

Measuring fluorescence and photosynthesis

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Photosynthesis measuring devices, based on chlorophyll fluorescence, have developed since the beginning of the 1990's. EARS, in Delft, the Netherlands, was the first company that developed a handheld instrument. This plant photosynthesis meter (PPM) has since then been improved and developed further. So far the portable instrument was still of substantial size. But in 2011 the miniPPM was launched. This instrument is based on a novel measuring method, has excellent measuring properties, but is not much larger than a mobile phone. Moreover the instrument is very affordable. As a result it is expected to reach a larger target group, in particular farmers and greenhouse growers. But the miniPPM is also very suitable for schools and in applied research. It is also a remarkable gadget for plant and nature lovers. This article explains the principles of measurement with and use of the instrument.

The photosynthesis reaction

The plant is composed of cells, which contain a large number of green grains, the *chloroplasts* (figure 1). In these chloroplasts there is a folded membrane, consisting of proteins and lipids, the *thylakoid*. Imbedded in the thylakoid there are clusters of several hundreds of *chlorophyll*-molecules, the so-called *photosystems* (figure 2). These provide the energy for the photosynthesis reaction. They function as antennae that capture light particles or *photons*. Photosystems absorb mainly blue and red light, while green light is scattered. That is why plants are green.

When the photosystem captures a photon, the light energy is transferred to an electron, which thereby enters into a higher orbit. This phenomenon is called *excitation*. In this state the electron is also called *exciton*. This state of excitation travels through the photosystem by resonance transfer. At one side of the photosystem and thylakoid, there is a specialized molecule that is called the *reaction centre*. This centre can bind the exciton. As a result an electrical potential difference is created across the membrane. So, the photosystems function like a photo-electrical cell that converts light into an electrical current. This current drives the photosynthesis reaction, a very complicated reaction chain which comes down to:



In fact there are two different photosystems, known as *PS2* and *PS1*, which are serially connected and pump the electrons to the required energy level. Therefore 2 photons are required to transport one electron. Consequently 8 photons are required to fix 1 molecule CO_2 in the form of 1 molecule glucose: CH_2O .

The chemical part of the photosynthesis reaction proceeds much slower than the light reaction. The time required for transferring an electron from the reaction centre is 10^{-4} s, while closure of the reaction centre by the exciton requires only 10^{-8} s. During the time that the reaction centre is closed, more excitations can take place. The excited state, however, has a restricted lifetime, and if the exciton does not meet a reaction centre, or if the reaction centre is already closed, then the exciton will finally fall back into a lower orbit. The excess energy mainly

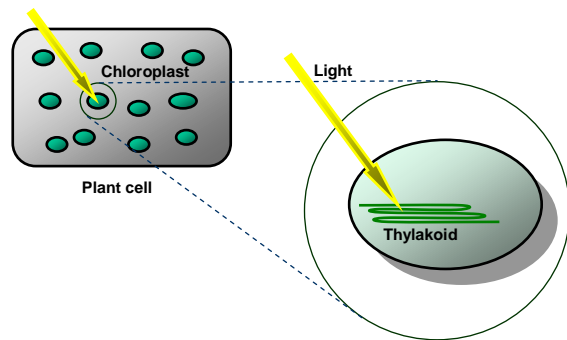


Figure 1: Schematic representation of the plant cell with chloroplasts and the thylakoid membrane inside.

dissipates into heat. Only in a very limited number of cases the excitation energy is emitted again as a photon. This is called *fluorescence*. The "color" of the fluorescence is shifted to longer wavelength. The absorbed photons are mainly blue to red (400-700 nm, also known as PAR: photosynthetic active radiation). De chlorophyll-fluorescence is mainly emitted in the far red and near infrared (660-800 nm).

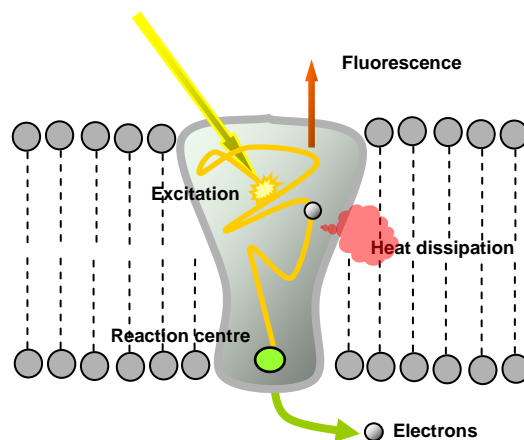


Figure 2: Schematic representation of a photosystem, showing excitation of an electron and the three alternative energy pathways: photosynthesis, heat dissipation and fluorescence.

