LIGHT POLLUTION AND POSSIBLE EFFECTS ON HIGHER PLANTS

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ABSTRACT. Light pollution is a well known problem for astronomic observation of the sky. In this study we aim to verify whether, besides this, it can also be regarded as a more general ecological concern. Preliminary data, reported in this study, seem to indicate that the presence of artificial light in the proximity of some trees can be responsible for impairment of both their photosynthetic efficiency and photoperiod. In the investigation of the physiological state of the photosynthetic apparatus of some selected trees we have used the method of the fluorescence induction which allows a rapid and quantitative measure of an eventual stress condition at the level of the photosynthetic apparatus of a single leave.

1. Introduction

Light pollution is a well known problem for astronomic observation of the sky. In this study we aim to verify whether, besides this, it can also be regarded as a more general ecological concern. Preliminary data, reported in this study, seem to indicate that the presence of artificial light in the proximity of some trees can be responsible for impairment of both their photosynthetic efficiency and photoperiod. In the investigation of the physiological state of the photosynthetic apparatus of some selected trees we have used the method of the fluorescence induction which allows a rapid and quantitative measure of an eventual stress condition at the level of the photosynthetic apparatus of a single leave. To illustrate the method we shall briefly go through the molecular structures and the mechanisms which are involved in this study.

2. Oxygenic photosynthesis

In higher plants algae and cyanobacteria, the molecular device which carry out the conversion of light energy into chemical free energy, is a very complex set of pigment-protein structures located in the thylakoid membrane inside a specialised cell organelle called chloroplasts which has dimension of about 5 x 10 μm. The thylakoid membranes are stacked in substructures known as grana which are interconnected by portions of

unstacked membranes called stroma membranes. The whole system defines a continuum internal space called lumen separated from the external space called stroma. The light-gathering apparatus is a complex of lipids and proteins imbedded in the thylakoid membrane of chloroplasts. A group of light absorbing molecules, or chromophores, is intimately associated with the membrane-bound proteins responsible for photosynthesis. The photosynthetic pigments are responsible for efficient capture of the solar radiation. They absorb electromagnetic energy over a range of frequency that spans the visible region and extends into the far infrared in the case of photosynthetic bacteria. Chlorophyll a and b are the major chromophores in green plants, carotenoids acts as accessory pigments and protectors against chlorophyll photo-oxidation. Within photosystems, photosynthetic pigments are located at distance and orientation such as to allow efficient energy transfer among each other in a process called exciton transfer. In each photosystem, the electronic excited state generated by the absorption of a photon by a pigment associated to one of the *antenna* protein is transferred to a nearby pigment until it reaches the *reaction centre*, where the excitation energy is converted into charge separation. An electron transport chain starts which terminates with the oxidation of a specific electron donor and the reduction of a specific acceptor.

3. Photosystem II

Photosynthesis in higher plants requires the cooperation of two distinct molecular assemblies known as *photosystem I* and *photosystem II*. The combined action of these two systems supplies the energy to accomplish three tasks: (1) oxidation of two oxygen atoms of two water molecules to molecular oxygen, (2) reduction of NADP+ to NADPH and (3) phosphorylation of ADP to ATP. Beside the two photosystems, where the so called *light reactions* take place, two other protein assemblies are necessary which are also embedded in the thylakoid membrane; these are the Cytochrome b6/f complex and the ATP syntase complex. Since the phenomenon of fluorescence induction depends almost exclusively on photosystem II, we shall describe the latter in some more details. Photosystem II (PSII) is the protein-pigment sovramolecular complex that carries out the light-catalysed oxidation of water and reduction of the plastoquinone pool, a set of plastoquinone molecules freely dissolved in the lipid bilayer. The primary chlorophyll donor of PSII is called P_{680} and is composed of a chlorophyll a dimer with an absorption maximum at 680 nm. After light absorption, an electron is transferred from the excited state of P_{680} to a pheophytin, which in turn reduces a bound plastoquinone molecule, called Q_A. $Q_A$reduces a second quinone molecule, Q_B

