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# Potential of estimating plant photosynthetic status from high resolution spectral data

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**ABSTRACT**

Rosema, A., Havinga, H.N.J., and Jans, .W.W.P. (2000) *Potential of estimating plant photosynthetic status from high resolution spectral data.*

*In earlier work the relation between (laser induced) chlorophyll fluorescence and photosynthesis has been studied and clarified. Since active laser scanning instruments will not be available in the near future, the present focus is on photosynthesis related changes in the plant spectrum which could be detected by imaging spectrometers. Such changes were anticipated to be the result of changes in the fluorescence evoked by solar radiation (centred at 685 and 730 nm) or in changes of xanthophyll stress pigment content (at 530 nm). Measurements have been made "in vivo" on a Ficus Robusta leaf and on a Pinus Sylvestris tree (Scots pine) with three instruments: the Laser Evironmental Ative Fluorosensor (LEAF-NL), the EARS fluorescence Plant Photosynthesis Meter (EARS-PPM), and the GER 2600 field spectrometer. On the tree also sap flow measurements have been done, using the trunk heat balance method. The fluorescence index (FI) and the xanthophyll index (XI), derived from the GER spectral data, show indeed a similar behaviour as the emitted chlorophyll fluorescence measured with LEAF-NL or EARS-PPM on the transition from low to high solar radiation. However factor analysis of measured tree spectra does not resolve corresponding spectral bands at 685, 730 and 530 nm. Under high radiation the tree shows a broad band increase in reflection with a maximum in the red, which is most likely due to increased scattering as a result of dehydration. Super-imposed three absorption bands are found at 625, 675 and 750, which disappear later during the afternoon. They are probably related to the reversible formation of stress products under high light. By comparing the sap flow measurements and the gross photosynthesis estimates on the basis of the fluorescence measurements, the validity of the Photosystem Deactivation Model, developed earlier, is reconfirmed. The findings in this study show an interesting perspective of using high resolution spectral imaging instruments for the assessment of photosynthesis and $CO_2$ fixation in plant canopies. It is recommended to continue this research both by monitoring on the ground and experiments with space borne imaging spectrometers.*

## EXECUTIVE SUMMARY

In a previous project the relation between the laser induced chlorophyll fluorescence, as measured with the LEAF instrument, and plant photosynthesis has been clarified (Rosema *et al.* 1996, NRSP-2/96-12). Under solar radiation limitation in $CO_2$ uptake is notified by a decrease in chlorophyll fluorescence. Although our understanding of chlorophyll fluorescence in relation to photosynthesis is developed to a level that it could be practically used, the required active laser scanning systems are not available and are not likely to be available in the near future.

Chlorophyll fluorescence, evoked by solar radiation, is also present in the plant spectrum, although superimposed on reflected radiation. In the present study we investigate the possibilities to extract such or similar photosynthesis related information from imaging spectrometer systems that may be available on air- or space borne platforms in the near future. The objective of this study is to provide experimental evidence that photosynthesis and photosynthetic stress related information is present in the plant spectrum and to propose a future approach to extract such information from high resolution spectral information.

Two different measuring experiments have been carried out: (1) in a garden on a *Ficus Robusta* leaf and (2) in the forest on a *Pinus Sylvestris* (Scots pine) tree. Simultaneous measurements taken with (a) the LEAF-NL laser induced fluorometer, (b) the EARS-PPM fluorescence photosynthesis meter and (c) the GER 2600 field spectrometer have been analysed. The LEAF-NL instrument measures in 4 wavelength bands, of which two, centred at 685 and 730 nm, correspond with fluorescence emission bands. The PPM, a modulated fluorometer, measures the fluorescence yield beyond 700 nm, and, using a saturating light pulse, the corresponding quantum yield of photosynthesis. The GER spectrometer measures the reflection spectrum between 350 and 2500 nm, with a sampling interval of 1.5 nm below and 10 nm beyond 1050 nm. In the case of the pine tree, also sap flow measurements have been carried out using the trunk heat balance method.

A fluorescence index (FI) and a xanthophyll spectral index (XI) have been defined, which are expected to be mainly sensitive for changes in the chlorophyll fluorescence and the concentration of xanthophyll, a stress pigment. A sun-screen placed over the ficus plant was removed for several hours during the mid-day period thus exposing the plant to full sunlight and introducing the $CO_2$ limited photosynthesis state. During the period of direct solar radiation the LEAF and PPM fluorescence decline. The FI and XI derived from the GER spectrometer, show a similar decrease, suggesting that the two indices are indeed reflecting the same photosynthetic phenomena.

Measurements on *Pinus Sylvestris* in the forest near Kootwijk show a similar picture. The decrease in the XI, however, starts at 11.30, almost 1.5 hr later than the decrease in the fluorescence and FI.

Using the Photosystem Deactviation Model (PDM, Rosema et al. 1998) the course of gross photosynthesis has been determined from the fluorescence measurements and has been compared with the sap-flow measurements in the tree stem (figures 19 and 20). These sap flow measurements are known to reflect evapotranspiration and $CO_2$ uptake very well, although with some phase difference because of the storage capacity in the stem and branches. This has been done on two different days (clear and cloudy) with considerably different photosynthesis conditions. On both day the time patterns of sap-flow and fluorescence derived photosynthesis correspond very well, although the sap flow is about 1 hr behind in phase.

A close look at the reflectance spectra measured on Pinus Sylvestris on a warm and sunny day reveals the development of some high resolution spectral phenomena around noon. They disappear again during the afternoon, when the light level decreases. We have used factor analysis to try and discriminate component spectra in these measurements. Factor analysis assumes the measured spectral data to be a weighed combination of component spectra, where the weight of each component spectrum changes in time. This technique appeared to resolve two component spectra (figure 23). The dominant component corresponds to the normal plant reflection spectrum. The secondary component represents the changes that occur under high light conditions. This secondary spectrum shows a broad band increase of reflection with a maximum in the red (600-700 nm) and 3 narrow absorption bands at 625, 675 and 750 nm, respectively. The broad band increase of reflection is most likely due to an increase of scattering as a result of dehydration. The narrow absorption bands are probably due to stress pigments that are formed under high light. Particularly the 10 nm wide band at 625 nm is strong. The active period of the phenomenon is between 12 and 15 hrs. It starts after the fluorescence has decreased as a result of photosystem deactivation and has already reached its lowest values. We have not been able to identify the nature of the pigments that cause these absorption bands. But they seam not related to changes in fluorescent emission or in xanthophyll content.

These findings offer an interesting perspective for the application of air or space borne imaging spectrometers for mapping photosynthesis and $CO_2$ fixation in plant canopies. The requirements for space borne instruments would be a sun-synchronous orbit at or shortly after noon, and a spectral resolution of at least 10 nm. The approach could be tested over large vegetated areas such as the Veluwe forest and Flevoland. There is a need to extend our knowledge of and experience with the observed phenomena. It is therefore recommended to continue research in this area in the framework of the user support program (GO-2) by carrying out longer monitoring experiments on trees and also on crops. It is also recommended to study these phenomena in the spatial domain by means of air- and space borne imaging experiments with high spectral resolution imaging equipment such as ENVISAT-MERIS and PROBA-CHRIS.

# 1 INTRODUCTION

## 1.1 General introduction

The BCRS project LEAF-95 has resulted in a strongly improved insight into the relation between laser induced fluorescence and photosynthesis. The results of this project have been published as BCRS report NRSP-2/96-12 (Rosema et all 1996) and as publications in Remote Sensing of Environment (Rosema and Zahn 1997, Rosema et al. 1998). During the LEAF-95 project measurements have been done with the Laser Environmental Active Fluorosensor (LEAF) on young Poplar trees, which were enclosed in a gas exchange room with glass walls. A new photosystem energy partitioning model has been developed that allows to estimate gross photosynthesis on the basis of measurements of chlorophyll fluorescence (F) and photosynthetic active radiation (PAR) with the LEAF instrument. There appeared to be a good correspondence between the output of this model and CO2 assimilation measurements carried out simultanously.

It was found that under photosynthetic stress, i.e. high PAR levels and/or limited CO2 uptake, plants regulate photosynthesis by de-activation of photosystems and this, quite unexpectedly, may lead to a strong decline of the chlorophyll fluorescence. During the afternoon values as low as 10% of the normal fluorescence have been observed and this was related to complete closure of plant stomata and a corresponding blocking of CO2 uptake.

The various LEAF projects carried out within the BCRS framework have brought our understanding of the relation between plant fluorescence and photosynthesis to a level, where it seems justified to orient ourselves gradually towards possible operational application of this know-how. Active laser scanning systems are, for practical and economic reasons, not expected to be readily available. For this reason the possibility of passive fluorescence detection with the CAESAR scanner has been explored earlier and was reported in BCRS report NRSP-2, 94-19 (Rosema, Roebeling and Van Persie 1994b). Such an approach was theoretically founded by means of canopy fluorescence simulation modeling (Rosema and Werner 1983, Rosema and Verhoef 1991, Rosema et al. 1991).

During the project CAESAR Experiment Forest Mode, only negative fluorescence index anomalies were found in the CAESAR data. Such an anomaly extended over several forest plots with different tree species, and after field inspection appeared to be associated with an elevation of the terrain. At that time a negative fluorescence anomaly was not expected nor understood. It was still assumed that stress would cause an increase of the chlorophyll fluorescence. However, after the LEAF-1995 project we know now that the opposite is true and the observations with the CAESAR scanner can now be tentatively explained: the negative fluorescence index anomaly most likely was an expression of drought stress due to the elevation in the terrain and related lower groundwater table. The present consistency of results from the two projects suggests a further continuation on this R&D path.

Another argument to follow this path is ENVISAT, which will be launched by ESA in a few years. A most interesting instrument on board of this satellite is MERIS, a programmable medium spatial resolution imaging spectrometer with a spatial resolution of 250 meter. The instrument would allow the passive fluorescence scanning methodology to be applied on national and regional scales. This, among others, could lead to a considerable improvement of our knowledge of actual CO2 exchange, in support of climate modeling and environmental studies.

Besides the fluorescence index approach both CAESAR and MERIS allow for the observation of another "hyper-spectral" phenomenon, which is related to the changes in the xanthophyll cycle pigments. Earlier laboratory experiments have shown that these pigments play a role in the regulation of the photosynthetic light reaction under stress conditions and that changes in these pigments cause a shift in the green reflection maximum around 530 nm (Demmig and Adams 1990, Thayer and Bjorkman 1990, Gamon et al. 1990, 1992, Penuelas 1995, Filella et al. 1995).

The suggested R&D traject, with the LEAF and CAESAR instruments as relatively operational research tools, will support the development of MERIS applications. It implies, however, that further applied research is necessary to develop from here to more operational CAESAR and MERIS "hyper-spectral" methods. The present short project serves to provide experimental evidence in support of the proposed R&D strategy.

The objectives of the LEICA project are:

1) to provide experimental evidence that changes in the laser induced chlorophyll fluorescence are indeed accompanied by changes in the spectral reflection at 650 and 685 nm (passive fluorescence index) and in the spectral reflection around 530 nm (xanthophyll cycle pigments).

2) to propose a possible future approach to passive, narrow band multi-spectral imaging of plant photosynthetic state, in particular the occurrence of stress due to drought conditions.

