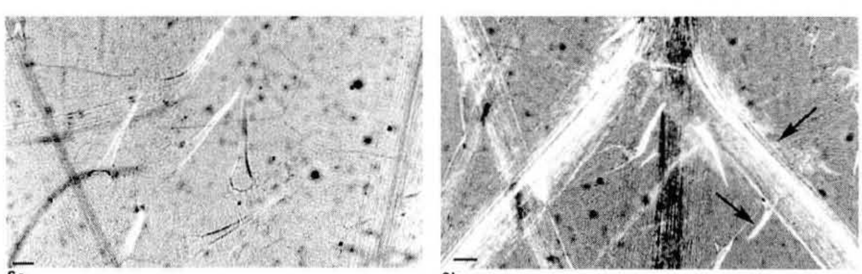
4a 4b

Figs 4a, 4b. *Myrtus communis* L.: there is a strong tendency towards sclerophylly, wall are more lignified, veins are closer together, some necrotic areas are evident, and there is greater secretion production in the leaves from the more coastal stations (4b) than in the controls (4a). In fig 4b: entire lamina leaf of sea aerosol station.



5a 5b 5c 5d

Figs 5a, 5b, 5c, 5d. *Nerium oleander* L.: once again there is an increase of protective hairs on the upper epidermis, subepidermic druses and the mucilages in leaves from near to the sea stations, but cells are only slightly deformed. The mesophyll is coarcted, the median rib is thicker and abundant particulated bedded on the cuticles. In Fig 5a: leaf control. In Figs 5b, 5c, 5d leaves of near to the sea and at a distance from the sea stations. In Fig. 5d: entire lamina leaf of near to the sea. Bars: 100µm.



6a 6b

Figs 6a, 6b. *Parietaria judaica* L: secretions are heavier and surface hairs are more profuse in leaves from the more polluted vehicle stations (6a) than in the clean stations (6b) and the cells are smaller, volumetrically coarcted. Bars: 100µm.

of alteration, except the cuticle (see *Citrus aurantium* L., Figs 1a, 1b), and almost all are prone to a higher (see *Myrtus communis* L., *Quercus ilex* L., Figs 4a, 4b; 9a, 9b) or lower (see *Sinapis alba* L., Figs 10a, 10b, 10c) sclerophyllous adaptations, as greater cell wall lignification and xylem density with some secundaries compound synthesis. In *Nerium oleander* L. leaves, from near to the sea station, unlike in the controls which are not subjected to the stress-inducing element, epidermis cells are collapsed and there are signs of necrosis. Stomata density is higher and some stomata are completely closed over by the coarctated mesophyll as further protection against stress. The oleander entire leaves have veins which can be defined as "camptodromous" (Hickey 1973) as the secondary veins do not end at the edges. In leaves from stressed environments eyespots form between the veins (Fig. 5d), the median rib is more lignified, the edges are thicker and there are crystals between the ribs. There is an increase of protective hairs on the upper epidermis, subepidermic druses and the mucilages in leaves from near to sea stations, but cells are only slightly deformed. In *Sinapis alba* L., 8-day-old cotyledons magnetic treatment induced the differentiation of supernumerary xylem elements along the basilar venation. The inductive magnetic treatment stimulated, respect to the control, an increase in xylem elements differentation because it induces a conspicuous differentiation of parenchimatic into xylem cells; control cotyledon (Fig. 10a mature venation of xylem pattern in entire cotyledonary lamina): treated cotyledon with supernumerary marginal veins (Fig. 10b);

Table 1.

| Name of species from environmental troubles | Thick cuticle | Stomata | Hair cover | More vein per surface unit area | Secondary metabolites | Displasic cells |
|---|---|---|---|---|---|---|
| *Citrus* | + | ± | - | + | + | - |
| *Cupressus* | + | ± | - | + | ++ | ± |
| *Ficus* | + | ± | - | + | ++ | + |
| *Myrtus* | ++ | ± | - | +++ | ++ | + |
| *N.oleander* | ++ | ++ | + | ++ | ++ | ++ |
| *Parietaria* | + | ± | + | + | ++ | + |
| *Pinus* | ++ | + | - | + | +++ | +++ |
| *Platanus* | ++ | ± | + | + | +++ | ± |
| *Quercus* | ++ | + | + | ++ | ++ | ± |
| *Sinapis* | + | + | - | ++ | ++ | ± |

**Cuticle**: presence or absence of a thick cuticle is represented by + or – respectively; ±represent some intermediate development of cuticle. **Stomata**: + and - represents sunken or superficial stomata respectively; ± represents an intermediate condition. Cryptas -- **Hair cover**: - represents a hairless, + is a hairy covering on both sides of the leaf; ± indicates presence of hairs on lower surface only. **Veins**: present +, abundant ++, very abundant +++. **Secondary metabolites**: present +, abundant ++, very abundant +++. **Displasic cells** : not present - , slightly present ± , present + , abundant ++ , very abundant +++ .