The centre of reaction is constituted of two intrinsic proteins called $D_1$and D_2 (respectively of 32 and 34 kDa); both present molecular portions which are exposed to the stromatic surface and can be phosphorylated. These two proteins, associated as eterodimers, provide the binding sites for: the photochemically active pigment of the reaction centre (P680), constituted of a chlorophyll a dimer, the primary acceptor, pheophytin a (a chlorophyll a without Mg), a first plastoquinon (Q_A), permanently linked to the D_2protein, and a second plastoquinon (Q_B) reversibly bound to the D_1protein. Schematically, the electrons transfer from the reaction centre to the primary acceptors ($Q_A$and Q_B), is shown in figure 2 (the times of passage from a form to the other have

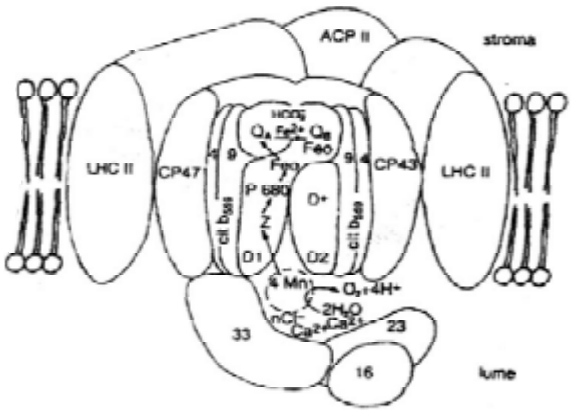

Fig. 1. Schematic model of PS II; as you can see, it is constituted of a reaction centre (P_{680}) and of an inner antenna (chlorophyll a, and of carotenoids) which are common to all organisms; an external antenna, LHC II, different depending on systematic groups (chlorophyll a or b). The polypeptides $D_1$and D_2coordinate both the electrons transport chain acceptors and donors from water to membrane plastoquinons. The OEE (Oxigen Evolving Enhancer) polypeptides belong to an enzimatic site which contains manganese whose function is to oxidise water to molecular oxygen (see also text).

been reported), (Krause and Weis, 1991). Through the same pathway, another electron doubly reduces Q_B, which is there protonated to Q_BH_2 (plastoquinol). In this form the complex brings its electrons to the cytochrome b6/f, and is replaced by another oxidised plastoquinon Q_B. The sites for the plastoquinons are located on the heterodimer D_1/D_2(respectively that of $Q_A$on protein $D_2$and that of $Q_B$on D_1). Other components of the reaction centre located on the $D_1$and D_2proteins, and which are probably involved in electronic transportation, are monomeric forms of chlorophyll a, a second molecule of pheophyitine a, a free radical D+, stable in the dark and identified as a residue of tyrosine on molecule D_2, a primary donor Z+ also consituted of a tyrosine residue present on D_1, and two molecules of bcarotene. The complex OEE (Oxygen Evolving Enhancer), constituted of three extrinsic proteins, respectively of 33000 dalton (OEE1), 23000 dalton (OEE2) and 16000 dalton (OEE3) is located in the stromatic part of the tylacoids, is strongly linked to the proximal antenna of PSII and to the proteins $D_1$and D_2through OEE1. In its catalytic site are also present four atoms of Mn. The complex has the function to reduce, through the catalytic cycle, the tyrosinic residue Z using H_2O as electron donor: it is capable of providing four electrons taken from it, gathering in turn four positive charges (4H+). The process develops through four consecutive stages, in each of which a redox process takes place with the formation of five consecutive S states (S0-S4), the last of which is sufficiently oxidised to be able to oxidate H_2O releasing oxygen in the tylacoidal lumen. The system returns to the initial state S0 (where the cycle restarts). The consequent release of protons in tylakoidal