## 1.2 Theoretical introduction

In the photosynthetic process carbon dioxide ($CO_2$) and water ($H_2O$) are converted into glucose ($CH_2O$) and oxygen ($O_2$). The energy provided for this reaction is light. The level of photosynthesis is often estimated by measuring $CO_2$-uptake of the plant. This approach, however, requires complicated instrumentation and is expensive and time consuming. Another approach is to measure the amount of light used to drive

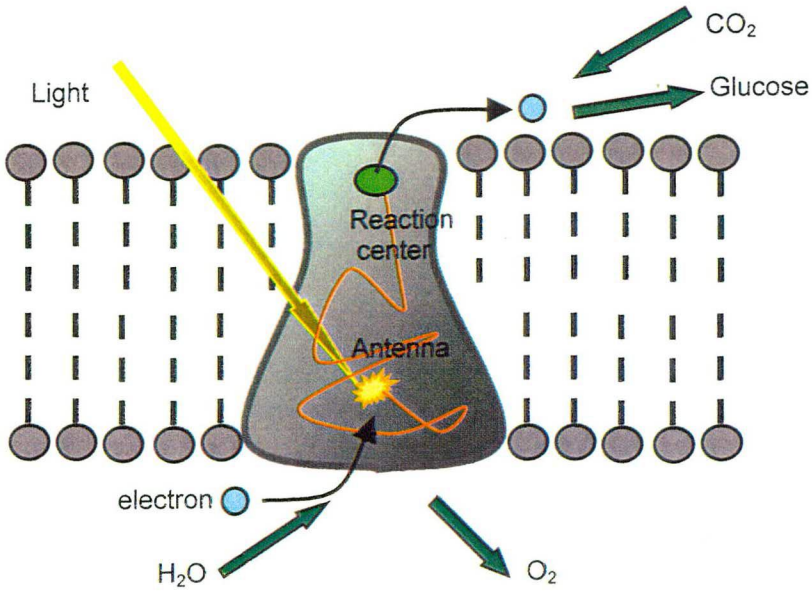


*Figure 1: Photosystem embedded in the thylakoid membrane with schematic representation of the light reaction of photosynthesis*

the photosynthetic reaction. This approach has been implemented in some specialised chlorophyll fluorometers, such as the WALZ-PAM and EARS-PPM.
The light reaction of photosynthesis takes place at the thylakoid membrane inside the leaf chloroplasts (figure 1). This membrane consists of fats and proteins, in which clusters of some hundreds of chlorophyll molecules are embodied. These so-called photosystems consist of a reaction centre and an antenna. When the leaf is exposed to light, the chlorophyll antennae absorb photons. On absorption of a photon an electron is excited. This "exciton" roams through the antenna. Its lifetime however, is short, in the order of $10^{-9}$ s. The exciton can loose its excess energy in three ways (figure 2):

1) the exciton is assimilated by the "reaction centre" (photosynthesis)
2) the exciton looses its extra energy while developing heat.
3) the exciton returns to a lower orbit, while emitting a photon (fluorescence)

In the dark the probability of these de-excitation pathways is roughly 82, 17.5 and 0.5%, respectively, for all plant species in good condition. Once the reaction centrum has been reduced by assimilating an electron, it remains closed for a relatively long time ($10^{-4}$ s) before opening again. During the period that the reaction centre is closed, all excitons in the antenna will loose their energy as heat or fluorescence and as a result heat dissipation and fluorescence increases more than fivefold. Genty et al. (1989) have proposed to calculate the probability of the photosynthesis pathway, or photosynthetic quantum yield ($\phi_P$), from the fluorescence as follows

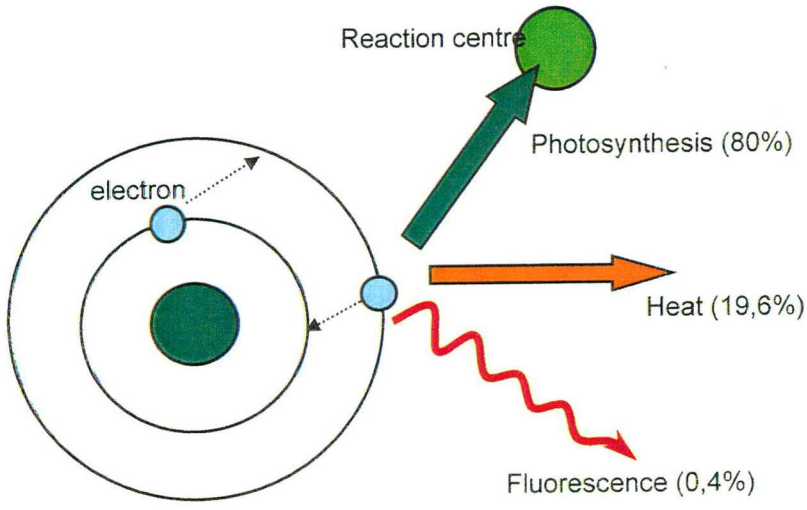


*Figure 2: Excitation and de-excitation energy distribution (in the dark)*

$$\phi_P = 1 - F / F_M \tag{1}$$

Where F is the fluorescent radiance, in short fluorescence, under the ambient light, and $F_M$ is the maximum fluorescence, which occurs after closing all reaction centres by means of a saturating light source. The fluorescent radiance is in principle expressed in $W/m^2.sr$. However all measurements of fluorescence will be uncalibrated and only relative levels will be used (like in equation 1) and therefore the dimensions are not important. Gross photosynthesis (P) is estimated with

$$P = \phi_P * PAR \tag{2}$$

Here PAR is the photosynthetic active irradiance (in micromole-photons/$m^2$). Since $\phi_P$ is dimensionless, P is expressed in the same units. P may be converted to micromole $CO_2$ or glucose if the conversion rates in plants are known. This will not be discussed discussed here.

Rosema et al. (1998) showed that the previous equation does not give good predictions of photosynthesis under conditions of high light, i.e. $CO_2$ limitation. In that situation photosystems are deactivated and both F and $F_M$ decrease, without an appreciable effect on the quantum yield $\phi_P$. It was shown that in that case the gross photosynthesis may be calculated with

$$P = \zeta * \phi_P * PAR \tag{3}$$

Where $\zeta$ is the photosystem deactivation factor. In that paper a method was presented to derive the course of photosynthesis from laser induced fluorescence measurements, in particular the LEAF-NL laser fluorometer.

The phenomenon of photosystem deactivation is very clearly present in fluorescence measurements of vegetation under high radiation (schematically shown in figure 3). It is a most interesting phenomenon because it marks the transition from light limited to $CO_2$ limited photosynthesis and therefore is a clear indicator of photosynthetic stress. It may give such information, while no visual signs are present.

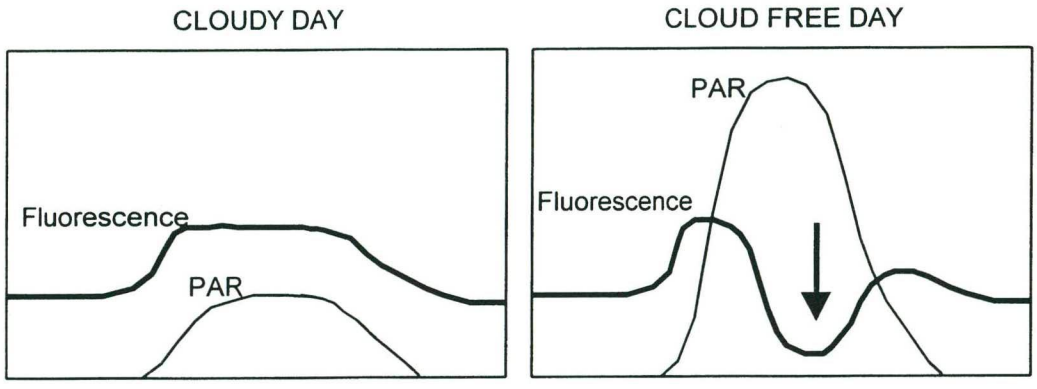


*Figure 3: Schematic course of phototsynthetic active radiation (PAR) and chlorophyll fluorescence on a cloudy and a cloud free day. The mid-day decrease in the fluorescence on the cloud free day marks the transition from the light-limited to the $CO_2$ limited photosynthesis phase. The decrease of the fluorescence is due to photosystem deactivation and is usually associated with stomatal closure.*

For this reason the chlorophyll fluorescence is a very interesting signal for application in remote sensing. Photosynthesis is key-information in many sciences and application fields, for example modelling of the global carbon cycle. Such information is also relevant to forest authorities as it may point to problems with tree growth due to drought and possibly air pollution, in particular ozone.

In remote sensing it has often been assumed that the part of photosynthetic active radiation that is absorbed by the vegetation ("APAR") is a measure of photosynthesis. This however is not true. Photosynthesis depends on the balance between radiation and $CO_2$ assimilation and the latter will depend on water availability, species, etc. The decrease in chlorophyll fluorescence is a clear indicator of $CO_2$ limitation of photosynthesis and the earlier developed Photosynthesis Deactivation Model (PDM, Rosema et al. 1998) in fact allows the calculation of gross photosynthesis from the daily course of fluorescence and PAR. This signal can be measured from a distance with laser induced fluorescence systems such as the Laser Environmental Active Fluorosensor (LEAF) system of BCRS.

A problem, however, is that active imaging techniques, using laser induced fluorescence technology, are not likely to be available in the Netherlands, as well as many other countries, in the foreseeable future. For this reason we want to

investigate the possible use of passive imaging techniques to obtain similar information.

It is clear that chlorophyll fluorescence is also evoked by solar radiation and is therefore present, mixed with reflected radiation, in the radiance of canopies under solar insolation. We therefore want to address the question if chlorophyll fluorescence information can be extracted from the vegetation reflection spectrum. The possibility of such passive fluorescence detection has been explored earlier by means of fluorescence and reflection modelling of leafs and canopies [Rosema and Werner 1983, Rosema *et al. 1991*]. Such chlorophyll fluorescence effects in the reflection spectrum would be centred around the wavelengths where chlorophyll fluorescence peaks, i.e 685 and 730 nm.

The vegetation reflection spectrum offers also another possibility to extract information on photosynthesis. Laboratory experiments have shown that xanthophyll pigments play a role in the regulation of the photosynthetic light reaction under high light and that changes in these pigments cause a shift in the green reflection maximum around 530 nm [Brugnoli and Björkman 1992, Demmig and Adams 1990, Filella *et al* 1995, Gamon *et al.* 1990, Penuelas 1995, Taylor and Bjorkman 1990]. It was also shown that these pigment changes are indirectly related to chlorophyll fluorescence changes.

## 1.3 Project set up

Two different measuring experiments have been carried out. The first experiment was done in a garden and concerned an individual leaf of *Ficus Robusta*. The second experiment was done on a *Pinus Sylvestris* tree, at a test site in the Veluwe forest near Kootwijk. Use was made of the following instruments:

- LEAF laser induced fluorometer (BCRS, Delft, NL)
- PPM fluorescence photosynthesis meter (EARS, Delft, NL)
- GER 2600 field spectrometer (GER, Millbrook, NY).
- Inframetrics 740 thermal scanner

At this test site also stem sap flow measurements have been carried out. These reflect evapostranspiration, stomatal closure and consequently $CO_2$ assimilation. The thermal scanner did not give useful results and these data will not be involved in the data analysis.

*Figure 4: Laser Environmental Active Fluorosensor (LEAF)*

# 2 MATERIALS AND METHODS

## 2.1 Laser Environmental Active Fluorosensor (LEAF-NL)

The Laser Environmental Active Fluorosensor (LEAF, see figure 3) is an instrument, developed for the BCRS by Nedinsco (Venlo), for remote sensing of the laser induced chlorophyll fluorescence of vegetation. For excitation a doubled Nd-Yag laser is used, with a 20 mJ pulse of 10 ns length at 532 nm wavelength. The radiance from the vegetation is collected by a telescope, split and deviated to four photomultipliers. The wavelength bands are selected by means of interference filters placed in front of the photomultiplier tubes. In the present set-up the following four bands are measured.

| Band nr | Short reference | Wavelength range |
|---|---|---|
| 1 | 650 nm | 640-660 nm |
| 2 | 685 nm | 675-695 nm |
| 3 | 730 nm | 720-740 nm |
| 4 | 785 nm | 770-800 nm |

The laser beam divergence is adjustable from 5-50 mrad. The detection field of view is automatically matched to the laser beam divergence. The radiance of the observed scene is measured once with the laser pulse (active measurement) and once without (passive measurement). In both cases different time windows are used to improve the signal to noise ratio. Grey filters in the optical path are used to adjust the light levels to the dynamic range of the instrument. The fluorescence evoked by the laser pulse is

obtained by subtracting the passive from the active measurement after correction for the length of the time window. Finally the fluorescence signal is normalised for the laser pulse intensity and for the transmission characteristics of the optical system, the grey filter in particular.

The LEAF instrument is completely controled by a lab-top microcomputer. Menu-driven software has been developed, which enables a fast and user friendly operation. The measured data are stored on floppy disk for off-line analysis.

At the measuring site in the forest neer Kootwijk, the LEAF instrument was placed on the ground in a "portacabine", just in front of an open window, in order to protect it from rain and possible low temperatures at night, which could cause condensation.

Annex 1, section 3 provides a discussion of ratio between the measuring time windows, which appeared to be different from the values provided by NEDINSCO.

## 2.2 Plant Photosynthesis Meter (EARS-PPM)

The EARS-Plant Photosynthesis Meter is a hand-held chlorophyll fluorometer. It uses a 7200 Hz modulated LED at 637 nm for excitation, with a 690 nm infrared cut-off filter in front. Chlorophyll fluorescence, after passing through a 700 nm infrared transmitting filter, is measured synchronically by means of a PIN-Si-photodiode, preamplifier, automatic gain control, synchronic detector and low-pass electronic filter. A 6V, 15W Halogen lamp, with near infrared blocking filter in front, is used to generate a saturating light pulse of about 5000 micromol-photons/$m^2$s (two to three times the level of full sunlight), which will close all photosystem reaction centres. The duration of this light pulse is automatically determined from the course of the chlorophyll fluorescence signal and is usually between 0.5 and 1.0 s. Two fluorescence measurements are carried out: one under ambient light (F) and one after closing all reaction centres with the saturating pulse ($F_M$). From these two measurements the photosynthetic quantum yield ($\phi_P$) is determined according to equation (1). In addition the PPM measures the photosynthetic active radiation (PAR in micromol-photons/$m^2$s) incident on the plant leaf by means of a blue enhanced Si photodiode with a 400-690 nm transmitting filter, which is measuring the light scattered from a white diffuser next to the leaf.