Table 2.

| Sample | Station | Pb (mg/kg) | Authors |
|---|---|---|---|
| *Citrus* | Lascari 7 (around Pa) | 0.78 | Crescimanno et al., 2000 |
| *Citrus*-control | Lascari 5 (around Pa) | 0.1 | Crescimanno et al., 2000 |
| *Cupressus* | Superstrada (Pa-Mondello) | 37.4 | Melati et al., 1996 |
| *Cupressus*-control | Orto Botanico (Pa) | 2.2 | Melati et al., 1996 |
| *Ficus* | P.za S. Francesco di Paola (Pa) | 10 | Alaimo et al., 2001 (in press) |
| *Ficus*-control | Orto Botanico (Pa) | 1.0 | Alaimo et al., 2001 (in press) |
| *Myrtus* | Via Emilia (Pa) | 3.4 | Robba et al., 2001 (in press) |
| *Myrtus*-control | Orto Botanico (Pa) | 0.3 | Robba et al., 2001 (in press) |
| *Parietaria* | Osped. Cervello (Pa) | 0.81 | Alaimo et al. 1997 |
| *Parietaria*-control | Via Emilia (Pa) | 0.59 | Alaimo et al. 1997 |
| *Pinus* | Via De Gasperi (Pa) | 24.2 | Alaimo, 2000 |
| *Pinus*-control | Orto Botanico (Pa) | 3.2 | Alaimo, 2000 |
| *Platanus* | Via De Gasperi (Pa) | 15.3 | Lipani et al., 2001 (in press) |
| *Platanus*-control | Orto Botanico (Pa) | 3.7 | Lipani et al., 2001 (in press) |

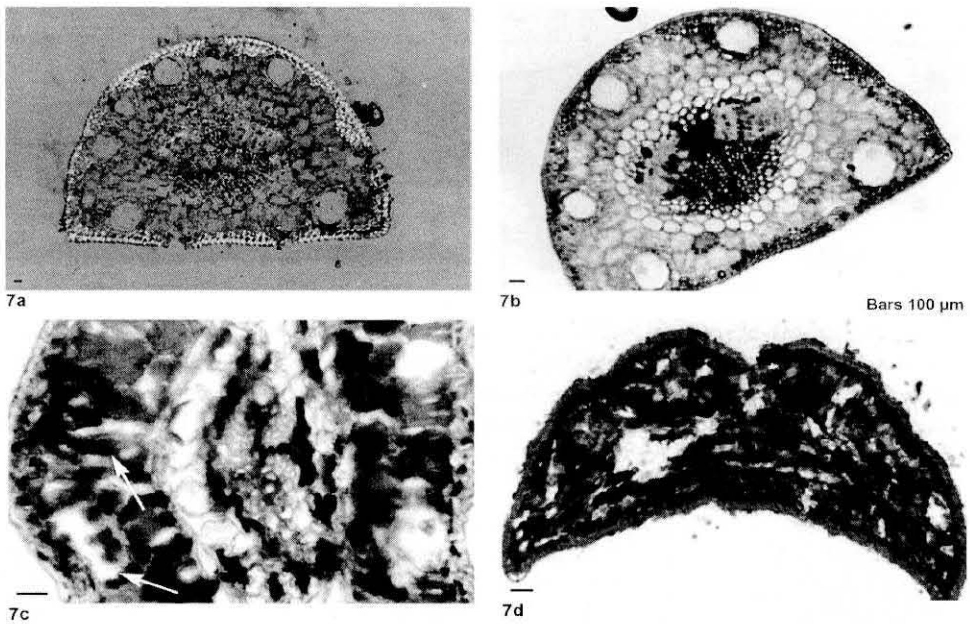
cross-sections reveal supernumerary marginal veins (Fig. 10c, 30mT x 15s). In the mature leaves disorganization of chloroplasts is described as one of the first events to appear, as a result of stress. Simultaneously the tannin appeared as a thin ribbon and scattered particles in the central vacuole, whereas normally it appears granular. In the second and third stage of cell injury, tannin appeared as a ribbon or disappeared completely, and no cytoplasmic constituents could be determined. Different stages of cell injuries are apparent in the same mesophyll tissue. The final disorganization and collapse of cell walls, accompanied by the spaces between cells widening, occurs to a great extent only when macroscopic symptoms

Table 3.

