

Ecophysiological Trend and Remote Sensing in *Platanus hybrida*

M. DE LILLIS, G. CATENA and L. COSTANZO

*Dipartimento di Biologia Vegetale, Università «La Sapienza», Roma
Istituto Superiore di Sanità, Roma*

ABSTRACT. — Two different samples of *Platanus hybrida* Brot. trees have been selected, in the urban area of Rome, on the basis of their photographic response to near infrared: a) individual plants showing white coloration, b) individual plant showing pinkish-red coloration. Some ecophysiological parameters were recorded: phenological phases, leaf chlorophyll "a" and "b" content, leaf organic carbon content, change in water balance and specific respiratory activity. Moreover the abaxial leaf surfaces were photographed using scanning electron microscope (SEM). The results point out that the different leaf reflectance in the two groups studied could be related to changes in specific respiratory activity and loss of turgidity of the epidermal cells of the lower leaf surface rather than to changes in chlorophyll content.

INTRODUCTION

The reflectivity of leaves has often been studied in relation to the structural and physiological characteristics of individual species (Clark, 1946; Woolley, 1971; Gausman, 1974; Grant, 1987). Various authors have reported that changes in leaf reflectance are associated with differences in chlorophyll content (Clark, 1946; Gausman *et al.*, 1974) and with intercellular spaces in the spongy parenchyma, which can vary during leaf ontogenesis or with the onset of pathological conditions (Clark, 1946; Gausman & Allen, 1973; Gausman, 1974; Grant, 1987). The spectral reflectance of a leaf in the infrared region can vary according to species, plant age, nutrient status and water content of the tissues (Murtha, 1978). One of the most effective methods of determining leaf reflectance, in large geographic areas, is aerial photography using colored films that are sensitive to near infrared. This method is useful in recognizing general categories of plants (e.g., evergreen, deciduous, conifers, broad leaf, etc.) and in revealing vegetation damage caused by various factors (Murtha, 1978).

Generally, the various types of foliage appear as different colors in the infrared photographs: red-magenta for broad-leaved species and red-violet for needle-leaved species. However, the color photographs sometimes show certain anomalies in the chromatic scale, such as the color white. Since now, the ecological meaning of the color white (or the absence of color) has not been adequately explained. Murtha (1978) suggests that the absence of color

observed when using colored films is associated with a decrease in leaf reflectance in the near infrared region and related to a yellowing of the foliage.

In order to further study this phenomenon, we examined photographs of *Platanus hybrida* Brot. trees lining streets in the urban area of Rome. These particular photographs show individual trees of various degrees of pinkish-red, alongside trees with white coloration — approximately 10% of the total trees — though there are no visible chromatic differences. This study's aim was to determine whether the absence of color in the infrared photographs may correspond to particular ecophysiological parameters which may influence leaf reflectance.

STUDY AREAS AND METHODS

The aerial photographs used for this study were taken by Dr. G. Catena of the *Istituto Superiore di Sanità* (National Institute of Health of Italy) during different seasons from September 1985 until 1990. The film used (Kodak Aerochrome Infrared 2443) is referred to as "false-color" because the colors that appear on the image do not correspond to visible reality: the radiations belonging to the green band of the spectrum are shown in blue, those of the red band in green, and the non visible radiation of the near infrared (between 0.75 and 0.9 microns) in red. From four different sites around the city, we selected images of individual trees that showed white coloration alongside trees with pinkish-red (referred to as "controls"). All specimens studied had a diameter ranging from 1 to 3 meters and a height of 15 to 20 meters; specimens analyzed were of comparable chronological age.

This study was carried out in the years 1990 and 1991 and the following parameters were recorded:

1) *Determining the phenological phases.* For each individual tree we evaluated the duration of the vegetative, flowering, and fructification periods. Observations were performed weekly, beginning with the appearance of buds and continuing until the complete fall of the senescent leaves.

2) *Measuring the content of chlorophyll "a" and "b"* (MacLachlan & Zalik, 1963). Every fifteen days from May to October leaves were collected in small plastic bags and transported in refrigerator bags to the laboratory for analysis, which was performed three times on fifteen samples from each tree.

3) *Measuring variations in water potential.* In early and late summer (i.e., June and September) we observed daily changes in water potential every two hours from 8.00 to 18.00. Ten small branches were cut from each tree and timely wrapped in aluminum foil to avoid water loss due to transpiration. Leaf water potential was immediately determined using a Scholander chamber in the nearby field laboratory.