## 2.3 GER spectroradiometer

Reflection spectra were monitored using a GER field spectro-radiometer with data acquisition from 350 to 2500 nm. The GER 2600 uses a fixed grating array-based design eliminating the need for moving parts with the exception of the shutter and chopper. The target to be scanned was accurately positioned with laser sighting. When the target's radiation enters the GER 2600 it is split into two beams. One beam is directed towards a 512-element silicon detector array in the wavelength range of

300 nm to 1050 nm with a sampling interval of 1.5 nm. The other beam utilises a 128PbS-detector array with a wavelength range from 1050 to 2500 nm with a sampling interval of 10 nm. The field of view of the spectrometer is $10^{o}$.

## 2.4 Sap flow measurements

The sap flow in the tree was measured using the trunk heat balance method with internal heating of tissues (Cermak et al. 1973, Kucera et al. 1977, Smith and Allen, 1996). A certain part of the tree was heated with constant power using five stainless steel electrodes inserted in the trunk and reaching the depth of the sapwood. Temperature was measured using eight compensating thermocouples (Cermak and Kucera, 1981). The measurement is done both on the east and the west side of the stem and then averaged. According to this method the measurement (Q) is expressed in kg sap per hour per unit of sap wood circumference ($kg.cm^{-1}.h^{-1}$). The tree sap flow rate S (kg/hr) is then obtained by multiplication with the sap wood circumference.

$$S = Q*(C - 2\pi*b) \qquad (4)$$

where $C$ is the tree circumference and $b$ the bark thickness.

The sap flow rate is, of course, closely related to the transpiration of the tree and the total evapotranspiration of the forest. However, because of the sap storage capacity inside the stem, the sap flow is usually delayed. It is known from sap flow and evapotranspiration flux measurements at the adjacent EUROFLUX measuring site "Loobos", that the sap flow is usually 1 to 2 hours behind in phase. The transpiration of the trees is regulated through stomatal closure, which has a simultaneous effect on $CO_2$ assimilation and consequently on the photosynthetic light reaction. We should therefore expect a similar delay of the sap flow relative to the cycle of gross photosynthesis.

# 3 DESCRIPTION OF EXPERIMENTS

## 3.1 Measurements on a Ficus Robusta leaf

The pot plant was placed outdoors and was screened by a sun umbrella. Only a few hours a day it was exposed to solar radiation for fluorescence measurements. The GER spectrometer was placed 0.7 m from the plant. Reflection spectra were measured on a spot ∅ 12 cm within the edges of a leaf (leaf 1). The measurements with the LEAF instrument were done on the same leaf, from a distance of 6 m with a 15 mrad field of view. The laser spot was ∅ 10 cm in diameter and the energy density about 1700 mJ/m$^2$. This energy density is sufficiently low to avoid notable disturbing effects of exciton annihilation and/or laser induced reaction centre closure (Rosema and Zahn 1997). The fluorescence of the leaf (F) was derived as the difference between the active and the passive measurement after correction for the time windows, and was normalised for the laser pulse intentsity. Only the fluorescence at 685 nm ($F^{685}$) and 730 nm ($F^{730}$) and the passive radiance at 730 nm ($R^{730}$) have been used in the analysis. In single shot mode, the signals measured with the LEAF instrument showed some oscillations. These were removed by measuring every two minutes and taking the average of six succeeding measurements.For further analysis these averages were used.

The EARS-PPM measurements were simultaneously carried out on a second leaf (leaf 2) which had a slightly different azimuth angle.

## 3.2 Measurements on Pinus Sylvestris tree

The tree on which the measurements were carried out is growing in a forest area of approximately 50 km$^2$. The site, called "Loobos" is located at the Veluwe (52°10'N, 5°44'O) in the centre of the Netherlands. The climate is moderately humid, the mean

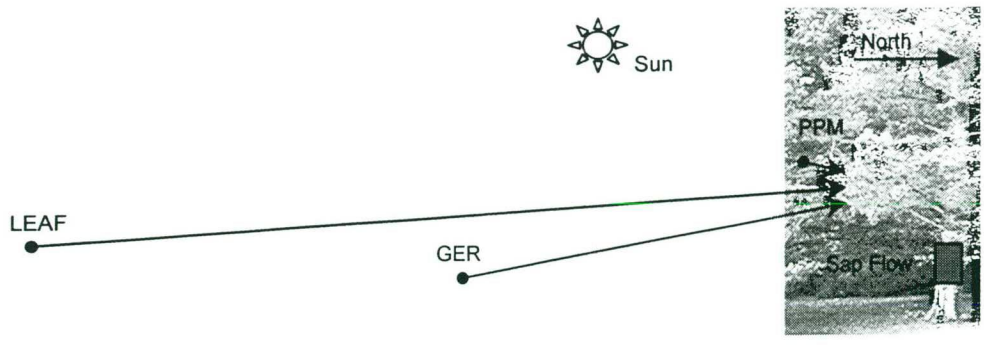


*Figure 5: Measuring set-up at the Loobos site*

air temperature in August is 16.2 °C, and the mean precipitation in July was 63 mm (measured at "Deelen"). The tree is planted on drift sand accumulations. The soil is a Dutch humus podsol covered with a litter layer of approximately 10 cm. Tree age is about 35 years. The tree height was approximately 8 m., the diameter at breast height (dbh) was 27.5 cm.

The GER spectrometer was placed 5 meters from the tree. Reflection spectra were measured on a crown spot of ∅ 0.9 m. This spot was oriented southward and was illuminated by the sun during a large part of the day. The zenith angle of the measurement was approximately 120°.

The laser induced fluorescence, as well as the passive radiance of the tree crown, were measured with the LEAF instrument from a distance of 13 m. The field of view was 25 mrad. The laser spot on the canopy was ∅ 35 cm and the energy density about 140 mJ/m$^2$. The zenith angle was approximately 100°.

The EARS-PPM fluorescence photosynthesis meter was installed to measure the fluorescence (F), the photosynthetic quantum yield $\phi_p$ and the photosynthetic active radiation (PAR) of a single twig, just besides the branch on which measurements of the LEAF and spectrometer coincided (see photograph).

Sap flow measurements were taken every minute and stored within data loggers. Averages for 15-minute intervals were determined. Two measuring points were installed on the opposite sites of the stem (east and west) slightly above breast height so as to compensate for internal variation of the sap flow.

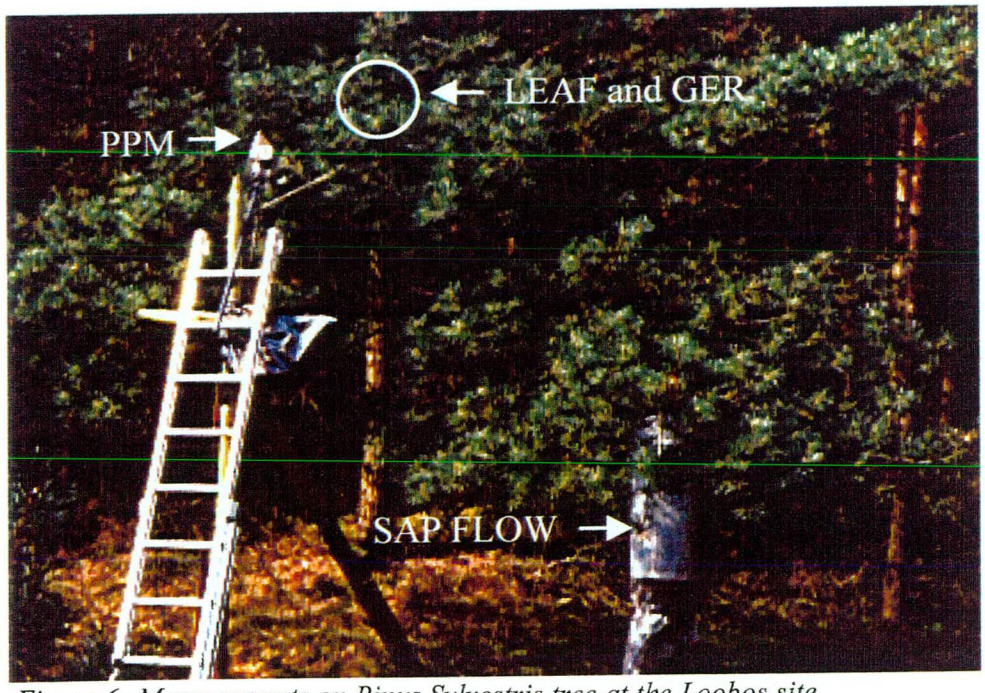


*Figure 6: Measurements on Pinus Sylvestris tree at the Loobos site.*

# 4 MEASURING RESULTS

## 4.1 Ficus Robusta leaf

Measurements were carried out in the period between 20 July and 5 August 1998. During this period, the weather conditions varied. Often the sky was cloudy. Only two sunny days were suitable for analysis. During these two days, 22 July and 3 August, the *Ficus Robusta* was sheltered by an umbrella and only exposed to solar radiation during a few hours around noon. The azimuth angle of the two leaves on which the measurements were done (leaf 1: LEAF and GER, leaf 2: EARS-PPM) was slightly different and for this reason the measuring results may show some difference in phase. When the *Ficus Robusta* was shaded by the sunscreen, the intensity of the incident radiation was low. Consequently the spectral radiance data measured with the GER spectrometer are low and have a relatively high noise content. The reflection indices derived from these measurements in the shadow show relatively large inaccuracies.

## 4.2 Pinus Sylvestris tree

LEAF, PPM and GER measurements were carried out in the period between 10 and 14 august 1998. Unfortunately, only the 11th and part of the 12th of August were sunny days. On the other days big cloud formations decorated the sky. The 14th of August, the sky was totally clouded. The sunny day, 11 August, is used for further analysis. The objective of the PPM measurements is to provide reference data to compare the active and passive signals from the LEAF instrument with. The branch on which the PPM measured and the spot on the tree crown where the LEAF and GER instrument measured, were not illuminated in the same way all day. Therefore some differences between the signals measured on branch and on canopy level are to be expected.

# 5 ANALYSIS OF THE MEASURING RESULTS

## 5.1 Spectral indices

Spectral indices serve to normalise the spectral radiance, measured with the GER spectro-radiometer, at the wavelength of interest. This is done to compensate for changes in light level and for broad band changes in the spectral radiance caused by other factors, for example light colour. Normalisation is done by means of a reference measurement at a wavelength close to the wavelength of interest but just outside the specific spectral band of the phenomenon studied. The fluorescence index (FI) is derived from the spectral radiance ($R^{\lambda}$) as follows:

$$FI = (R^{685} - R^{640}) / R^{640} \qquad (5)$$

685 nm is a peak wavelength of fluorescence. The 640 nm band is situated symmetrically on the other site of the chlorophyll absorbtion maximum around 660 nm. There is no notable fluorescence at this wavelength while light absorbtion and scattering are expected to be similar. Thus a decrease in the fluorescence index would indicate a decrease of the chlorophyll fluorescence.

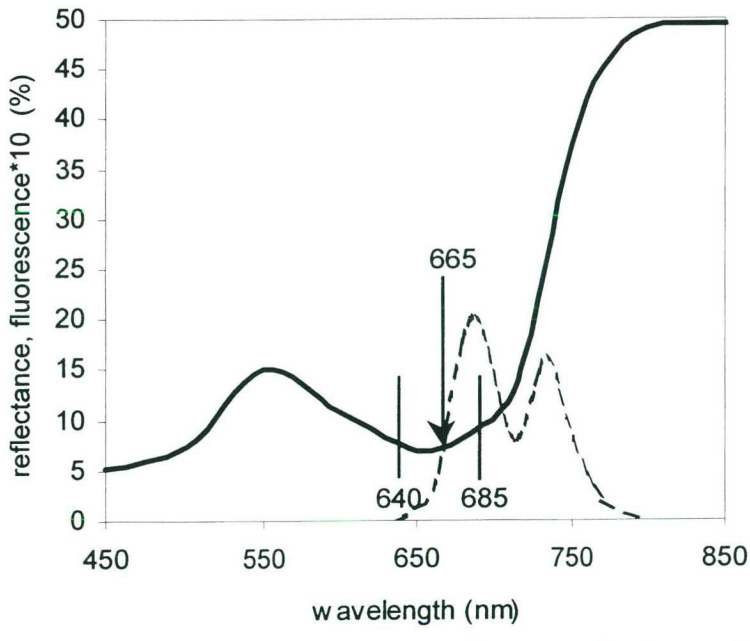


*Figure 7: Schematic representation of a tree reflection and fluorescence spectrum, the latter 10 times exaggerated. The graph also shows the position of the chlorophyll absorption maximum (at 665 nm) and the two wavelengths from which the fluorescence index (FI, eq. 5) is derived*

Changes in the xanthophyll pigments are known to cause changes in the reflection spectrum around 531 nm [Gamon *et al.* 1990]. The xanthophyll index (XI) will be calculated as follows:

$$XI = (R^{531}-R^{490}) / R^{490} \qquad (6)$$

The 490 nm reference wavelength is supposed to be outside the influence of the xanthophyll pigment changes. When both indices, FI and XI, change simultaneously, the phenomena causing these changes are supposed to be directly or indirectly related.