Photosynthesis yield

There are innumerable photosystems in the plant. In the dark, all reaction centers are open. When there is some light, equilibrium develops between the supply of photons to and the flow of electrons from the photosystems. At low light only a small part of the reaction centers is closed, but when the light level increases, the number of closed reaction centers increases too. As a result more excitons meet a reaction centre that is already closed. When this is the case they can only lose their excess energy by heat dissipation or fluorescence. In other words the light utilization for photosynthesis, also called the *photosynthesis yield* or *light use efficiency* (Φ_P) decreases, while the heat yield (Φ_H) and fluorescence yield (Φ_F) increase proportionally. Their sum remains 1 or 100%.

In the dark all reaction centers are open and the photosynthesis yield (Φ_P) reaches its highest value, but does not reach 100%. There remains a certain chance that excitons fall back before they have met the reaction centre. The maximum photosynthesis yield, measured in the dark, is about 82%. Of the remaining 18% about 17.5% is dissipated into heat and about 0.5% is emitted as fluorescence.

At *light saturation*, for example, created with a very intense light pulse, all reaction centers are closed. In that case the photosynthesis yield is zero ($\Phi_P=0$). In this state the heat dissipation and fluorescence have increased more than five-fold.

From the previous we conclude that the chlorophyll fluorescence is a very weak but variable signal. It varies between about 0.5 and 2.5% of the impending light energy. The fluorescence can under normal conditions not be observed by eye, as this weak signal is submerged in the ambient light. The dynamics of the fluorescence is however considerable and may give information on photosystem closure and photosynthesis. Whereas the fluorescence is so weak and submerged in ambient light, we have to use a trick to measure it.

Measurement of fluorescence and photosynthesis

This trick consists of using a special *excitation light source* that is used to elicit and recognize the fluorescence. To this end the excitation light source is *modulated*, which means that the source is turned on and off with a known frequency. The fluorescence, elicited by this source, is also modulated, contrary to the fluorescence resulting from the ambient light. We may measure the fluorescence with a light sensitive diode. Subsequently we use electronics to separate the modulated part of the signal from the constant part. The modulated fluorescence (F), measured in this way, is elicited by a light source of fixed intensity, and thus is a measure of the fluorescence yield of the plant (Φ_F).

In addition it is necessary to separate the excitation light spectrally from the fluorescence, otherwise reflected excitation light could be measured by the



Figure 3: The plant photosynthesis meter (PPM-100) with excitation light source (left), saturation light source (middle) and sensor for measuring the fluorescence (right).

sensor, and could be mistaken as being fluorescence. To this end, optical filters are used. In front of the excitation light source a PAR filter is placed, which transmits only 300-700 nm. In front of the photo-diode an infrared long pass filter is used which transmits only infrared radiation longer than 700 nm.

We are not particularly interested in the measurement of chlorophyll fluorescence, but more in the information on the photosynthesis yield (Φ_P). To extract this information, an additional measurement is done. This measurement is carried out in very high, saturating light, created by a separate light source. This source throws light of intensity equal to about 2 times sunlight on the leaf. As a result all photosystems are closed in a short time: 0.5-1 s. Full photosystem closure is detected by the fact that the fluorescence reaches a maximum value (Φ_{Fm}). At the same time the photosystem yield becomes equal to zero ($\Phi_P=0$).

In summary, the full PPM measurement consists of two subsequent partial measurements. The first one measures the fluorescence yield in ambient light (Φ_F), while the second one measures the maximum fluorescence yield (Φ_{Fm}). For these two partial measurements we have:

$$\Phi_P + \Phi_H + \Phi_F = 1 \quad (1)$$

$$\Phi_{Hm} + \Phi_{Fm} = 1 \quad (2)$$

In addition it is known that the ratio of heat dissipation and fluorescence is constant:

$$\Phi_F / \Phi_H = \Phi_{Fm} / \Phi_{Hm} = c \quad (3)$$

From these three equations one may easily derive:

$$\Phi_P = 1 - \Phi_F / \Phi_{Fm} = 1 - F / F_m \quad (4)$$

If a measurement in this way is carried out in the dark on a dark adapted plant, i.e. when all photosystems are open, then this relation is written as:

$$\Phi_{P0} = 1 - \Phi_{F0} / \Phi_{Fm} = 1 - F_0 / F_m \quad (5)$$

For all natural plant species the maximum photosynthesis yield in the dark appears to be almost the same, 82%.