| *Nerium oleander* STATION | Cl – | Na + |
|---|---|---|
| | *ppm* | *ppm* |
| Petrosino (TP), near sea | 8190 | 3420 |
| Petrosino (TP), far from sea | 3000 | 1750 |
| Foro Italico (PA), near sea | 4380 | 1720 |
| Foro Italico (PA), far from sea | 2710 | 1850 |

Table 4.

| *Nerium oleander* STATION | Cl – | Na + |
|---|---|---|
| | *ppm* | *ppm* |
| Petrosino (TP), near sea | 8190 | 3420 |
| Petrosino (TP), far from sea | 3000 | 1750 |
| Foro Italico (PA), near sea | 4380 | 1720 |
| Foro Italico (PA), far from sea | 2710 | 1850 |



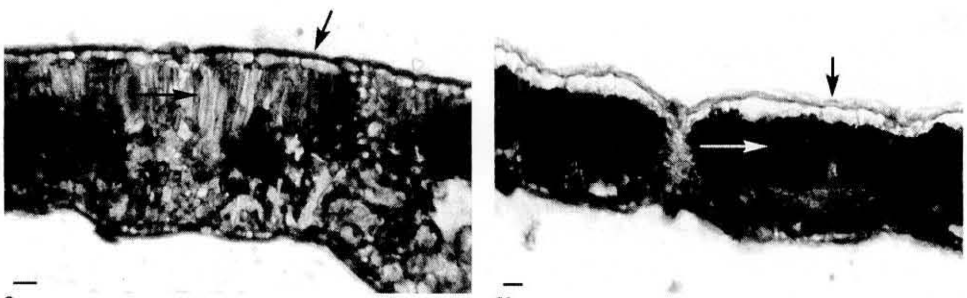

Figs 7a, 7b, 7c, 7d. *Pinus pinea* L: no phenols in the not much polluted leaf (7a); initial phenols deposits (7b); cell changes and necrosis in distal leaf polluted portion: red phenols entirely fill vacuoles cells (7c); cell changes in distal leaf polluted portion : leaf lacunae and red phenols (7d). Bars: 100µm.

also exist. The structural data show that individual leaf surface decreases, whole the thickness of the leaves. Histochemical tests show a marked increase of phenolics in polluted or costal sites or inside a magnet in the laboratory. These substances, present primarily in the leaves of plants growing in stress conditions, have been identified mainly as polyphenols.

They accumulate in the vacuoles, especially those of the upper epidermal layer and the palisade mesophyll or between the wall of the epidermal cells and the cuticle (Table 1). This is a research on morphological and microscope observations, concerning environmental troubles effects on several plants; we've also compared these effects with those observed at the chemical level (trace Pb concentrations in dry matter) (Tables 2, 3, 4). All data are collected in urban area. After examining the different structures of the leaves sampled in Palermo the leaves' chemical composition was determined to guage contamination. Concentrations of trace elements were significantly higher in leaves taken from polluted sites, and also in leaves with a high resin content, a lighter covering of hairs and more membranous surface.

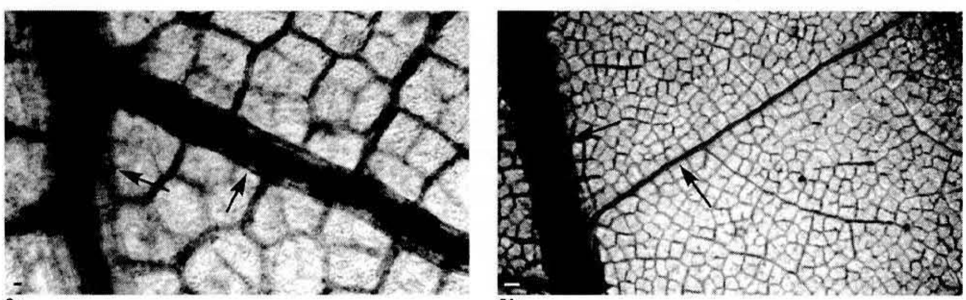
## Discussion

The sclerophylly strategy is best developed in air polluted; also characteristic leaf rolling influences air pollution interception. However also coastal vegetation uses this



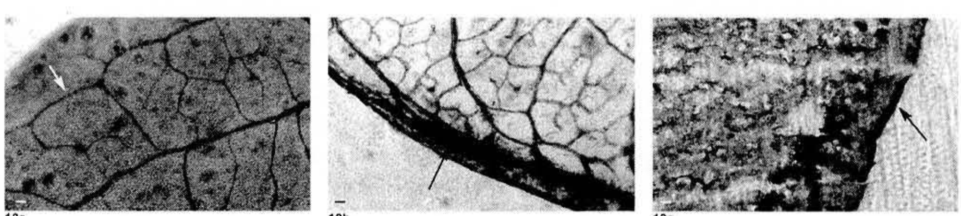
8a 8b

Figs 8a, 8b. *Platanus hybrida* Brot.: foliar chemical composition was determined as an indicator of contamination of the plants examined anatomically concentrations of heavy metals (Pb) were significantly higher at the polluted site. Citological changes observed in plants from polluted sites are most likely caused by pollutants: cells are not deformed, but red phenols fill vacuoles. Control (8a), polluted samples (8b). Bars: 100μm.