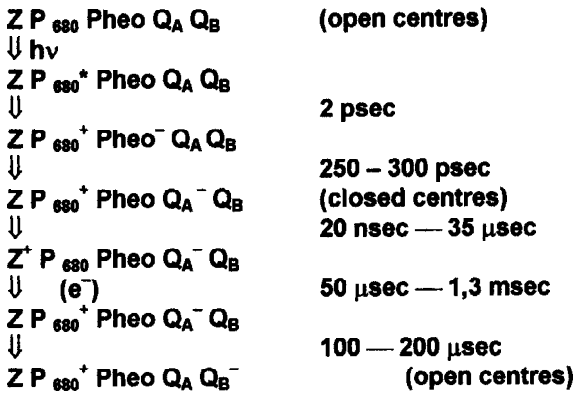

Fig. 2. Electrons transfer from the reaction centre to the primary acceptors ($Q_A$and Q_B).

lumen contributes in generating a protoni gradient in the membrane which is exploited by ATPases complex to produce ATP. The inner antenna of PSII transfers excitation energy from the external antenna complexes to reaction centre. Structurally it is constituted of two pigment-protein complexes with about 30 molecules of chlorophyll a; called CP 47 and CP 43 (chlorophyll-protein complex 47 and 43); the second also presents a site of phosphorylation, whose functional role is still uncertain. The inner antenna, is also involved in the stabilization of the reaction centre structure. The external antenna presents characteristics which depend on the systematic groups. In organisms such as clorofites, euglenofites and higher plants the complex of the external antennae is constituted of proteins associated with chlorophyll a, b and xantophylles; the number of chlorophyll molecules can be about 200 units per photosystem, nevertheless the dimensions of the complex can vary depending on the intensity and spectrum of light and temperature. The most important antenna complex in these organisms is LHC II (light harvesting complex II), made of different oligomeric components constituted of proteins of various nature. Actually, LHC II is divided in two subunities the inner of which is tightly linked to the photosystem's nucleus and contains almost exclusively polypeptides of higher molecular weight, while the second possesses proteins with slightly lower molecular mass; and high level of phosphorylation which allows a reversible dissociation that generally occurs following sudden environmental variations, in light intensity and temperature.

4. Fluorescence

Chlorophylls absorb blue (about 420 nm) and red (about 660 nm) light, transmitting and reflecting green. Most of the absorbed energy is conveyed to the reaction centres and transduced into charge separation. However, a small part of it is reemitted, as fluorescence, by chlorophyll a of the antenna system. The emitted fluorescence is in

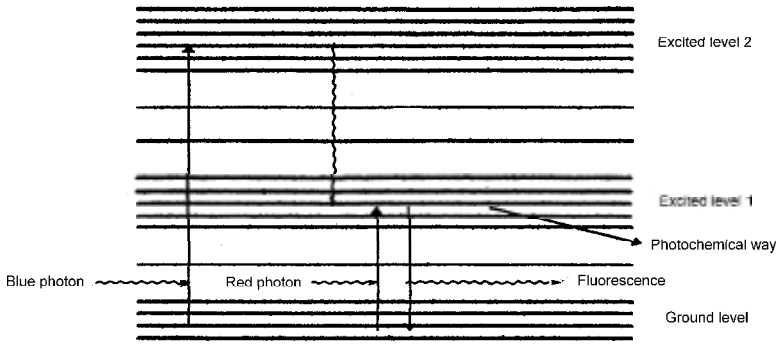

Fig. 3. Chlorophyll excitation by light. The importance of fluorescence emission by photosyntetic complexes (in our case PS II), resides in the possibility of analyzing its intensity variation depending on the time and therefore of evaluating in an indirect way the photochemical events of the photosynthetic system.

the red and far red region of the spectrum and is easily visible when a concentrated solution of chlorophyll is illuminated. In the case of chlorophyll in solution, the fraction of excitation energy dissipated as fluorescence can get to 30 % of the absorbed light, while in vivo this fraction is limited to about 3-5 %.