4) *Measuring variations in leaf resistance.* In June and September, daily changes in leaf resistance were measured *in situ* every 2 hours from 8.00 to 18.00 (that is, simultaneously with the measuring of leaf water potential) with a ΔT porometer, which was calibrated every 2 hours. Ten measurements were taken for each reading in ten different leaves.

5) *Measuring the organic carbon content of the leaves.* We measured organic carbon content in a sample of 20 leaves during the full vegetative period (July) in three replicates. Leaf material was dried in a 105°C oven for 2 days following the procedure described by Anne (1945) modified for leaf material.

6) *Measuring specific respiratory activity.* Specific respiratory activity was measured at night at temperatures ranging from 24°C to 26°C, in the month of July, with the Infrared Gas Analyser ADC Portable System. Twenty measurements were taken for each sample.

7) *Photographing the lower surface of the leaf using a scanning electron microscope (SEM)*. At least three samples for each plant were taken in early and late summer (June and September). About 0.5 cm² of each leaf was cut and fixed with adhesive tabs onto aluminium stubs to observe the abaxial surface. After three days of dehydration over phosphorus pentoxide the stubs were coated with about 40 nm of gold. The observations were carried out using a Cambridge 200 Scanning Electron Microscope (SEM) and the representative portions were photographed at magnitude of about 500X.

RESULTS

The leaf content of chlorophyll "a" in the control group (trees with red coloration on the infrared film) ranged from 0.58 mg/g to 1.02 mg/g (Figure 1). At the beginning of May, in the one-month old leaves, the chlorophyll "a" content did not exceed 0.83 mg/g. From the middle of May to early June, it peaked at 1.02 mg/g and then progressively decreased until October, not going above 0.58 mg/g in the senescent leaves. The chlorophyll "a" content in the

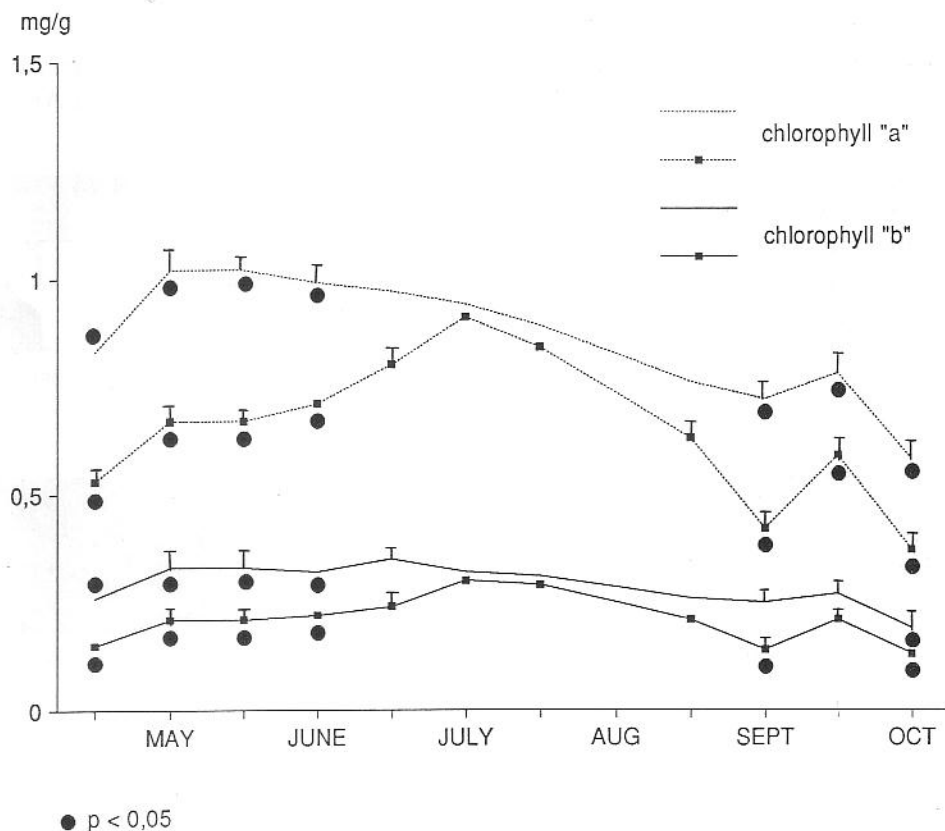


Fig. 1 – Leaf content of chlorophyll "a" (dotted line) and "b" (solide line). Trees with white coloration(■).

trees showing white under infrared photos varied from 0.37 mg/g to 0.91 mg/g in May, the leaf concentration was 0.53 mg/g, reaching the maximum value of 0.91 mg/g in mid-July, and finally, decreasing to 0.37 mg/g in the senescent leaves. Significant differences ($p < 0.05$) of chlorophyll "a" concentration between the two groups were reached from May to mid-June and from mid-September to October.