The measurement of the photosynthesis yield as described here, is implemented on the PPM-100 photosynthesis meter.

Measuring of photosynthesis rate

Although the measurement of photosynthesis yield has a number of important direct applications, it is not so easy to interpret this information in terms of growth, i.e. weight increase of plants. For this reason a next step is made with the PPM-200: measuring the *photosynthesis rate* (P). To this end an additional sensor is added to the instrument, which measures the light level on a small white, diffuse reflecting square next to the leaf. In front of this sensor a PAR filter is placed that transmits only the photosynthetic active radiation in the 400-700 nm wavelength band.

The light intensity on the square, and thus on the leaf, is measured in micromole-photons/m²s. This is a measure of the number of photons incident on the leaf per unit of time and surface. The photosynthesis rate (P) is obtained by multiplying the photosynthesis yield with the incident photosynthetic active radiation or PAR:

$$P = \Phi_P \cdot PAR \text{ (micromol/m}^2\text{s)} \quad (6)$$

The photosynthesis-light curve

It was already argued that, in the above equation, Φ_P itself is also depending on the light level and decreases with increasing light intensity. We can now try to further our understanding of the relation between photosynthesis and light by theoretical means. To this end we consider the equilibrium between exciton supply to and electron drain from the reaction centers. First it is noted that all reaction centers are open in the dark at $\Phi_{P0} \cong 0.82$, while all reaction centers are closed in saturating light and $\Phi_P=0$. Thus the degree of reaction centre closure (C) may be given by::

$$C = 1 - \Phi_P / \Phi_{P0} \quad (7)$$

The rate of exciton delivery to the reaction centers is proportional to the number of open centers: 1-C and the incident radiation. Thus exciton supply to the reaction centers is (1-C)*PAR. On the other side of the reaction centers, the drain of electrons is proportional to the degree in which reaction centers are closed (C). The corresponding electron flow for photosynthesis may be quantified by assigning an effective resistance (r) to the reaction chain. The drain of photons from the reaction chain is then equal to

C/r. Now, the closure of the reaction centers will set in such a way that there is equilibrium in supply and drain of electrons, and thus:

$$(1-C) PAR = C/r \quad (8)$$

By elimination of C from (7) and (8) we then find:

$$\Phi_P = \Phi_{P0} / (1 + r.PAR) \quad (9)$$

Now we obtain the following expression for the electron flow or photosynthesis rate:

$$P = \Phi_P PAR = \Phi_{P0} PAR / (1 + r.PAR) \quad (10)$$

The suggested relation between photosynthesis and light is hyperbolic. Such shape is also found by measurements with the photosynthesis meter. At high light intensity the photosynthesis reaches a maximum value which equals:

$$P_m = \Phi_{P0} / r \quad (11)$$

We call the maximum photosynthesis rate also the *growth power* of the plant species. The growth power is specific for each species because different species have different effective resistance r. In addition the value of r may increase if due to water shortage the plant stomata are closed and consequently the CO₂ uptake is reduced.

Photosystem deactivation

The earlier mentioned maximum fluorescence yield (Φ_{Fm}) can be considered a measure of the number of photosystems that contribute to photosynthetic electron transport. It appears however that, with increasing light level, this maximum fluorescence yield decreases. From this fact we have to conclude that the number of photosystems that contribute to photosynthetic electron transport decreases with increasing light level. This phenomenon is called *photosystem deactivation* or *photo-inhibition*. It is considered to be the consequence of excess excitation energy, which causes damage to (parts of) the photosystems. This effect causes a relative decrease in photosynthetic electron flow which, however, does not follow from the earlier presented calculation of the photosynthesis yield:

$$\Phi_P = 1 - \Phi_F / \Phi_{Fm}. \quad (12)$$

By deactivation, Φ_F en Φ_{Fm} are both affected in the same way and consequently there is no influence on the calculated photosynthesis yield, which apparently represents only the yield of active photosystems. To account for the effect of deactivation on photosynthesis, we multiply equation (6) with deactivation factor Q, thus obtaining for the photosynthesis expression:

$$P = Q \cdot \Phi_P \cdot PAR \text{ (micromol/m}^2\text{s)} \quad (13)$$

The deactivation factor Q may be obtained from the value of the maximum fluorescence yield in the current measurement (Φ_{Fm}), divided by a similar reference measurement at a moment that no