9a 9b

Figs 9a, 9b. *Quercus ilex* L.: in the entire *Quercus ilex* L. lamina the small veins (minor venation) form a network among the large veins within the mesophyll. The branchings of these veins subdivide the mesophyll into a series of small polygons, with the ultimate branches extending into the areole. Minor venation has supernumerary xylem elements in polluted station respect controls. Large bundle sheath of sclerenchimatons elements, are more frequently found in the mesophylly of polluted leaves (9a), together with some isolated sclerenchymatic fibers and with pluricellular hairs. Bars: 100μm.



10a 10b 10c

Figs. 10a, 10b, 10c. *Sinapis alba* L.: magnetic treatments induced the differentiation of supernumerary xylem elements in *Sinapis alba* L. cotyledon. The exposure time required to induce the stimulus is 15 and 30 seconds with 30mT: control cotyledon (10a); treated cotyledon (10a); treated cotyledon with supernumerary marginal veins (10b, 10c). Bars: 100μm.

strategy, as well as young plants subjected to magnetic treatment to improve culture condition, from our observations it can therefore be concluded that abiotic stress in general triggers a certain degree of sclerophylly, which can be defined as the emblem of plants' response to distressed environments.The plants respond in the same way to various types of natural or man-made stress. In order to evaluate effects on plants, we need to compare plants from "distressed" environments with samples from control areas (urban sites, suburban areas, in parks or gardens). Abiotic stress affecting plants in disturbed environments in urban and suburban areas of the city can derive from water, salt, nutritional and thermal agents as well as from pollution.

Our research has shown that these elements provoke a different tendence towards leaf sclerophylly, the result of a number of metabolic and structural alterations which include a reduction of cell volume, a tendence towards vein lignification, thickening cuticles, crystal accumulations, frequent trichomes, sunken stomata with high density, relatively thick lamina and various sclerous tissues in relatively small leaves. Cuticle morphology, wax stratification, number and distribution of cuticle ectodesmata, ion-binding sites, cuticle thickness and number or density of trichomes protect the leaves to a greater or less extent against stress, and also pubescent leaf surfaces accumulate much more than leaves which are smoth and waxy in coastal and polluted environments. Secondary metabolites have a role in protection and detoxification processes (Bussotti & al. 1998). Whatever the kind of stress responsible for cell modifications, the effects on both broad-leafed plants and conifers are fairly simîlar.

This research focuses on how the leaves of biomonitor plants how plants behave in a disturbed environment. Plants react in different ways to air pollution depending on their leaf type, in particular, the leaves' surface coating and segregating tissues. Type of leaf surface is an important factor, whether it be membranous, waxy, leathery or even sclerosed, depending on modifications in the walls of the upper and lower epidermic strata; the leaf's piliferous coating; the position, size and density of the stomata; cell division capacity, i.e. the ability to construct biochemical or anatomical barriers in response to microlesions and injuries or to external chemical, physical or mechanical stimuli. The way pollen aggregates on linear and resinous, or smooth and waxy or sclerosed leaves with a fine or dense covering of hairs differs from how it collects on leathery leaves with a cuticle full of wax and profuse segregating tissues. Degree of sclerophylly is also significant in diagnosing a plant's tolerance of abiotic stress caused by atmospheric pollution. Cytological alterations observed in trees from polluted sites are most likely caused by the environmental stress. The strategy of sclerophylly, for example, is best developed in air polluted and also characteristic leaf rolling influences interception of air pollution, but coastal vegetation also uses this strategy, as well as young plants subjected to magnetic treatment to improve cultivation conditions. Foliar chemical composition was determined as an indicator of contamination of the plants examined anatomically. Concentrationsof heavy metals (Pb) were significantly higher at the polluted site. Citological changes observed in plants from polluted sites are most likely caused by pollutants and foliar mineral composition was used as an indicator of health condition and contamination of the plants examined anatomically.

We can therefore conclude from our observations that abiotic stress factors in general tend to trigger sclerophylly, which may be considered the emblem of plants' response to troubled environments. Plants respond in the same way to a number of natural and man-made stress-inducing elements.

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