The seasonal trend of the leaf concentration of chlorophyll "b" (Figure 1) was similar to that for chlorophyll "a". In the control group, the concentration varied from 0.20 mg/g to 0.35 mg/g; the maximum value of 0.35 mg/g was reached in mid-May and remained more or less constant until the end of June. In October, the concentration decreased to the minimum value of 0.20 mg/g. For the white trees, the chlorophyll "b" content peaked at 0.30 mg/g in mid-July and decreased to 0.13 mg/g in October. Significant differences ($p < 0.05$) of chlorophyll "b" between the two groups were reached from May to July and from mid-September to October.

The sequence of phenological phases, simultaneously observed with the variations in chlorophyll content, showed a similar trend in all the trees examined (Figure 2a). The vegetative period began in early April, with the appearance of new leaves, and ended with their abscission after the end of November. Flowering lasted approximately 2 months, beginning in mid-April

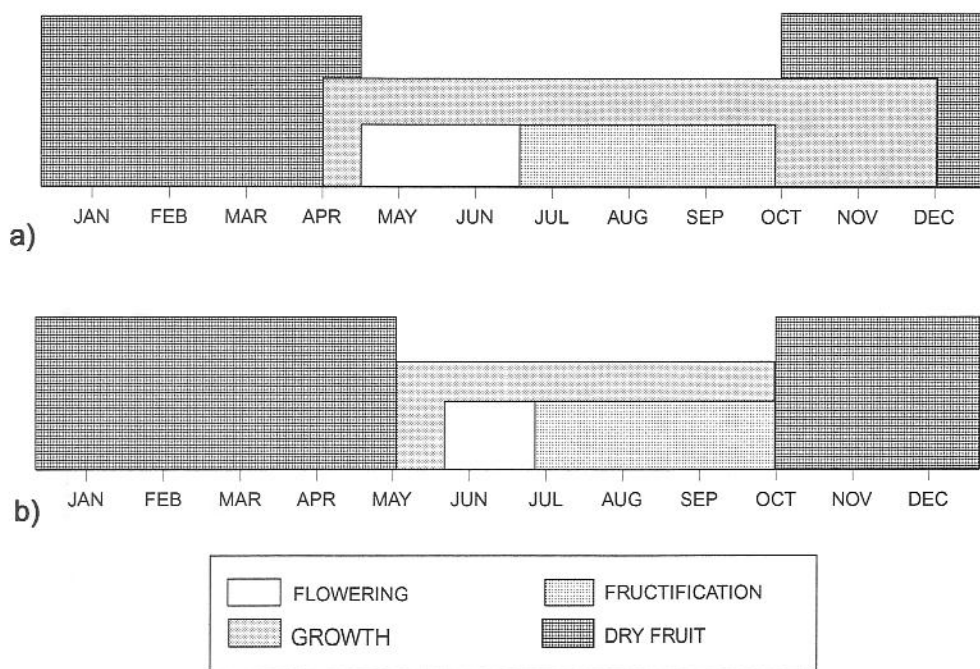


Fig. 2 – Sequence of phenological phases for all trees studied (a); sequence of phenological phases in an individual white tree (b).

and continuing until mid-June. Fruits appeared in mid-June, matured in October, and remained on the plant until the following vegetative season. A single white tree deviated from this phenological sequence (Figure 2b): leaf development began in early May and leaf abscission occurred at the end of September, indicating a shorter vegetative period. Moreover, though the fructification phase was similar to that of the other trees, the flowering was shorter and much fewer flowers and fruits were produced.

During the vegetative period (from June to September), the two groups showed a similar trend with respect to water balance. In June (Figure 3a), the leaf water potential for the control group ranged from -1.10 MPa at 8.00 to -1.22 MPa at 18.00, representing a decrease of 11% over the entire day. During the same days in June, the water potential in the white trees decreased by 15%, ranging from -1.08 MPa at 8.00 to -1.24 MPa at 16.00. By contrast, leaf resistance showed variations in all of the examined trees (Figure 3a). In the control group, the resistance at 8.00 was 5.70 s/cm, progressively decreasing to 3.35 s/cm (18.00), showing a reduction of 41%; in the white trees, leaf resistance values were significantly lower only at 8.00 and 10.00,