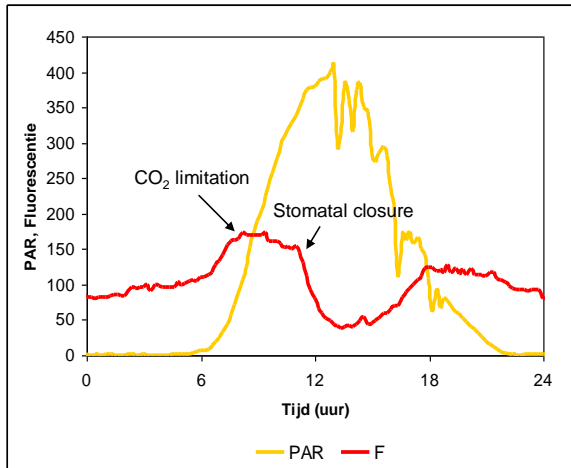


Figure 4: Typical daily course of light level (yellow) and plant fluorescence (red) during a sunny day. Already 2 hours after sunrise at 8:00 the plant becomes subject to CO₂ limitation: deactivation compensates excitation and the fluorescence flattens. Around 11:00 stomatal closure is evident from a sudden dramatic decline in the fluorescence, caused by strong deactivation.

deactivation has taken place yet (Φ_{Fm}), for example just before sunrise:

$$Q = \Phi_{Fm} / \Phi_{Fmr} \quad (14)$$

Does deactivation imply damage ?

Photosystem deactivation could be considered as damage to the plant. This is only partly true. Deactivation is also a necessary antagonistic element in the control of photosynthetic electron transport. It facilitates that excess excitation energy is converted into heat in a controlled way, and does not cause permanent damage to the plant.

Deactivation starts already at low light and dims electron flow. Measurements show that the value of Q decreases linearly with reaction centre closure (see equation 7). The photosynthetic electron flow rate reaches a maximum value when it is in balance with CO₂ uptake. If the light level increases further, electron flow has to be contained by increasing deactivation. If, due to water shortage, also the leaf stomata are closed, deactivation increases dramatically and may reach levels of 80-90% ($Q=0.1-0.2$).

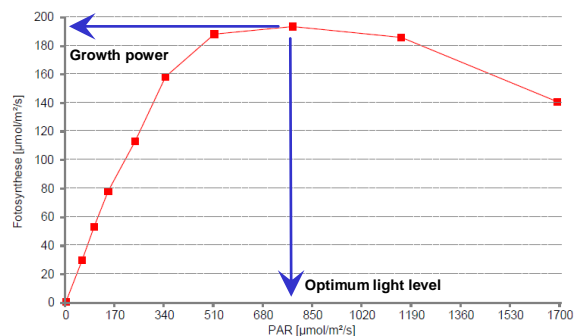
In direct sunlight, deactivation due to CO₂ limitation may already start in the morning, as shown in figure 4. In this case, with some drought stress present, stomatal closure occurs before noon and deactivation becomes very strong. Repair of photosystems may require a number of hours and usually takes place in the late afternoon and evening. This means that in case of strong de-activation around noon, Q will be relatively low in the afternoon, implying that photosynthesis and crop growth are not optimal. In horticulture and farming this means loss of production and economic damage. For this reason light screens are used in horticulture to protect a product against

excessive light. This however raises a new question: what is the best light level to close the light screens? If screens are closed too early, then one can expect loss of growth. Are screens closed too late, then growth is also sub-optimal and there is risk of light damage (burning). Using the PPM photosynthesis meter it is possible to answer these questions. Particularly the PPM-300 version, to be discussed hereafter, is a useful instrument for this purpose.

Measuring photosynthesis-light curves

The PPM-300 is not only suitable to measure the photosynthesis rate, like the PPM-200, but in addition has the possibility to measure in an automatic way the complete photosynthesis-light curve (PLC) of plants. To this end the saturation light source of the instrument is not only used to provide saturating light pulses, but also creates a specific ambient light level on the plant. By means of a pre-programmed measuring protocol, a complete light cycle may be stepped through, while the corresponding photosynthesis measurements are done after adaptation of the plant to each light level.