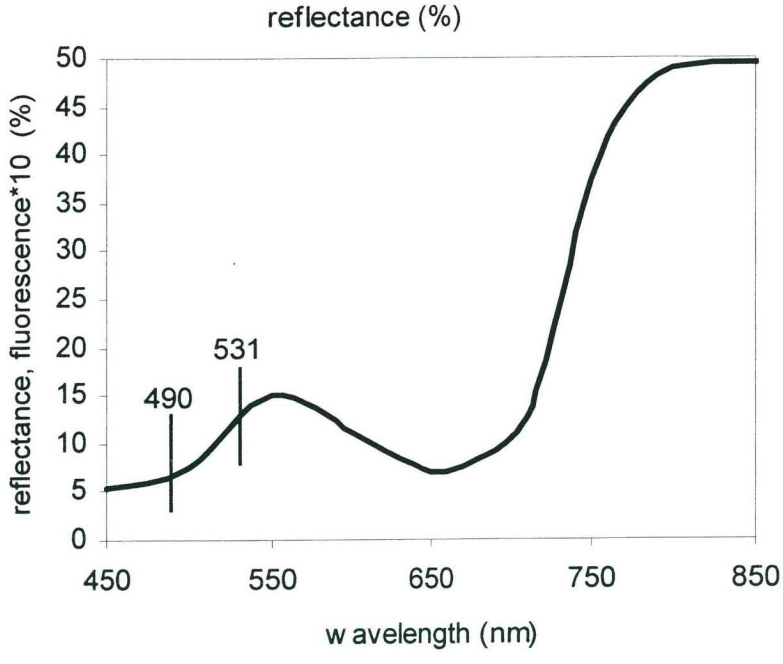


*Figure 8: Schematic representation of a tree reflection spectrum. The graph shows the position of the wavelengths used in deriving the xanthophyll index (XI, eq. 6)*

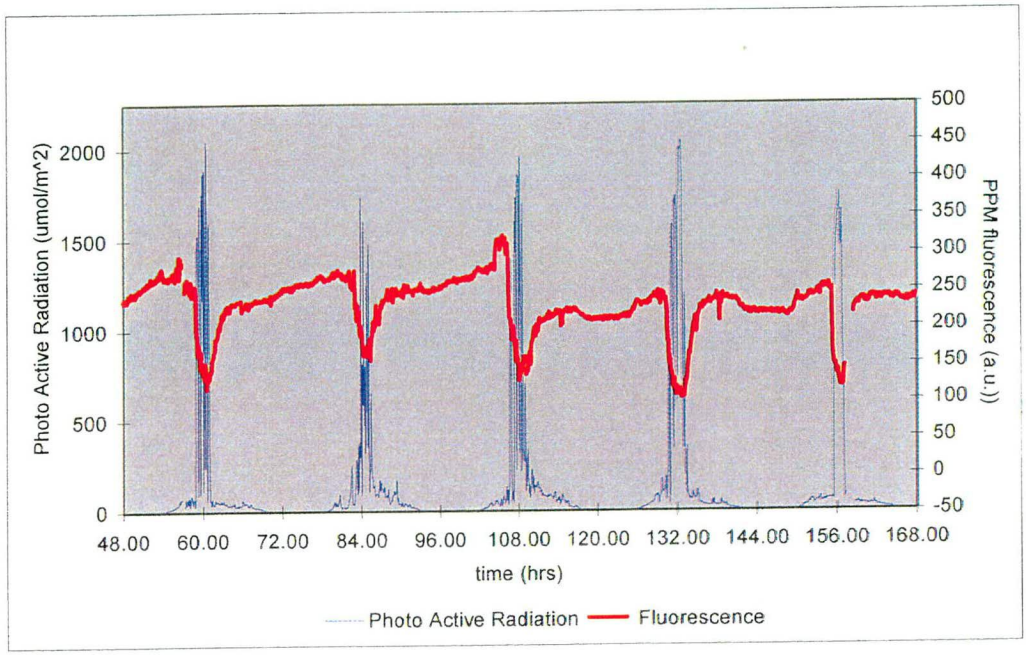


*Figure 9: Daily cycles of EARS-PPM measurements of chlorophyll fluorescence and photosynthetically active radiation on a Ficus Robusta leaf, Delft 22-26 July 1998.*

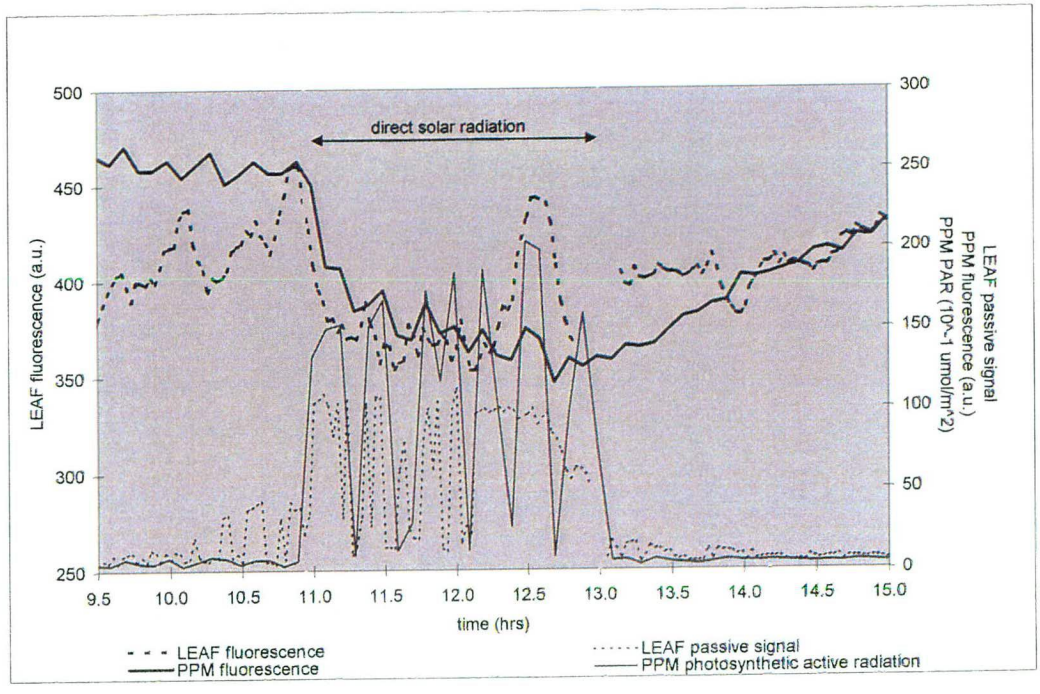


*Figure 10: PPM and LEAF measurements of fluorescence and light level on a Ficus Robusta leaf, Delft, 22 July 1998. The leaves were screened by a sun umbrella except for the period indicated as "direct solar radiation"*

## 5.2 The daily cycle

### *5.2.1 Ficus Robusta leaf -*

The daily cycle of fluorescence measured by the EARS-PPM chlorophyll fluorometer (figure 9) shows the expected course, which is known from earlier experiments (Rosema et al. 1998). Fluorescence increases during the night, which indicates the restoration of the plant from the previous day. After daybreak the fluorescence first tends to increase further. When the sunscreen is removed, indicated by the high PAR values, the fluorescence almost immediately declines and reaches values as low as 50% of the value in the shade. When the light level decreases again, particularly after replacing the sunscreen, the fluorescence increases during the following hours and gradually reaches its normal dark level during the night.

The reversible decline of the fluorescence signal during the period of high radiation is attributed to photosystem deactivation (Rosema *et al,* 1998). Photosystem deactivation is assumed to be a change in the photosystem by which it looses its ability to emit fluorescence and to transport electrons. This electron transport regulation mechanism indicates that the photosynthesis is $CO_2$ limited. The photosynthetic light reaction tends to run faster than $CO_2$ can be assimilated. It is a situation of photosynthetic stress, characterised by stomatal closure and increased heat development.

Figure 10 shows the course of both the PPM fluorescence and the LEAF laser induced fluorescence on 22 July. Also the passive signals, indicating the light level are shown. Both the PPM and LEAF measurements show a similar decrease in the fluorescence when the *Ficus Robusta* was exposed to the sun. Figure 11 shows a similar parallel behaviour on 3 August. But there are also some differences. For example on 22 July LEAF shows an increase in fluorescence before the sunscreen is taken away, although the PPM fluorescence shows a constant or slightly decreasing course. This can be explained by the difference in position and orientation of the two leaves the PPM and LEAF measured on. This is clear from the fact that the LEAF passive signal, indicating the light level, is increasing, while the PPM light level remains constant. The fluorescence measured with LEAF shows a temporary rise and subsequent decline at the end of the period of solar exposure, which we cannot explain. Just hereafter, the instrument had a drop out. This drop out has occurred on more occasions, always at exactly the same time of the day. It is therefore supposed to be a bug in the LEAF steering software.

On 3 August, the course of the LEAF and PPM fluorescence is again similar. Minor differences occur at the beginning and the end of the unshaded period which are again related to small differences in radiation. After shading the *Ficus Robusta* again, both PPM and LEAF fluorescence recover to a level near the morning shade level. Differences during this course may also be due to the somewhat different course of the light levels during that period. Just before 13 hrs again a drop out of the LEAF

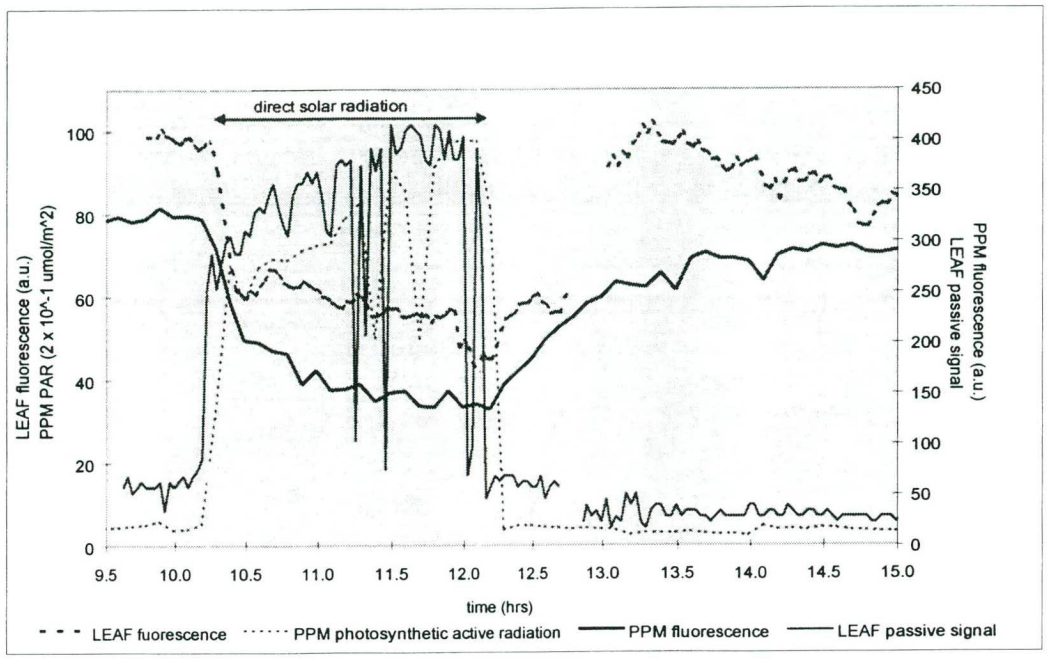


*Figure 11: PPM and LEAF measurements of fluorescence and light level on a Ficus Robusta leaf, Delft, 3 August 1998. The leaves were screened by a sun umbrella except for the period indicated as "direct solar radiation"*