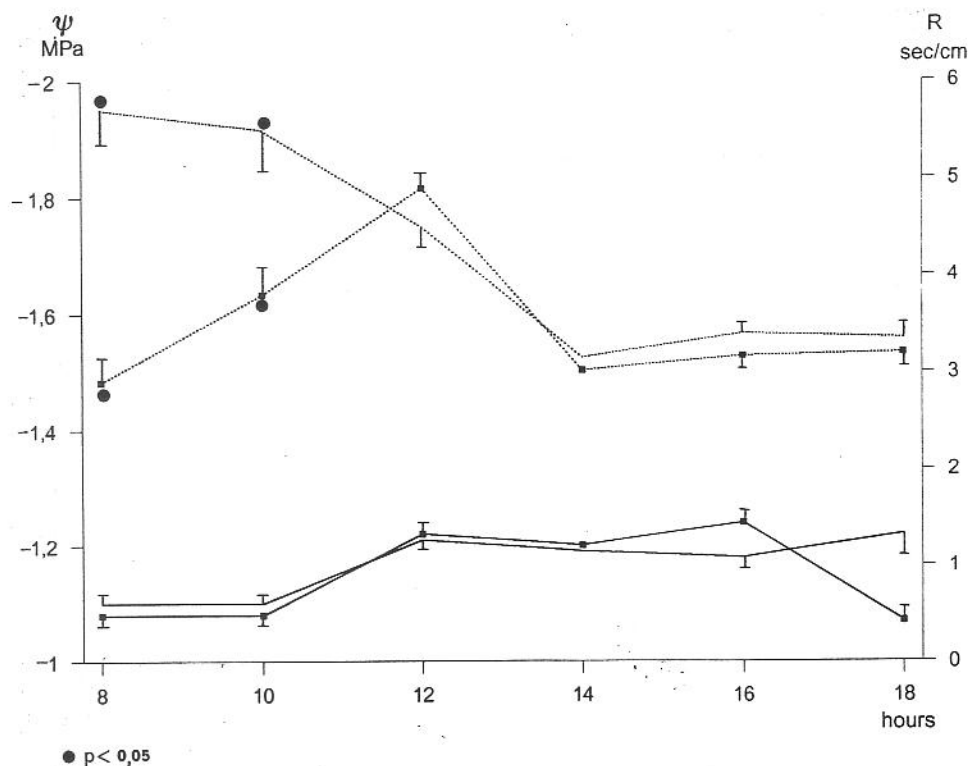


Fig. 3a – Water balance measured in the month of June. The solid line indicates the values for water potential; the dotted line indicates the values for leaf resistance. Trees with white coloration (■). Mean values \pm standard error are given.

ranging from 2.90 s/cm to 3.80 s/cm, respectively. In the latter samples leaf resistance increased by 69% from 8.00 to 12.00, peaking at 4.88 s/cm, and then decreased by 35% to the minimum values of 3.20 s/cm (18.00).

The water potential measured in September (Figure 3b) did not greatly differ in quantitative terms from that measured in June; yet there were major variations in the trends. In the control group, water potential ranged from -1.07 MPa to -1.70 MPa. It decreased by 29% between 8.00 and 12.00 (-1.32 MPa and -1.70 MPa, respectively) and then increased by 37% between 12.00 and 18.00 (-1.70 MPa and -1.07 MPa, respectively). In the white individuals water potential ranged from -1.10 MPa to -1.85 MPa, decreasing by 21% between 8.00 and 12.00 (-1.53 MPa and -1.85 MPa, respectively) and then increasing by 41%, to -1.10 MPa.

The leaf resistance of the control group increased by more than 100% between 8.00 and 16.00 (3.18 s/cm and 7.32 s/cm, respectively). Among the white trees, leaf resistance values were lower, ranging from 1.89 s/cm at 8.00 to 4.20 s/cm at 16.00, increasing more than 100%. At 12.00 and 16.00 significant