The Figure 3 schematically illustrates the process of energy dissipation as fluorescence.

5. The Kautsky effect

Figure 4 allows to analyse the kinetics of fluorescence induction (Kautsky effect, 1931), going from darkness conditions to light conditions (Krause and Weis, 1984; Briantais et al., 1986). If a leaf is kept in darkness (or under low intensity light) for a few minutes and then is illuminated with intense attinic light, fluorescence grows within fractions of second, to diminish again after a few seconds or minutes.

It is possible to identify two components in the registered signal: $F_\circ$, registered in conditions of darkness and F_V , the variable component which is observed when the system is illuminated. $F_\circ$ (O), which is the starting signal, is the initial fluorescence which comes from chlorophyll molecules excited in the antenna of PS II when the excitation light is on but its intensity is not sufficient to make a significant number of $Q_A$reduced; its level is determined as the time zero emission when the system has been kept in the darkness as to to guarantee the almost complete oxidation of Q_A. From this point on the fluorescence curve presents a growth to a I level (inflection), then a brief phase of fluorescence decline D (dip) followed by a peak P. The remaining time course is characterized by a comparatively slow fall to a T (terminal) level, going through an

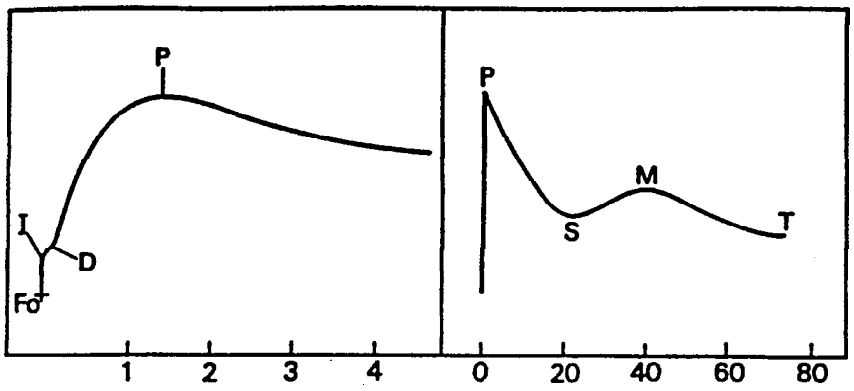

Fig. 4. Kinetics of fluorescence induction; in this representation the OIDPSMT terminology is used (from Lavorell and Etienne, 1977). In this picture O is designated as $F_\circ$.

almost stable S state. It is hypothized that the fluorescence growth from state $F_\circ$ to I and the following standstill in D mirrors the imbalances between the reduction and re-oxidation of Q_A. The maximum value P is reached after about one and a half second of illumination with intense light. This highest possible value for P is Fm which is obtained when the light intensity is sufficient to saturate the system. The relationship F_V /$F_\circ$ (where F_V is equal to F_m -$F_\circ$) can vary from 4 to 6 for intact leaves. The following passage from P to T requires much more time if compared to the previous passages. The fluorescence emission speed is influenced by a series of factors that can modify it and that involve different mechanisms which compete with each other when the chlorophyll molecules de-excite back to the fundamental state. These mechanisms are the energy transfer to neighbouring molecules, the internal conversion and the energy conversion in a photochemical reaction. The fluorescence emission intensity can be expressed by the following relation between rate constants:

$$F = Ia\frac{k_F}{k_F + k_D + k_P} \tag{1}$$

where k_F= fluorescence emission k_D= internal conversion k_P= photochemical conversion hence the fluorescence output is:

$$\Phi = \frac{F}{Ia} = \frac{k_F}{k_F + k_D + k_P} \tag{2}$$

It varies from a minimum figure Φ_0, when all the reaction centres are open ($Q_A$oxidised), to a maximum figure Φ_m, when they are closed ($Q_A$reduced):