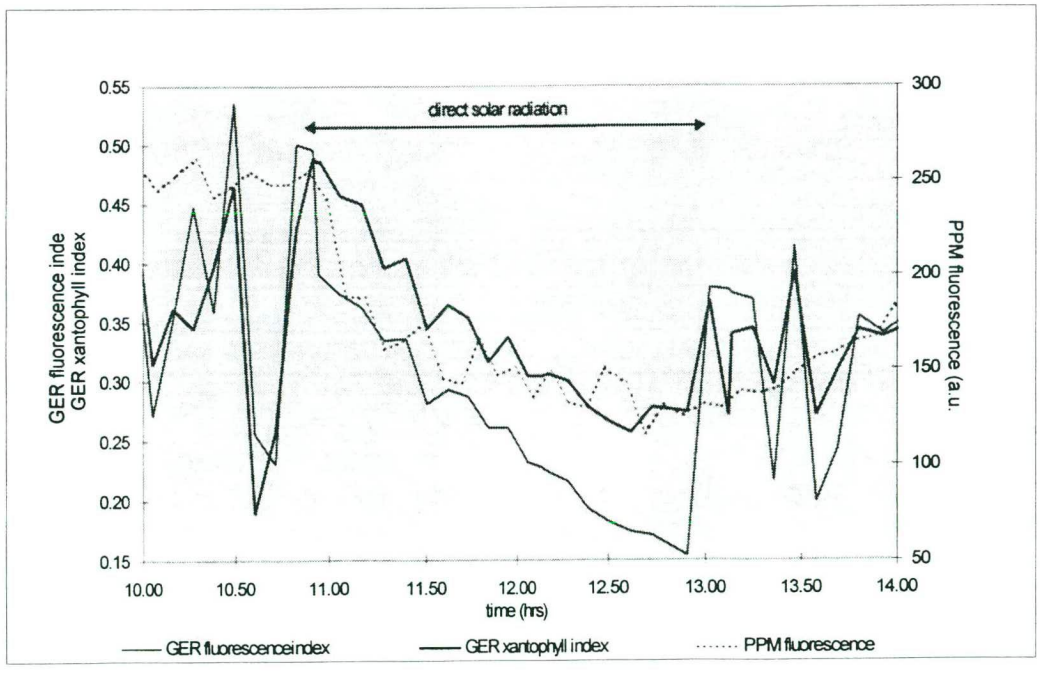


*Figure 12: Fluorescence index and xanthophyll index calculated from GER spectral radiance compared with the PPM fluorescence as measured on Ficus Robusta, Delft, 22 July 1998.*

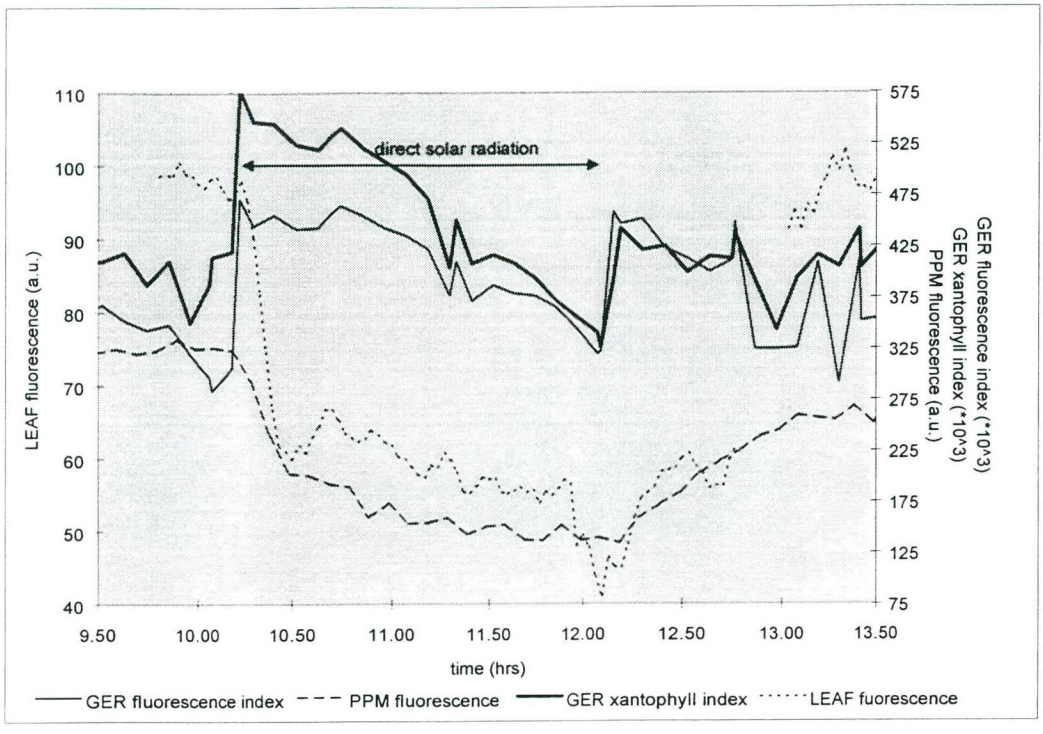


*Figure 13: Fluorescence and xanthophyll index calculated from GER spectral radiance compared with LEAF and PPM active fluorescence measurements, Delft, 3 August 1998.*

instrument occurred, which was due to earlier mentioned software bug.

The fluorescence index (FI) and xantophyll index (XI) have been calculated from the multi-spectral reflection spectra measured with the GER field spectrometer and are plotted together with PPM and LEAF fluorescence in figure 12 and 13. The fluorescence and xantophyll indices seem to follow the course of the active fluorescence measurements quite well during the period of direct solar radiation. During the shade periods the indices fluctuate broadly, which is attributed to noise in the radiance measurements.

### *5.2.2 Pinus Sylvestris tree*

On 11 August, a clear day, the LEAF and PPM fluorescence measurements show a different course (figure 14). The PPM fluorescence shows a decrease in the fluorescence during the period of high radiation (PAR), as expected, while the LEAF fluorescence shows little variation.

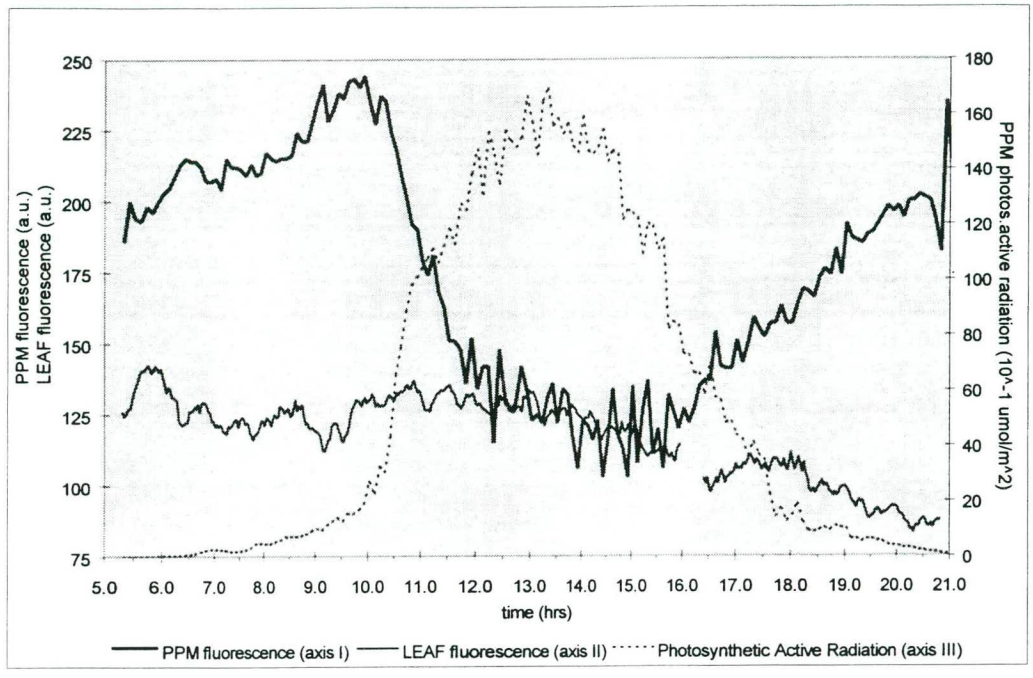


*Figure 14: Measurements of LEAF fluorescence and PPM fluorescence and PAR on Pinus Sylvestris, Kootwijk, 11 August 1998.*

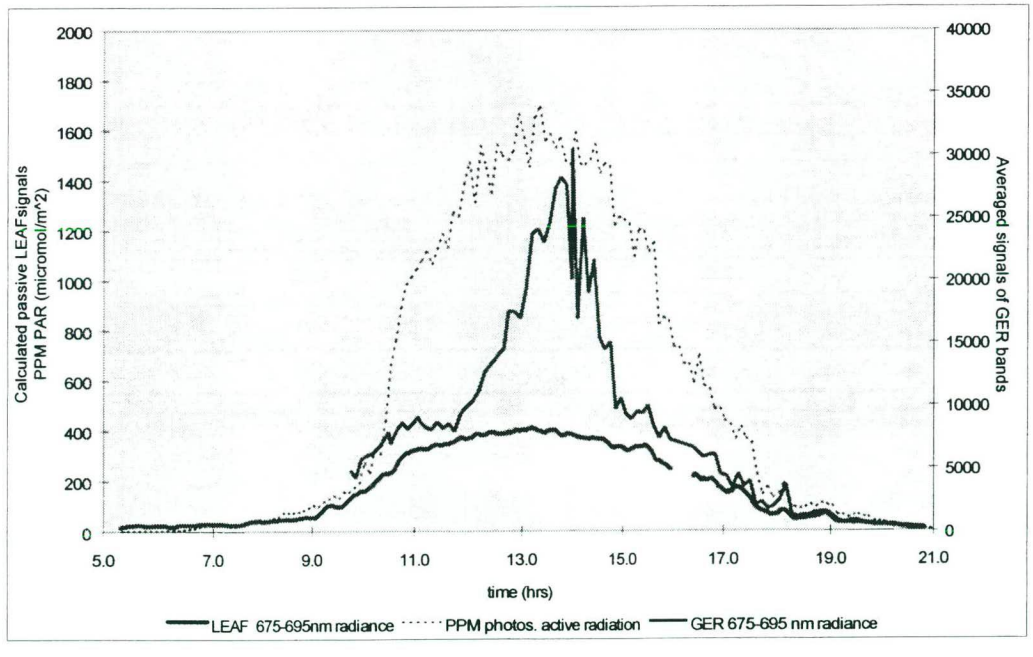


*Figure 15: Passive signals from LEAF and GER instruments in two corresponding wavelength bands and the PPM photosynthetic active radiation, measured on Pinus Sylvestris, Kootwijk, 11 August 1998*

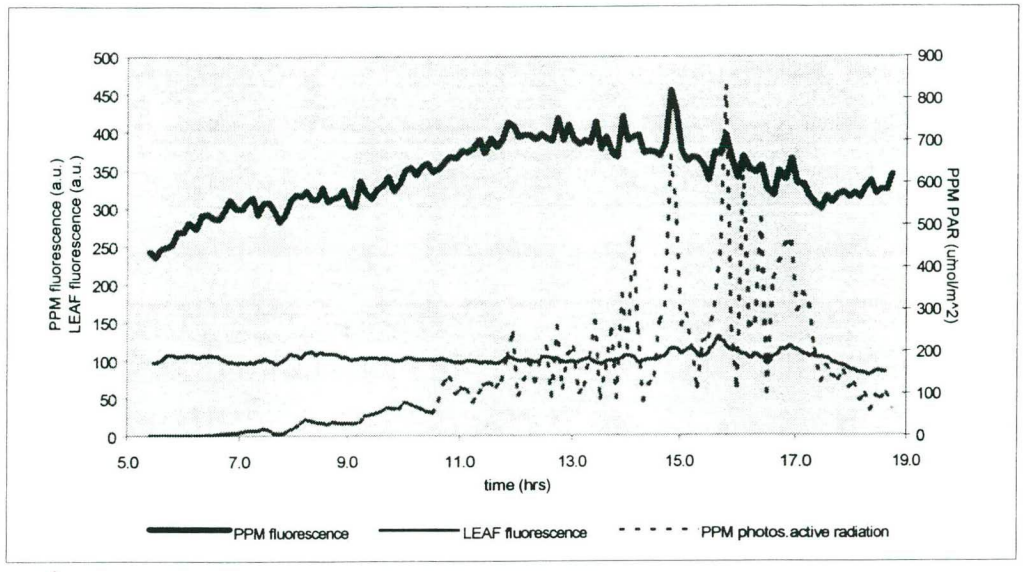


*Figure 16: Measurements of LEAF fluorescence and PPM fluorescence and PAR on Pinus Sylvestris, Kootwijk, 14 August 1998.*

To explain this difference, the passive LEAF signals of bands 1- 4 and the averaged GER reflectance in the wavelength bands corresponding to the LEAF spectral bands were compared with the PPM PAR measurements (figure 15). GER and PPM measure high radiance on the midday period, whereas LEAF measures a lower and quite flat light intensity. It is therefore concluded that the GER and LEAF measurement spots did not coincide. In fact it was noted at the end of this day during the field campaign, that LEAF was shooting in a "hole" of the tree crown. This may explain the deviating behaviour of the LEAF fluorescence on this day.