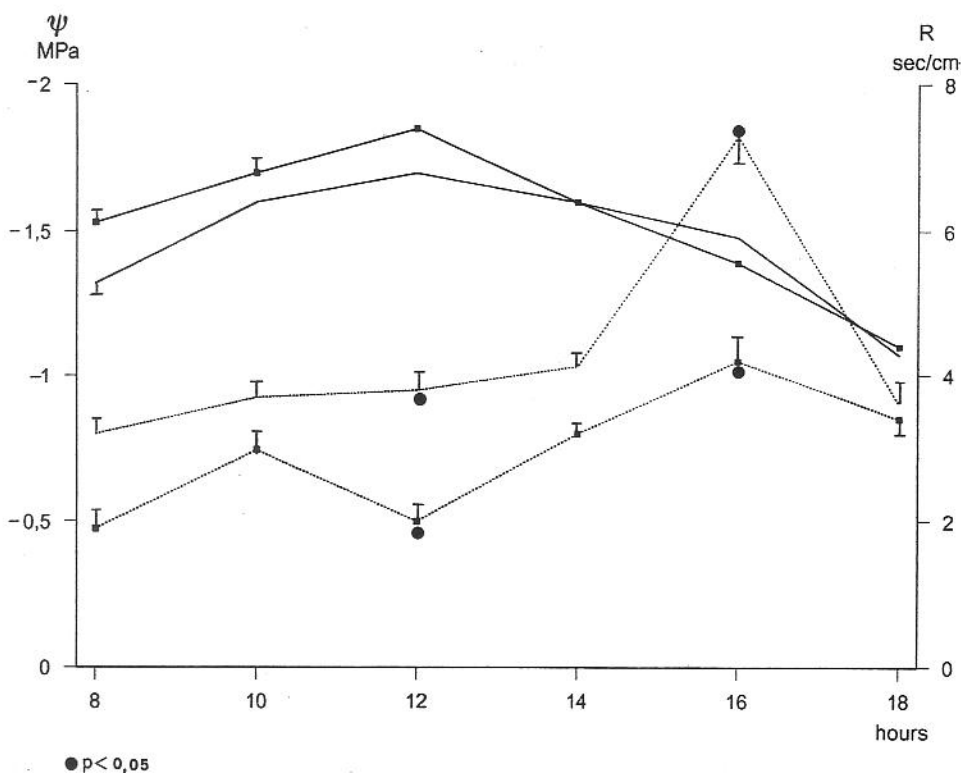


Fig. 3b – Water balance measured in the month of September. The solid line indicates the values for water potential; the dotted line indicates the values for leaf resistance. Trees with white coloration (■). Mean values \pm standard error are given.

differences ($p < 0.05$) in leaf resistance between the two samples were recorded.

In the full vegetative period (July) the concentration of organic carbon in the leaves was not greater than 46% in the white trees and increased by 22%, in the control group, which reached 56% of organic leaf carbon content (Figure 4); significant differences ($p < 0.05$) concerning leaf organic carbon content were recorded between the two samples. In the same month (July) the specific respiratory activity was $2.61 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ for the control group and more than 57% greater for the white trees, reaching $4.10 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ (Figure 5); significant differences ($p < 0.05$) were recorded between the two groups.

The lower leaf surface (Figure 6), photographed through the SEM in early and late summer (June and September), showed marked differences when comparing the white trees to the controls. Infact, the epidermal cells of the control appeared to be perfectly turgid, while those of the white trees were completely collapsed. With respect to the stomata and the epicuticular waxes, no differences were evident: the guard cells appeared to be equally turgid and the epicuticular waxes were not damaged in any of the trees studied.

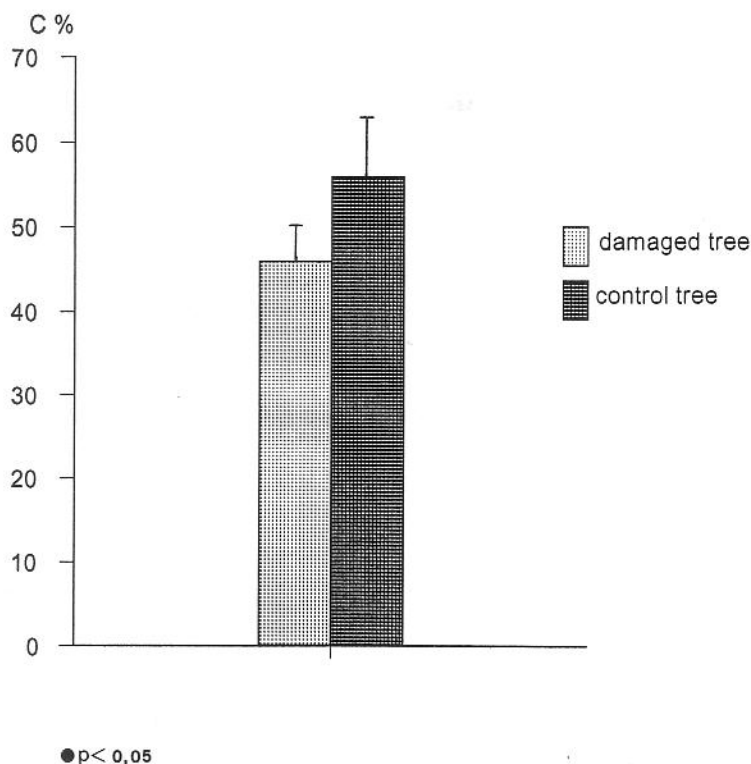


Fig. 4 – Organic carbon content in the leaves. Mean values \pm standard error are given.