$$\Phi_0 = \frac{F_0}{Ia} = \frac{k_F}{k_F + k_D + k_P} \tag{3}$$

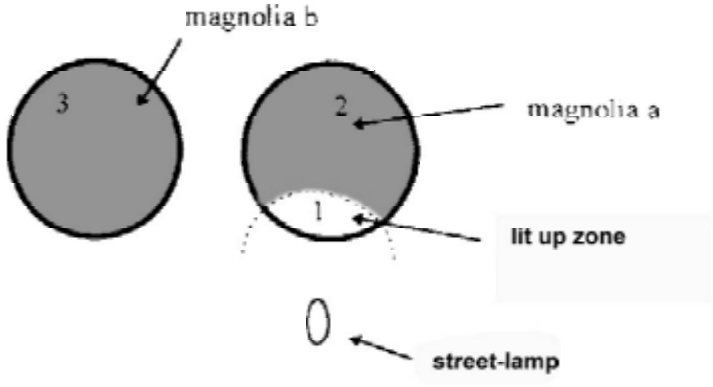

Fig. 5. Entrance of the Botanical Gardens in Padua and location of magnolias: numbers 1,2,3 indicate the sampling areas.

$$\Phi_m = \frac{F_m}{Ia} = \frac{k_F}{k_F + k_D + k_P} \tag{4}$$

In the first case $k_P >> k_F + k_D$, while in the second case $k_P = 0$. From these equations it is possible to get derive the quantum yield for the photochemical process at the reaction centre:

$$\Phi_P = \frac{k_F}{k_F + k_D + k_P} = \frac{(\Phi_m - \Phi_0)}{\Phi_m} = \frac{F_m - F_0}{F_m} = \frac{F_\nu}{m} \tag{5}$$

This is given by the easily measurable parameter $\frac{F_\nu}{m}$, which can thus be used to evaluate the physiological state of the photosynthetic system and in particular PS II.

6. Materials and methods

The samples used for analysis and fluorescence measures are constituted of small discs of magnolia leaf (diameter about 13 mm), taken from the Botanical Gardens of Padua at weekly intervals and always at the same hour of the day (about 9.00 am). The two chosen plants (Magnolia grandiflora L.) are at the entrance of the Gardens (see figure 5) planted in 1800.

The reasons for this choice were the following: i) the Gardens could give logistic support to the sampling of leaf specimens, placed to the height of 3-3,5 meters; ii) in the Botanical Gardens there is a station of meteorological data linked with the regional network surveying, capable of supplying continuous registration of temperature, humidity, radiation etc.; iii) since the plants are evergreen it is possible to collect data all over the year; iv) a portion of one of the two magnolias is illuminated by a street lamp (milk white light), a 125 watts mercury vapour bulb, placed at the height of 6,30 metres and at a minimum distance from the plant of about 2,20 metres: the light flux in this area is about 157 $watt/m^2$. The street lamp is also set by an automatic lighting system, called

"crepuscular", which turns the light on 20 minutes after sunset and off 20 minutes before sunrise. The samplings were made in three different areas of the trees, so as to have the data referring to the first plant, in illuminated and dark regions (used at control) and to the second which is not illuminated. Sample leaf discs were placed into separate boxes in order to guarantee their permanence in the dark until the measurement, taken at the Biology Department and measured with a fluorimeter (the interval of time between samplings and measurements was of about 15/20 minutes). The experiment lasted one year (from October 1994 to October 1995) with an interruption during the summer (from half of July to the end of August). (1) In the beginning the specimens where 10 for all the three areas chosen for the samplings, later, in order to avoid deterioration of the esthetical appearance of the plants we decided to reduce samplings respectively to 5 for the two non illuminated parts, keeping the number of ten for the illuminated area.

7. Measured parameters

Performed measurements considered the analysis of fluorescence emission of the sampled leaf samples, therefore the main parameter that we got from the registered curves was:

$$\Phi_P = \frac{F_\nu}{m} \tag{6}$$

Φ_P indicating photosyntetic efficience.