On 14 August the sky was fully clouded and the PPM fluorescence shows only a weak cycle (figure 16). At the same time, however, the LEAF fluorescence shows almost no change. A possible explanation of this difference could be as follows. The PPM measures on a single twig. The needles of this twig are more or less flattened against the transparent socket of the measuring instrument in a plane perpendicular to the measuring axis. The photosynthetic active radiation is measured on a white diffusor in the same plane on the inside of the socket. The LEAF instrument measures a crown area of about 35 cm in diameter. The orientation of the needles within this spot is more or less random, while at the same time on a cloudy day the incoming light is largely diffuse. This creates a mixture of needle exposure conditions which, on the average, may lead to an almost invariable excitation and fluorescence level during a large part of the day.

On the sunny day, 11 August, the GER fluorescence index follows the PPM fluorescence quite well (fig. 17). Both signals decrease in the morning after 10.00 hrs and increase again during the afternoon. The GER xantophyll index is also shown.

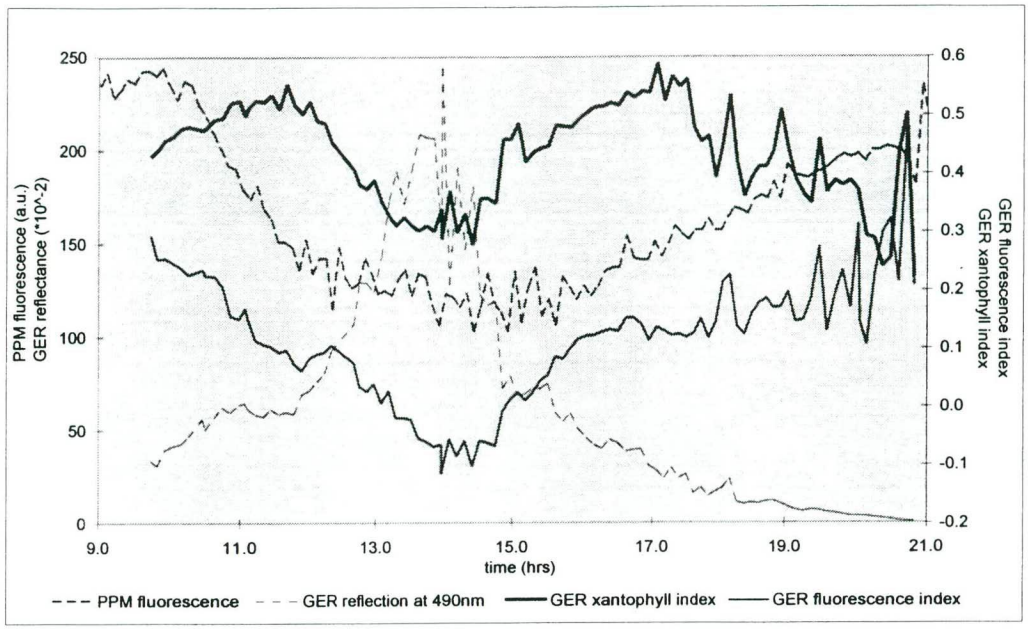


*Figure 17: Fluorescence and xanthophyll indices derived from the GER spectral reflectance in comparison with the PPM fluorescence and the light intensity as indicated by the GER reflection at 490 nm, measured on Pinus Sylvestris, Kootwijk, 11 August 1998.*

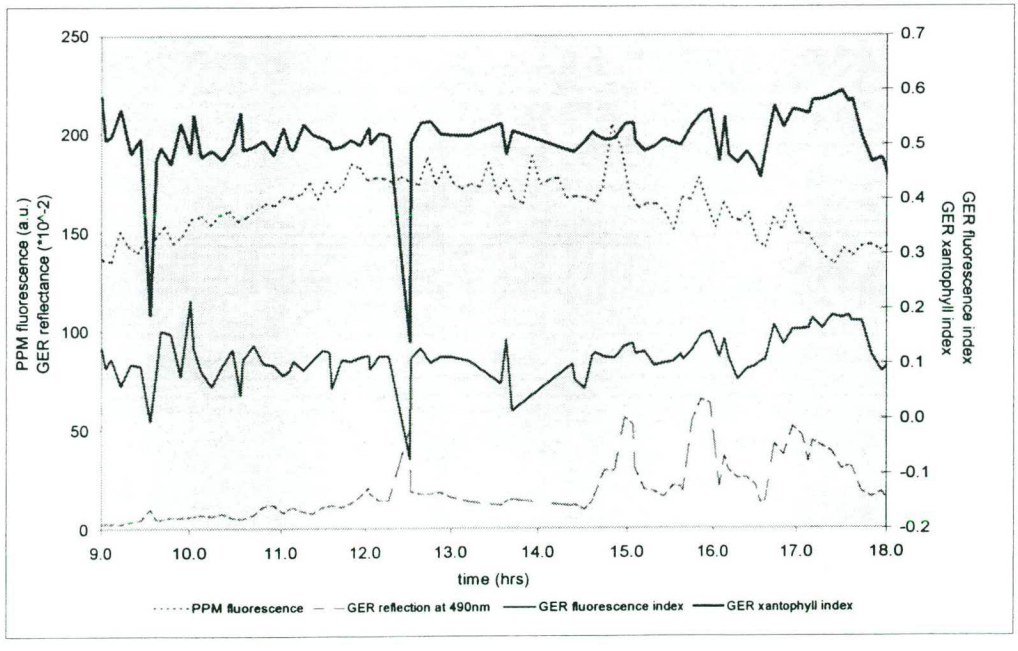


*Figure 18: Fluorescence and xanthophyll indices derived from the GER spectral reflectance in comparison with the PPM fluorescence and the light intensity as indicated by the GER reflection at 490 nm, measured on Pinus Sylvestris, Kootwijk, 14 August 1998.*

In the morning the decrease in the xantophyll index starts 1.5 hr later, but the fluorescence index and the xanthophyll index start increasing again 1 hr after the solar maximum. In this graph the solar radiation intensity is indicated by the GER 490 nm reflection. After 17.0 hrs there are strong fluctuations in the two indices which result from the noise component in the low GER signal.

On the cloudy day, August 14th, the two indices do not show much change except for some temporary disturbances or noise, which are probably due to the low light levels (figure 18).

The sap flow measurements on the Scotch pine tree offer an additional possibility for validation. This measurement is closely related to stomatal closure and consequently to the CO2 assimilation or gross photosynthesis. The Photosystem Deactivation Model (PDM) has been used to derive the course of gross photosynthesis from the PPM fluorescence measurements [Rosema *et al.*, 1998].

$$P = \zeta * \phi_P * PAR \tag{7}$$

The quantum yield $\phi P$ is calculated with

$$\phi_P = 1 - F / F_M \tag{8}$$

$\zeta$ ("zeta") is the fraction of active photosystems. It is calculated as the ratio of the actual fluorescence (F) and the fluorescence expected when no photosystem deactivation would occur (F*)

$$\zeta = F / F^* \tag{9}$$

Without photosystem deactivation, the relation between the fluorescence and the quantum yield is linear. The coefficients of this linear relation can be derived from a plot of F versus $\phi P$.

$$F^* = a - b * \phi_P \tag{10}$$

Figure 19 shows the course of the photosynthetic active radiation, the sap flow and the gross photosynthesis on the sunny day: 11 August. Sap flow starts about 2 hours after dawn and ends about 2 hrs after sunset. In the morning it increases and reaches a maximum value at 11.30 hrs. The sap flow then gradually decreases towards the end of the day. The gross photosynthesis also shows a strong increase in the early morning and reaches a maximum 0.5 to 1 hour before the sap flow. It then decreases in a gradual way, like the sap flow does. The correspondence in the sap-flow and the gross photosynthesis pattern is remarkable. The phase delay of about one hour in the sap flow, compared to the gross photosynthesis, is the result of water storage and depletion in the tree stem and leaves.

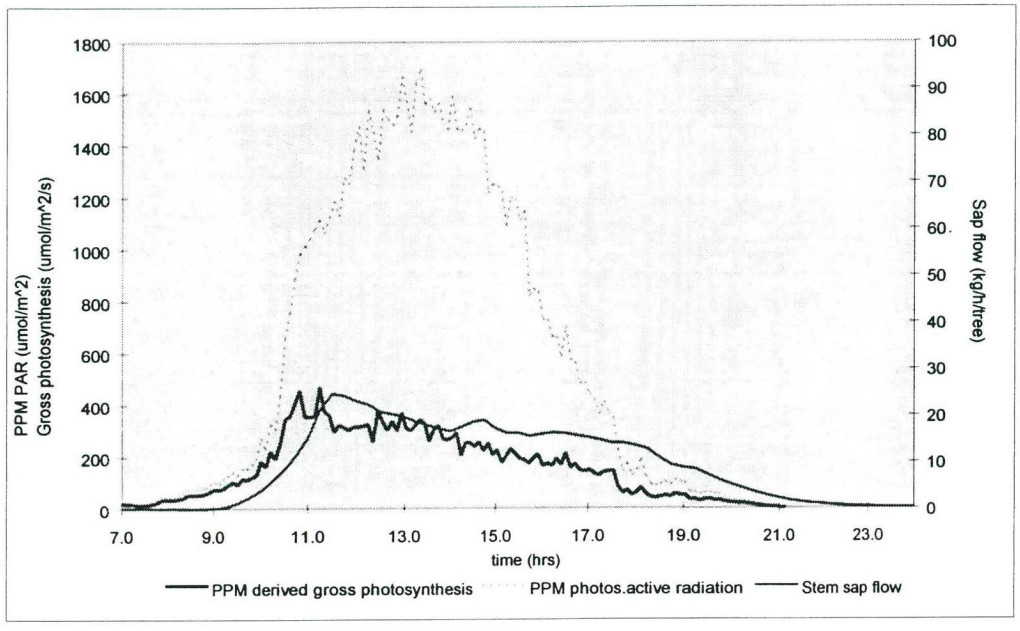


*Figure 19: Stem sap flow compared with gross photosynthesis and photosynthetic active radiation (PAR) derived from the PPM, measured on Pinus Sylvestris, Kootwijk, 11 August 1998*

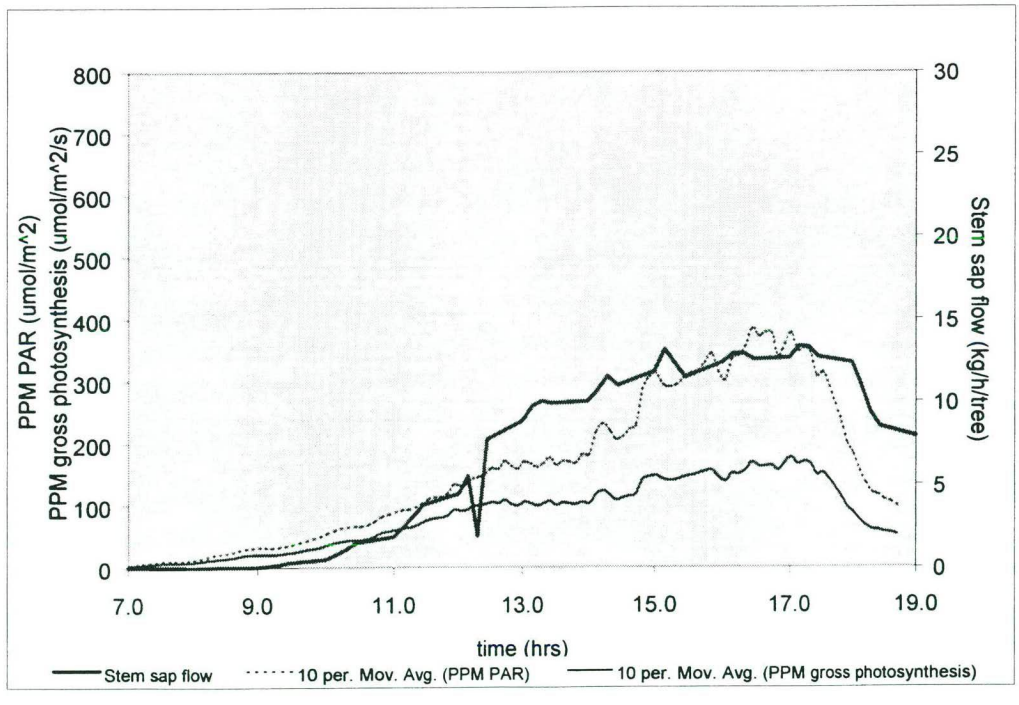


*Figure 20: Stem sap flow compared with gross photosynthesis and photosynthetic active radiation (PAR) derived from the PPM, measured on Pinus Sylvestris, Kootwijk, 14 August 1998. Moving averages of PPM data are also shown*

On 14 August (figure 20) the patterns of sap flow and gross photosynthesis are also similar, but, on this cloudy day, different from the patterns on 11 August. In this case the gross photosynthesis derived from the PPM fluorescence reaches a maximum at 17 hrs and then the decreases again. The sap flow is maximum at about the same time, but its decrease is delayed by about one hour and, occurs only after 18.00 hrs. It is known that the sap flow measurements, with some delay, reflect the CO2 assimilation. The sap flow measurements demonstrate experimentally that de fluorescence measurements and the gross photosynthesis estimates derived from these, do indeed reflect the photosynthesis of the tree.