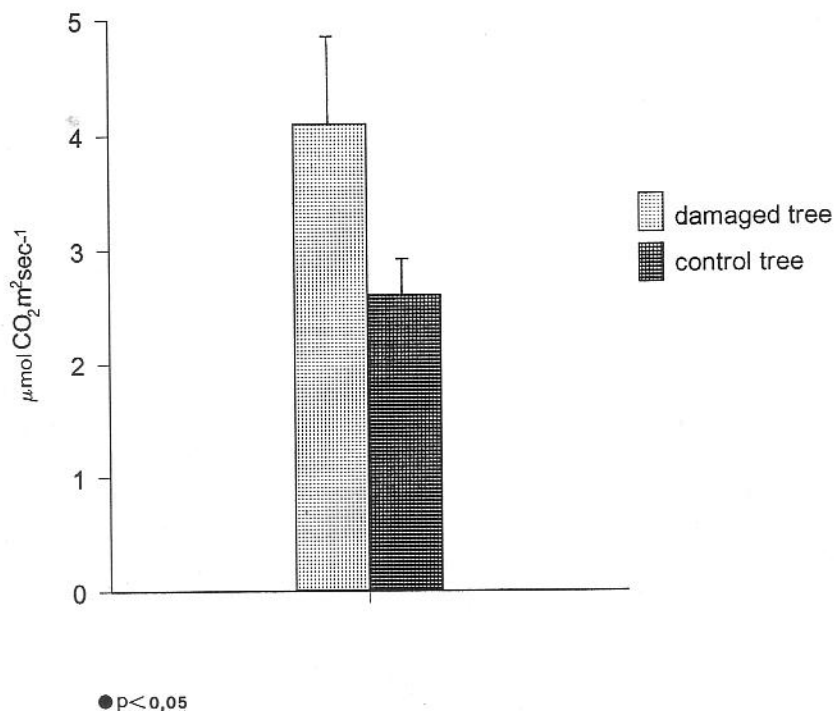


Fig. 5 – Specific respiratory activity. Mean values \pm standard error are given.

DISCUSSION

The white coloration observed in the trees studied may be associated with a different reflectance mostly in the near infrared band, to which the films used are sensitive, since in the visible band only slight variations in the green tonality were noted. Infact leaf reflectance in the infrared region is much higher (about five times) than in the visible band (Murtha, 1978).

The absence of color initially led us to hypothesize that a pathological condition influencing leaf reflectance was present. However, the infections observed in these trees, caused by *Corythuca ciliata*, are present in most of the city's platan trees.

Generally, the high leaf reflectance seen in infrared photographs is basically related to leaf chlorophyll content (Wagner, 1980; Gausman *et al.*, 1974). However, our results did not confirm this finding. The content of chlorophylls "a" and "b" measured in this study tended to show higher concentrations in the red trees. Nonetheless, this difference was not constant over time and was only significant in the early and late vegetative period (from May to July and from September to October). Thus, no relation could be found between the different leaf reflectance, which is a response that was