In practice the instrument and the plant are placed in an (almost) dark room (a box or cloth could also be used). The plant is allowed to adapt during a short period to the dark. At the end of that adaptation period the first measurement of Φ_F , Φ_{Fm} and PAR is done and the corresponding photosynthesis rate (P) is calculated. Thereafter a higher light level is created and the cycle of adaptation and measurement is repeated. This continues until in some 6 to 10 measurements the full light range from 0 to some 2000 micromole/m²s has been completed. Finally an automatic measuring report is generated with all data and a plotted curve of the photosynthesis rate as a function of light level. The latter is shown in figure 5. This photosynthesis-light curve (PLC) is the most important growth characteristic of a plant species or variety. There are two very important figures that may be derived from this curve: (a) the growth *power* of the plant, i.e. the maximum photosynthesis rate, and (b) the preferential, *optimum light level*, i.e. the light level that creates maximum growth and above which the plant should be protected from receiving more light.



Photosynthesis light curve measured with the PPM300. Growth power is 190 micromole/m²s, about 25% of the optimum light level. Growth power corresponds to a dry weight increase of 1.9 gram/m² hr.

Calculation of dry matter production

The photosynthesis rate of a plant, and more specifically the growth power, are measured in micromole per square meter per second. We may convert this to dry matter production, i.e. the increase of plant dry weight. Suppose the growth power is 200 micromole/m²s. We have seen earlier that 8 photons are required to bind one molecule of CO₂ as glucose (CH₂O). The molecular weight of the latter is 12+2*1+16=30 microgram/micromole. Consequently glucose production is 200*30/8 = 750 microgram/m²s or 2,7 gram/m²hr. This is the gross production. About 25% is used again to provide energy for respiration and maintenance processes in the plant. Thus the net dry matter production may be estimated as 0.75*2.7= 2 gram/m²hr. In conclusion we have the following rule of thumb:

$$100 \text{ micromole/m}^2\text{s} \rightarrow 1 \text{ gram/m}^2\text{hr}$$

Applications

Already in the 1990's a number of interesting applications have been developed on the basis of measured photosynthesis yield (Φ_P). Provided these measurements are carried out at a fixed light level, they can predict the *life of pot plants and cut flowers*. They may also be used to judge the *quality of green vegetables and fruit*.

The PPM may also be used for *selection and breeding* from seed and cuttings. In Canada the PPM has been applied extensively to select spruce seedlings on the basis of their *cold hardiness*. To this end the seedlings were subjected overnight to freezing temperatures and were thereafter measured. Seedlings showing a relatively high photosynthesis yield are selected for further breeding.

Another practical application of the instrument is for measuring *herbicide efficacy*. It is difficult to apply herbicides in the right, limited dose. If too much herbicide is used there is risk of pollution of ground water and damage to nature. Moreover there can also be substantial growth retardation of the crop. In the 1990's, Plant Research International in Wageningen, the Netherlands, has developed the *Minimum Lethal Herbicide Dose Method (MLHD)* on the basis of the EARS-PPM instrument. Through lab and field trials, tables have been developed that specify the minimum dose as a function of weed development (number of leaves). These dosages, however, are that low, that the farmer cannot see the effect of the treatment. Would he come back two weeks later, finding the weed still vital, then the economic damage is large. The solution to this problem (which exists with all low dosage techniques) is to measure the photosynthesis yield of the weed plants 2 days after spraying. If the photosynthesis yield is then equal to or lower than 20%, the farmer can be assured that the treatment has been sufficient. He may also use the instrument to measure and follow possible growth retardation of his crop as a result of the treatment. In the period 2005-2009, a consortium, lead by EARS, has introduced the MLHD technology successfully in China. In maize a reduction of herbicide use was

reached of 50-70%. At the same time the maize yield increased with almost 7%, implying a large economic benefit to the farmer.

An interesting application of the plant photosynthesis meter is *controlling light and water* in horticulture. In cooperation with DLV-Plant a system to control light screening on the basis of PPM measurements was developed, tested and demonstrated in roses, Schefflera and Spathyfillium. In this way plants could effectively be protected on the basis of their own well-being and without need for the grower to interfere.

The miniPPM

EARS has recently developed a new measuring approach in which excitation, saturation and conditioning light are united in a single source. This new instrument, the *miniPPM* has excellent measuring qualities. The miniPPM will replace previous instruments, but is much lower in price. In this way the measurement of photosynthesis and dry matter production comes within reach of a much wider target group, such as farmers, greenhouse growers, community parks departments. Also gardeners and nature lovers will find it an interesting tool to broaden their knowledge. The PPM is also very suitable for biology lessons at school.



Figure 6: The new miniPPM. This innovative instrument is small, economic and accurate. Press the button and the photosynthesis measurement proceeds within 1 second.

Further information

For more information on the application of fluorescence-photosynthesis measurements, please contact:

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