The sap-flow pattern on 14 August (figure 20), a cloudy day with temperatures up to 22 °C, can be considered as the normal, unrestricted transpiration pattern. 11 August, however, was a day with high radiation and high temperatures, resulting in a very high sap flow, up to 20 kg/tree/hour, in the morning (figure 19). These conditions have apparently caused stomatal closure around 11.00 hrs, and a gradual decrease of transpiration during the rest of the day.

## 5.3 Component spectra

A closer look at the reflection spectra of Pinus Sylvestris on 11 august shows that an absorption phenomenon in the spectrum develops around noon in a small wavelength band between 621 and 633 nm (figure 21). The absorption band disappears again later that afternoon. This absorption phenomenon has a specific time course, which differs from the time course of the normal spectral reflection pattern of the plant. The normal and a-normal component may be separated by means of factor analysis.

Factor analysis is a statistical technique to study the structure of multivariate data. The objective of factor analysis is the de-composition of a set of data that is a linear, but variable combination of a number of components or "factors. When applying factor analysis to the reflection spectra measured with the GER instrument, the normalised component spectra or "factor scores" will be resolved together with the time course of their weight or "loading" in the mixed spectrum. A brief outline of the involved mathematics is given in annex 2.

The factor analysis method used in the present study is based on a method used to analyse data sets on water pollution [Bopp *et al.*, 1981; Kuik *et al.*, 1993a, 1993b; Havinga, 1998; Verlaan, 1998]. Factoring is continued until the residuals behave like random error, i.e. are normally distributed [Havinga, 1998]. The number of significant component spectra found in our data set of Pinus Sylvestris was 2.

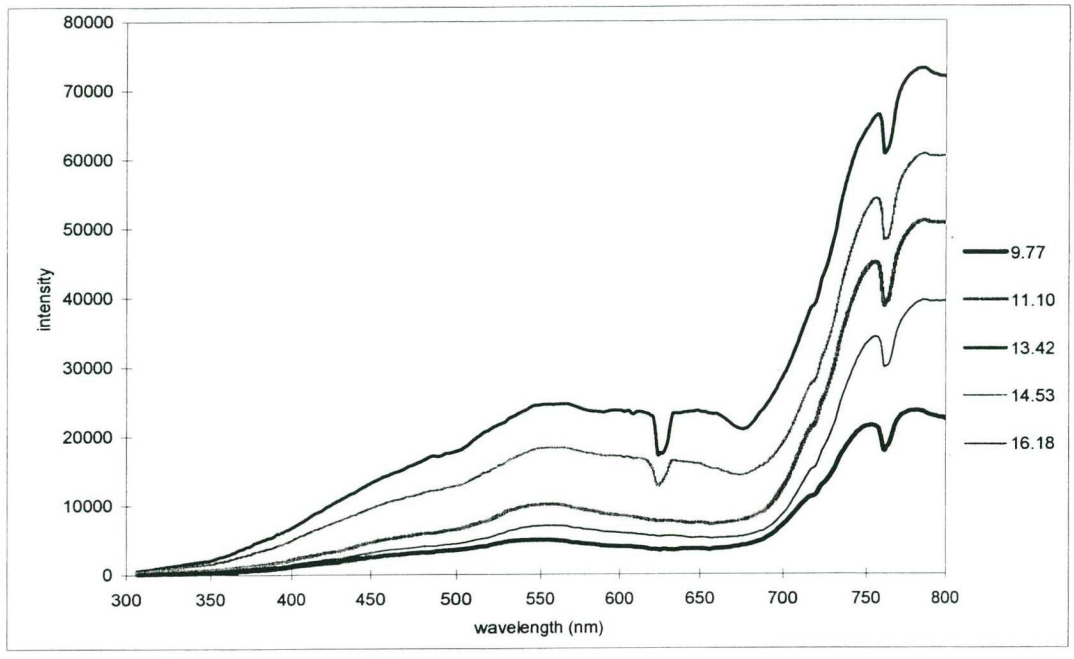


*Figure 21: GER reflectance spectra measured on the Pinus Sylvestris crown, Kootwijk, 11 August 1988*

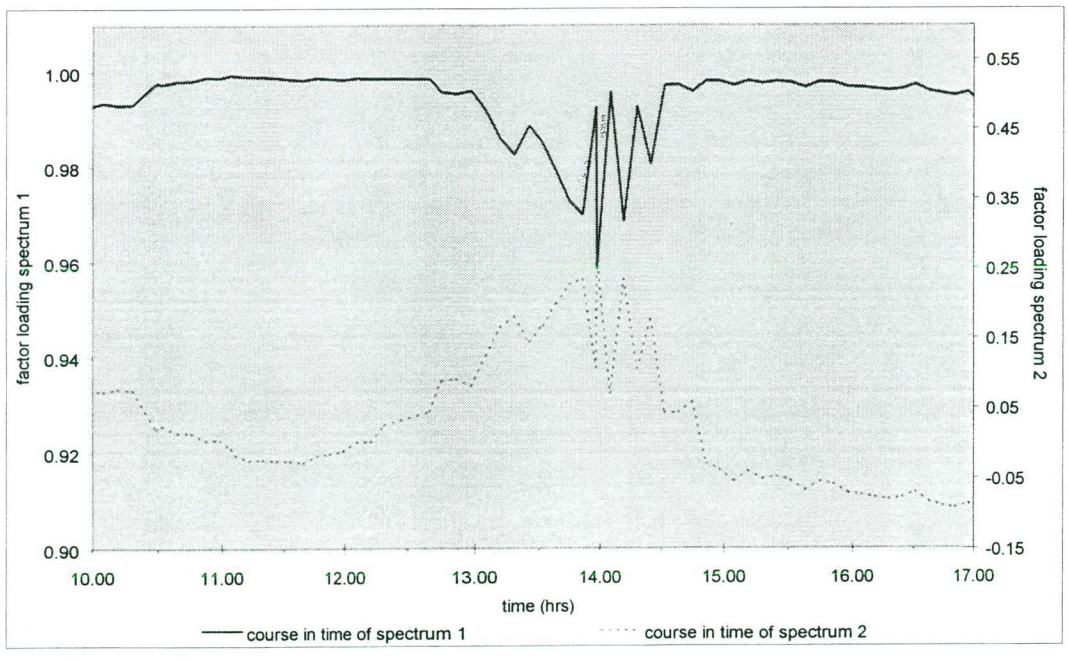


*Figure 22: Course in time of the "loading" of the first and second component spectrum of Pinus Sylvestris, Kootwijk, 11 August 1988. The normalised component spectra are shown in figure 15.*

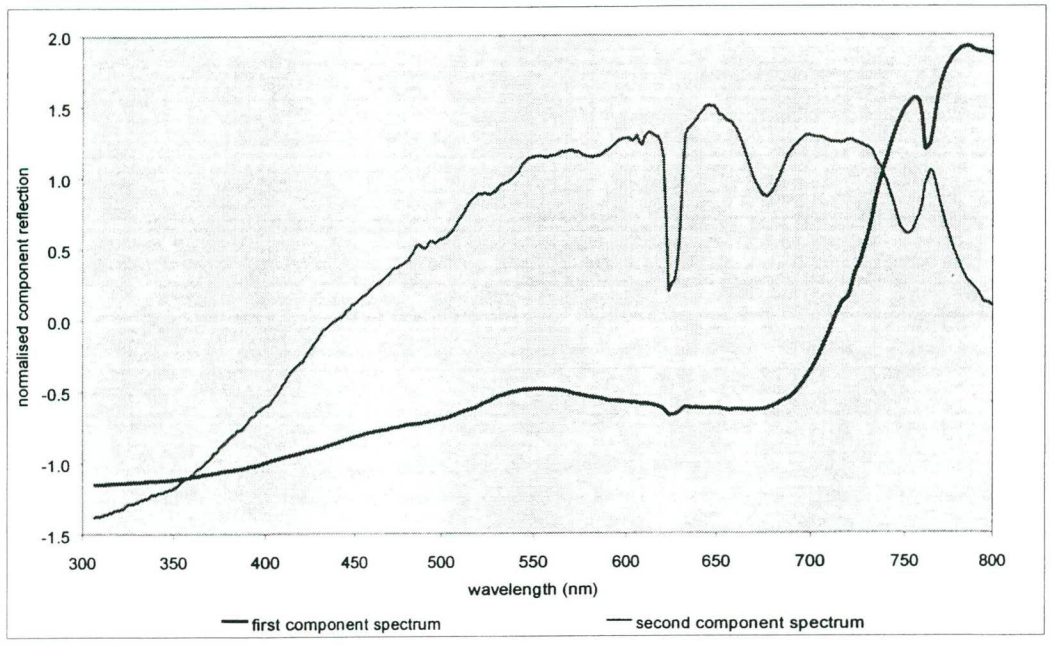


*Figure 23: First and second normalised component spectrum of Pinus Sylvestris, Kootwijk, 11 August 1988*

The courses in time of the weight or "factor loading" of the 2 component spectra are presented in figure 22. The normalised component spectra are shown in figure 23. The factor loading in figure 22 indicates to what extent a component contributes to the total spectrum as a function of time. The maximum loading is 1.

The shape of the normalised first component spectrum (figure 23) is very similar to the normal reflection spectrum of the tree. It represents 98% of the information in this spectrum. The second component spectrum influences the tree reflection during the midday, between 12 and 15 hrs. This spectrum shows three absorption bands of which that around 625 nm was already noted in figure 21. Two additional absorption bands are indicated as well, centred around 677 and 750 nm respectively. The absorption bands are embedded in a broad increase of the reflection with a maximum in the red and near infrared region (600-700 nm). This effect could partly be due to bleaching of chlorophyll and related pigments. But, in view of the broad spectral range, might as well result from changes in leaf scattering due to dehydration.

The active period of the second underlying spectrum is between 12 and 15 hrs (figure 22). There is a delay of 1.5 hr compared to the decline in chlorophyll fluorescence (figure 17 ). In fact the spectral changes seem to start after the PPM fluorescence has decreased by about 50% and has reached its lowest values. The PPM fluorescence remains low and only starts increasing again after 15 hrs, when the influence of the second component spectrum has disappeared.

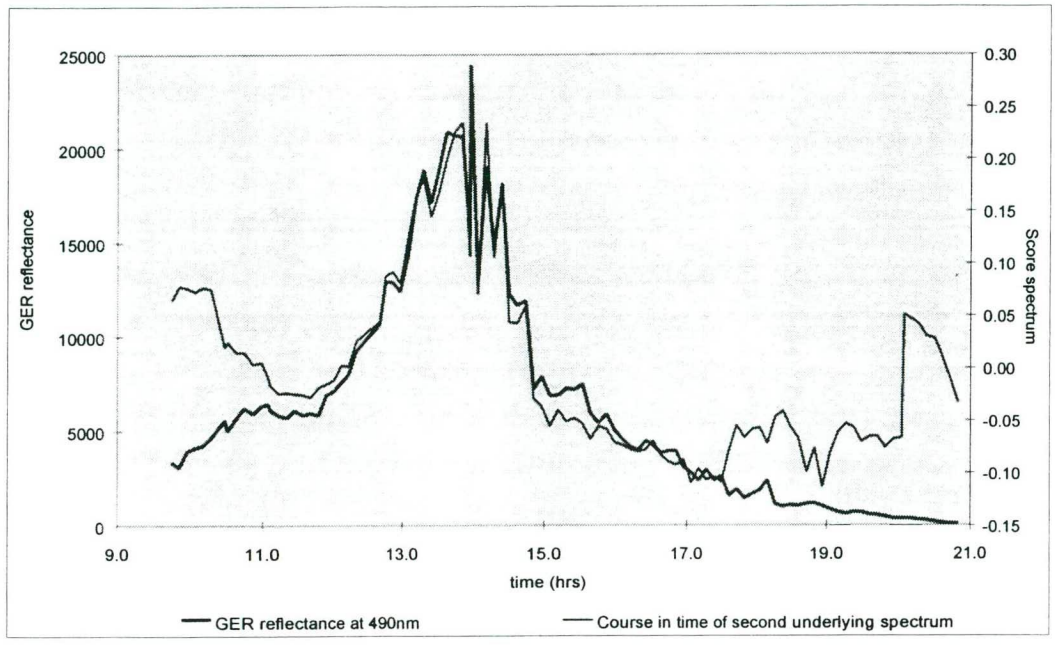


*Figure 24: Time course of 490 nm reflectance and of second underlying spectrum, based on GER reflectance measurements on Pinus Sylvestris, Kootwijk, 11 August.*

Figure 24 shows that the course of the second component spectrum coincides with that of the 490 nm wavelength intensity during a large part of the day. Here the 490 nm measurement indicates the light level. It is therefore concluded that the change in the spectrum is related to the intensity of the illumination of the tree. Measurements on a 50% grey reference panel were also made but did not show similar spectral changes as measured on the tree. Therefore the relation with the light intensity must be indirect and the spectral changes observed reside in the tree and not in the illumination.