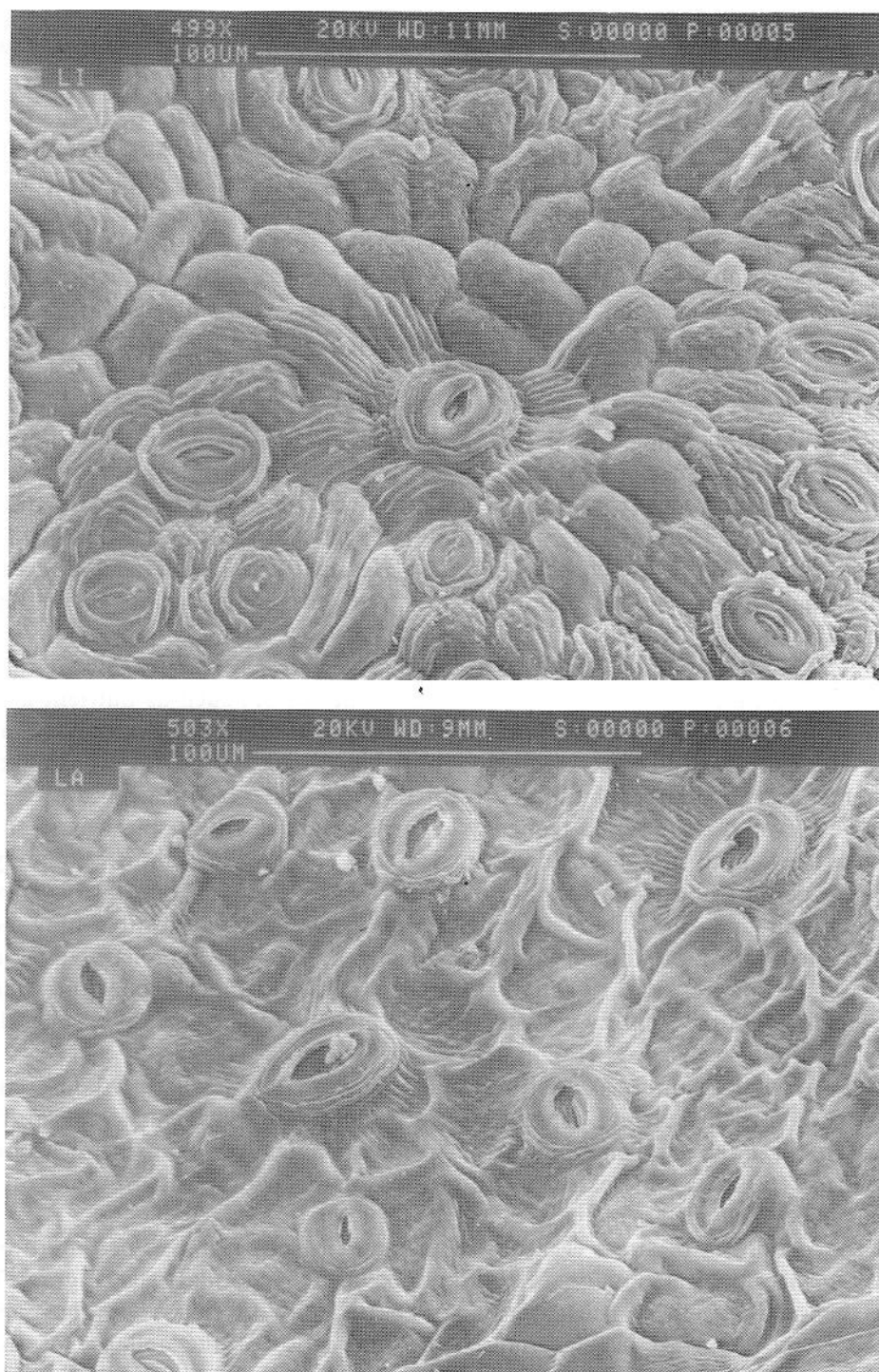


Fig. 6 – Above, SEM photographs of the lower leaf surfaces of the controls. Below, SEM photographs of the lower leaf surfaces of the comparison group.

constant over time, and the different concentrations of chlorophyll, which were variable over time.

Figure 1 shows the seasonal trend of chlorophylls "a" and "b" in the two groups studied. It can be noted that there are no significant differences between the two groups: the observed seasonal variations, in both cases, are related to the different phases of growth and maturation of the leaf. Chlorophyll "a" and "b" trends were similar: both peak simultaneously, corresponding to the maturation of the leaf, though the variations in the chlorophyll "b" content are more gradual. A difference between the two groups can, however, be noted in the period at which leaf chlorophyll peaked. All the white trees showed a particularly evident delay (with respect to the controls) in reaching the maximum concentration of leaf chlorophyll. This could be due to differences in phenological behavior i.e., a delay in leaf maturation may cause chlorophyll concentration to peak later. However, a delay in leaf development and earlier senescence (with respect to the control group) was observed in only one white tree (Figure 2b), which showed particularly little flowering and fructification, indicating chronic suffering (Heath, 1980; Lorenzini, 1983); the phenological behaviour of the other white trees was similar to that of the red trees (Figure 2).

Other studies have described a correlation between leaf reflectance and stress conditions due to natural or antropogenic factors such as nutrient and water stress or air pollution (Murtha, 1978). Daily and seasonal water stress of the trees under study was evaluated by measuring the leaf water balance. There were no significant differences in the leaf water balance for the trees studied; even if there was a tendency towards greater leaf transpiration in the white trees, both at the beginning and at the end of the summer but only during certain hours of the day, indicated by the trends of leaf resistance (Figure 3a and 3b). The tendency towards greater transpiration may be associated with a condition of stress, which may be also demonstrated by a greater specific respiratory activity (Larcher, 1983). In fact, in our study the specific respiratory activity was significantly higher in the white trees, suggesting a condition of stress (Figure 5).