8. The fluorimeter

In order to measure the fluorescence emitted by the leaf samples a special fluorimeter was used, called PAM (photoamplitude modulate chlorophyll fluorimeter, figure 6), made by Schreiber et al. in 1986. The PAM, compared to a normal fluorimeter, uses a technique based on an in-phase coupled amplifier (lock-in amplifier), which allows to measure the excited fluorescence from a low intensity modulated light source (observation light), in presence of a second continuous or flash light (attinic light), much stronger (can be up to 100 times stronger than the observation light) which has the task of inducing photochemical reactions. As only fluorescence induced by the observation light is modulated, it is the only one that gets amplified. With this technique, in fact, fluorescence induced by attinic lights, doesn't get amplified and does not disturb the measure.

9. Results

The results obtained suggest that the presence of a light source near plants can be the cause of a partial decrease of their photosyntetic efficiency. However it is a quantitative evaluation of the level at which artificial light is responsible of such decrease and if this can be considered pathological for the leaves. In fact, there could be other polluting factors which interfere with such efficiency, also considering that the samples analyzed were taken in the open field, which prevents, differently from laboratory conditions, a successful monitoring of environmental conditions that may influence measurements.

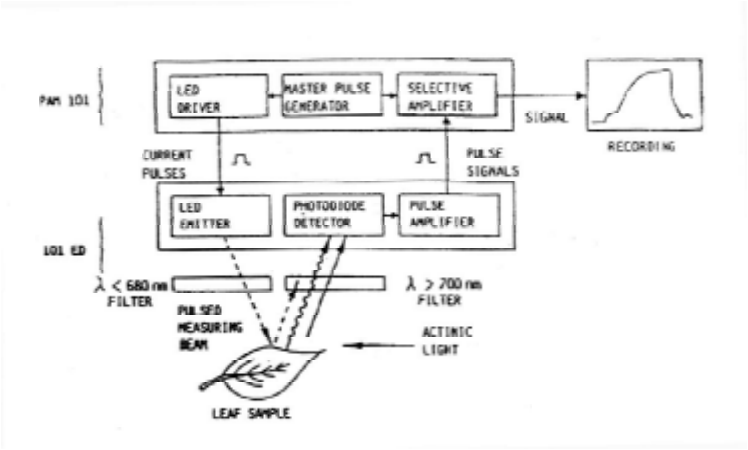

Fig. 6. PAM fluorimeter scheme (photoamplitude modulate chlorophyll fluorimeter) used in fluorescence measurements.

10. Daily averages

The fluorescence curves obtained from each specimen allowed to calculate the constant Φ_P daily average for each sampling area (obtained by arithmetic averages of each single value) and represent its time dependence. Chart 1 and table 2 show how: there is substantial coincidence between non illuminated magnolia a and magnolia b (specimen magnolia) while a significant difference exists between the illuminated part and the non illuminated one of the same magnolia a. It can be hypothesized that one or more factors contribute to the variation of this parameter: it is possible to exclude meteorological changes, such as temperature, humidity, wind strength and direction, etc., as the examined plants are all in the same place. Probably the presence of the street lamp near the plant, constantly on every night of the year, can have determined physiological imbalances (e.g., low chlorophyll production). This phenomenon should also be more evident if the area is illuminated at closer distance. In the same chart another interesting phenomenon is evident: from the first days of February to the first days of April the daily average of the measured parameters tends to diminish progressively; from April (minimum value) the tendence is inverted with a fast reclimbing, until it reaches, in mid-June, values which can be comparable with those of October. This trend is the same for the three areas of the considered plants, but is evident for the one which is illuminated. An explanation for this observation could be that in April magnolias prepare themselves to let the older leaves fall (which are about two years old), where the senescence process is accentuated; therefore it is very probable, compared to other times of the year, to find leaves whose photosyntetic efficiency is lower.