The greater catabolic activity was also suggested by the observed differences in the organic carbon content which, in the white trees, was significantly lower than in the controls (Figure 4). The lower content of organic leaf carbon in these trees may indicate that a greater quantity of photosynthates are consumed through an increase in respiratory activity.

A condition of stress is also seen in the SEM photographs of the lower leaf surfaces, which show notable differences in the epidermal cells (Figure 6): in the controls, these cells appear to be perfectly turgid, while they are completely collapsed in the white trees. The cause of the collapse of the epidermal cells of the white trees is not clear, though in general, pollutants such as NO_2 , O_3 , and SO_2 are involved in this type of damage.

Though the response to SO_2 and O_3 of different species could be very

different (Darrall, 1989), it could be remind that sulfur dioxide and ozone are thought to be responsible for increased transpiration, as measured in the white trees.

Black and Black (1979a, 1979b), based on experiments on *Vicia faba* L. plants, report that low concentrations of sulfur dioxide ($50\text{--}500\ \mu\text{g}/\text{m}^3$) as yearly average concentration measured in the city of Rome, i.e. $57\ \mu\text{gr}\ \text{m}^{-3}$ (AA.VV. 1991), can cause extensive loss of turgidity and the collapse of epidermal cells shortly after exposure. The mechanism responsible for this damage to the epidermal cells has still not been clearly explained. The preferential damage with respect to these cells probably depends on the fact that the guard cells hinder direct absorption as a consequence of the presence of a thicker protective cuticle (Black & Black, 1979a). An increase of the opening of stomata has also been noted. This increase would be caused by an alteration of the turgor balance between epidermal cells and guard cells (Biscoe *et al.*, 1973). The epidermal cells are no longer capable of controlling the uptake of solutes (responsible for the increased turgidity) by the guard cells. Consequently epidermal cells lose control of the stomatic opening causing transpiration to increase (Squire & Mansfield, 1972).

O_3 concentration in the city of Rome reaches 22 ppb (mean value) during summer if peak values (150 ppb) could be measured along with some hours of the day (Altieri *et al.*, 1994). Even though the response of different species to ozone could be different according to the responsiveness to ozone of each species (Darrall, 1989), yet some authors demonstrated O_3 would be responsible of the decreased stomatal control, due to the alteration of permeability of the cell membranes, both at low and high concentration (Evans & Ting, 1974; Reich & Lassoie, 1984).

Thus, it can be hypothesized that the trees showing white in the infrared photographs could suffer from "invisible damage", caused by low concentrations of pollutants and possibly indicated by reduced reproductive power, induction of early senescence, and increased respiration (Lorenzini, 1983; Heath, 1980).

CONCLUSIONS

The results point out that the different leaf reflectance in the two groups studied could be related to changes in specific respiratory activity and loss of turgidity of the epidermal cells of the lower leaf surface rather than to changes of chlorophyll content. The selective damage to the epidermal cells, with respect to the stomatic cells, may be due to the presence of pollutants in the air, in particular SO_2 in low concentrations, which may favour a condition of stress, shown by an increase in respiration. Actually, the variations in turgidity are probably not limited to the epidermal cells but are also present in the cells of the deeper layers of the spongy parenchyma, causing changes in the

intercellular air spaces. Therefore, the different reflectance is also probably associated with an increase in the dimensions of the intercellular spaces in the entire mesophyll.

This findings suggest false-color aerial photos may show plant physiological changes caused by a condition of stress. These photographs can reveal eventual plant damage through changes in the "extra-visible" spectral reflectance, which occur prior to changes in the visible spectra. This allows earlier recognition of the damage, before or without becoming visually evident (Weber & Polcyn, 1972; Murtha, 1978; D'Andrea & Nevini, 1976; Catalano *et al.*, 1986; 1988).

RIASSUNTO

Due differenti gruppi di *Platanus hybrida* sono stati scelti sulla base della loro risposta all'infrarosso fotografico, nella città di Roma: un gruppo con colorazione bianca e l'altro con colorazione rosso violacea. Sono stati registrati alcuni parametri ecofisiologici: le fasi fenologiche, il contenuto fogliare di clorofilla "a" e "b", il contenuto di carbonio organico fogliare, il bilancio idrico e l'attività respiratoria specifica. Inoltre sono state fotografate al SEM le superfici fogliari inferiori. I risultati evidenziano che la differente riflettanza fogliare nei due gruppi esaminati può essere correlata a differenze nell'attività respiratoria specifica e alla perdita di turgore delle cellule epidermiche della superficie fogliare inferiore piuttosto che a differenze del contenuto di clorofilla fogliare.

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