Table 1 -

plant	annual average	standard deviation
magnolia a	0.7251	0.0062
magnolia b (control)	0.7182	0.0053
illuminated magnolia a	0.6006	0.0133

Table 2

date of sampling	magnolia a in the dark	magnolia b (control)	illuminated magnolia a
10 04 94	0.7371	0.7335	
10 11 94	0.7850	0.7638	
10 18 94	0.7844	0.7290	
10 24 94	0.8022	0.7604	
10 31 94	0.7576	0.6496	
11 08 94	0.7776	0.7428	
11 16 94	0.7624	0.7395	
11 22 94		0.7792	0.6545
11 29 94		0.8058	0.5612
12 06 94		0.7648	0.6963
12 13 94	0.7782	0.7757	0.6464
12 20 94	0.7477	0.7808	0.7051
12 29 94	0.7601	0.7542	0.6147
01 04 95	0.8075	0.7651	0.5470
01 10 95	0.7292	0.7000	0.5445
01 17 95	0.7232	0.6508	0.5101
01 24 95	0.7396	0.6669	0.6526
01 31 95	0.7684	0.6926	0.6318
02 09 95	0.7500	0.7180	0.6240
02 16 95	0.7280	0.7210	0.6248
02 21 95	0.7310	0.7175	0.5230
02 28 95	0.7487	0.7220	0.6310
03 07 95	0.7416	0.7188	0.4792
03 14 95	0.7095	0.6303	0.3712
03 21 95	0.7280	0.6598	0.6990
03 29 95	0.6537	0.6176	0.5022
04 05 95	0.6682	0.7504	0.4426
04 11 95	0.7030	0.7801	0.5160
04 20 95	0.6889	0.7205	0.4710
04 27 95	0.7460	0.7686	0.5290
05 04 95	0.6996	0.7371	0.6420
05 09 95	0.6993	0.6532	0.6485
05 16 95	0.7980	0.7538	0.6158
05 23 95	0.6879	0.7146	0.5902
05 30 95	0.7380	0.7547	0.5922
06 14 95	0.7674	0.7880	0.7127
06 27 95	0.7440	0.7812	0.7249
07 12 95	0.7930	0.7490	0.7410
09 21 95	0.6463	0.6986	0.7267
09 27 95	0.7182	0.7390	0.5547
10 05 95	0.7467	0.7195	0.6158
10 12 95	0.7255	0.6222	0.6974
10 19 95	0.7632	0.6248	0.7205

11. Annual averages

From all the daily averages of photosyntetic efficiency (Φ_P) an annual average for each magnolia has been drawn (see table 1). In order to calculate it, one has used statistical relations which kept in mind that every single figure could be the result of a different number of samplings; therefore a weighed average has been obtained. The figures relating to the dark area of magnolia a and of magnolia b can be compared, that is to say they are similar, while those relating to the illuminated area of magnolia a were clearly lower. The standard deviation of the first is lower compared to the second, pointing out the higher variability of the single specimens taken from the latter area.

12. Conclusions

As pointed out above, it is possible to hypothesize that artificial light influences the process of transferring of light energy from the antenna system to the PS II reaction centre. It was not possible to determine how this influence manifests itself biochemically, and it is also difficult to quantify the eventual damage at this level. It is important to point out that if F_m and $F_\circ$ diminish proportionally, so as F_V /F_m remains constant, a variation of energy absorption can be hypothesized; if instead it is only F_m to diminish, this could indicate the instauration of a quenching phenomenon the nature of which is however unknown. In our research, for the illuminated magnolia together with a substantial diminishing of F_m , a growth of $F_\circ$ has been found; indicating a higher energy dispersion by the PS II antenna as fluorescence, and a lower energy transfer to the reaction center.

In general, the illuminated area of the plant seems to have a lower photosynthesis activity, as it probably absorbs and uses less natural light, compared to the non illuminated area.

Another possible explanation for the lower efficiency of the illuminated area is a lower content of chlorophyll with respect to non-illuminated areas; this would consequently determine a lower absorption by the antenna systems and therefore also a reduced maximum fluorescence emission.