## 6 DISCUSSION

It is shown in this study that the gross photosynthesis, calculated from the fluorescence measurements with the Photo-system Deactivation Model, corresponds well with the sap flow pattern in the stem. This experimental evidence confirms the relation between chlorophyll fluorescence and photosynthesis, demonstrated in a previous study (Rosema et al. 1998) on the basis of simultaneous $CO_2$ assimilation measurements.

It is also demonstrated that the changes in chlorophyll fluorescence during the daylight period are accompanied by changes in the fluorescence index (FI) and the xantophyll index (XI), which are derived from the reflection spectrum. A causal relation seems likely, but is difficult to prove. Factor analysis of measured spectra resolves only two component spectra of which the first represents the spectral reflection of the leaf at low light levels, while the second component spectrum

represents the changes that occur in the spectrum under high light. The second component spectrum shows, in the case of Pinus Silvestris, 3 absorption bands, which are centred at 625, 677 and 750 nm. They are superimposed on a broad band increase of reflectance in the green, red and near infrared wavelength region (figure 23).

The absorption band at 625 nm is most likely originating from a stress pigment, that is generated during the high light period and disappears thereafter. We have not yet been able to identify it.

One could suppose that the absorption bands centred at 677 and 750 nm are related to the decrease of the chlorophyll fluorescent emission during midday. Such a decrease, however, is expected to have its main point at 685 and 730 nm. Moreover, the chlorophyll fluorescence decline measured by the PPM starts at least 1 hour earlier that these spectral changes. It is therefore not likely that these bands are related to changes in chlorophyll fluorescence.

Changes in the xanthophyll index, which are calculated from the 531 and 490 nm radiance, seem not to be related to a local absorption phenomenon but merely result from the broad band increase of the radiance during high radiation. A similar broad band increase of reflection is usually observed in yellowing leaves. It might therefore be associated to a temporal decrease in chlorophyll and other photosynthetic pigments. This phenomenon could directly or indirectly be related to the photo-system deactivation, that causes the chlorophyll fluorescence to decline under $CO_2$ limited photosynthesis. A change of xanthophyll pigment composition could just be a part of this broad band phenomenon.

## 7 CONCLUSIONS AND RECOMMENDATIONS

On clear days the daily course of photosynthesis usually has two characteristic phases: (1) the light limited phase, when there is insufficient light to fix all available $CO_2$ and (2) the $CO_2$ limited phase, when the available light exceeds the $CO_2$ assimilation. The availability of $CO_2$ for photosynthesis is regulated by the plant stomata, which close when insufficient water is available.

The $CO_2$ limited phase is a period of stress. The excess light cannot be used for photosynthesis and causes heating and chemical changes inside the plant tissue. This period of stress is characterised by a decrease of chlorophyll fluorescence due to photo-system deactivation, a relatively slow process that balances electron transport with CO2 availability. During the late afternoon and night the de-activated photo-systems are repaired and the photosynthetic capacity of the plant is restored.

A model has been developed (Photo-system Deactivation Model: PDM), which allows the estimation of gross photosynthesis levels on the basis of fluorescence measurements. This model was validated earlier under greenhouse conditions with

simultaneous $CO_2$ measurements (Rosema *et al* 1998). In the present study we have reconfirmed the validity of this model in the field, using sap flow measurements on a Pinus Sylvestris tree.

The present research was intended to provide evidence that stress related changes in the chlorophyll fluorescence are accompanied by changes in the spectral reflection. Such changes in the spectral reflection were expected at wavelengths related to fluorescent emission (685, 730 nm) and changes in xanthophyll pigments (530 nm). We have found substantial and reversible changes in the reflection spectrum, which however do not fit with our initial expectations. The relatively narrow absorption bands at 625, 675 and 750 nm suggest the development of stress related products as a result of excess light. The broad band increase of reflection between 400 and 800 nm could be related to overall pigment bleaching or an increase of scattering due to leaf dehydration.

These findings offer an interesting perspective for the application of "hyper spectral" scanning instruments or "imaging spectrometers" in relation to the monitoring of the photosynthetic status of vegetation. This could particularly be useful for studies of $CO_2$ fixation in canopies by remote sensing. The spectral resolution required would be in the order of 10 nm.

The investigation of the potential of this approach from space will initially not put too high demands in terms of spatial resolution, as long as the approach could be tested over large vegetated areas, such as the Veluwe forest and the IJsselmeerpolders. A specific demand is the time of observation. This would preferably require a sun-synchronous orbit around or shortly after noon. It is proposed to further investigate this research path in the framework of the User Support programme (GO-2).

1) The findings in the present project are unmistakable but still based on a limited data set. There is a need to extend our experience with and knowledge of the phenomena discussed. It is therefore recommended to carry out the monitoring of fluorescence and multi-spectral reflection during longer periods on trees and also on other types of vegetation which are of social interest (agricultural crops).

2) It is also recommended to study these phenomena not only in the temporal, but also in the spatial domain by means of air- or space-born imaging experiments with high spectral resolution imaging equipment. Possible space-born instruments that could be used for this purpose are ENVISAT-MERIS and PROBA-CHRIS.

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# Annex I Functioning of the LEAF instrument

## I.1 Acceptation test

The LEAF instrument was send back to NEDINSCO by the previous user for maintenance. The instrument was thereafter delivered to EARS. After reception the LEAF instrument was tested according to the existing BCRS protocol H970-0042A. The findings were reported to BCRS on 29 juli 1998 and are briefly summarised here.

The calibration panel was missing and could not be retrieved. The camera and viewing aid on the instrument did not provide a sharp image of the measuring object and the functioning of the ring projector could not be verified. The cause of this defect is not clear.

The shape of the laser pulse was round. The laser intensity varied by 15%. During a 5 hourly measuring session the laser power decreased with 45%. The diameter of the laser spot was larger then expected on the basis of the specified laser divergency. This deviation was +24% at 20 mrad and +38% at 40 mrad. The pulse diameter did hardly decrease at specified divergencies below 20 mrad. These findings are similar to earlier tests.

Compared to earlier experiments the steering software has been changed in such a way that the fluorescence is not directly calculated from the active and the passive measurement. The current version produces the original passive and active measuring values. Calculation of the fluorescence from these two signals has to be done by the user afterwards. This involves a factor that mutually scales the two signals (see section I.3). The fluorescence values calculated after scaling showed a good intensity level when compared with earlier experience.

The reproducibility of the measured fluorescence, after correction for the laser power, measured on a plant at 10 m distance, was unexpectedly good. This result suggested that there was no need for averaging measurements to eliminate oscillations, as observed during earlier measuring campaigns.

## I.2 Functioning during project execution

Contrary to our findings during the acceptation test, the single shot measurements showed oscillations as observed in earlier measuring campaigns. For this reason measurements were taken every 2 minutes and the oscillations were eliminated by taken the average of six succeeding measurements.

On several days the LEAF-NL instrument stopped spontaneously at 12.50 hrs and had to be restarted. The reason is not clear. The fact that this stop occurred several times at the same time suggests a software problem.

## I.3 Empirical determination of the time window correction

Each measuring action with the LEAF instrument consists of two measurements, one with the laser pulse ("active measurement") and one without laser pulse ("passive measurement").

The active and passive signal is detected during different time intervals by means of an electronic shutter. The electronic shutter is open during a certain time window and the entering radiation is divided in four beams by beam-splitters and detected by four PMT's, each having a specific narrow wavelength filter in front. So, the time window is the same for all four PMTs. The laser induced fluorescence (F) is derived from the active measurements (A) and the passive measurement (P) by

$$F = A - (\Delta t_A/\Delta t_P)*P$$

$\Delta t_A$ and $\Delta t_P$ are the time windows of the active and the passive measurement respectively.

Active and passive signals have been measured from a non-fluorescent 50% grey panel under different light intensities, while the laser pulse was shielded. So no laser-induced fluorescence can be measured (F=0). Then the ratio between the two time windows follows from

$$(\Delta t_A/\Delta t_P) = A / P$$

According to the data provided by NEDINSCO the time window is supposed to be

$$\Delta t_A/\Delta t_P = 0.95/6.5 = 0.1462$$

The following table present the values that were obtained with this experiment from a series of measurements under different light intensities by means of a linear fit.

| PMT | Wavelength band (nm) | $\Delta t_A/\Delta t_P$ | $R^{2*}$ |
|---|---|---|---|
| 1 | 640-660 | 0.1677 | 98 |
| 2 | 677-695 | 0.1677 | 98 |
| 3 | 720-740 | 0.1658 | 98 |
| 4 | 770-800 | 0.2090 | 86 |

* $R^2$= square fit (%)

The measured active and passive time window ratio is higher than the ratio specified by NEDINSCO. PMT 4 shows a ratio, which is very different from the others. A possible explanation could be non-linearity of the PMT. Signals of PMT 4, however, were not used in the present work. The calculated ratio's have been used for processing the LEAF data.

## I.4 Conclusion

Several projects have been carried out with the LEAF instrument during the 2nd National Remote Sensing Programme (NRSP-2). The measurements with this instrument have been essential for our understanding of the relation between chlorophyll fluorescence and plant photosynthesis. As a result the Photosystem Deactivation Model was developed which enables the estimation of gross photosynthesis from series of fluorescence measurements. The functioning of the LEAF instrument, however, was never without problems. The most important ones are mentioned in the previous sections of this annex. For this reason the instrument can only be handled effectively by experienced users. For the same reason the instrument is not ready for market introduction, as was originally intended.

## Annex II Factor analysis

Factor analysis has been applied to a data set of wavelengths in the range 300 -2500 nm measured from 10-21 hrs. A brief outline of the mathematics involved is presented here.

Consider spectral reflectance measurements of $n$ wavelengths measured $q$ times. The intensity of the $w$-th wavelength ($w = 1 \ldots n$) of the $t$-th spectrum ($t = 1 \ldots q$) measured at time $t_t$ is $s_{tw}$. To avoid scaling problems as a result of different light intensities, it is convenient to transform the spectra to standardised variables $z_{tw}$ by

$$z_{tw} = \left(s_{tw} - \bar{s}_t\right) / \sigma_t \tag{1}$$

where $\bar{s}_t$ and $\sigma_t$ are the mean and standard deviation of the spectrum at time $t_t$, respectively:

$$\bar{s}_t = \sum_{w=1}^{n} \frac{s_{tw}}{n} \qquad \sigma_t = \left( \sum_{w=1}^{n} \left(s_{tw} - \bar{s}_w\right)^2 \Big/ (n-1) \right)^{1/2} \tag{2}$$

The $z_{tw}$ are assumed to be a linear sum of $m$ common factors, with $m \leq q$, which account for the observed correlation's between the wavelengths, and a unique contribution which is specific for each individual wavelength:

$$z_{tw} = \sum_{k=1}^{m} a_{tk} f_{kw} + d_t u_{tw} \tag{3}$$

or in matrix notation:

$$Z_{q.n} = F_{q.m} A^T_{m.n} + U_{q.n} D_{n.n}$$

The coefficients $a_{tk}$, forming a $q.m$ matrix $A$, are the loadings of the factors. They represent the correlation of spectra $s_t$ with factor $k$ and are therefore indicative for the relative daily course of factor $k$. The coefficients $f_{kw}$ forming an $m.n$ matrix $F$, symbolise the contribution of factor $k$ to wavelength $w$. Correctly normalised, they can be interpreted as the contribution of component $k$ to the measured reflectance of wavelength $w$. The product $d_t u_{tw}$ represents the residual error of wavelength $w$ at spectrum $t$ which is not accounted for by the $m$ common factors. For a given wavelength $w$ the $z_{tw}$ can be conceived as a column vector with the $q$ standardised spectra as its components. Equation 3 can then be written as

$$\begin{pmatrix} z_{1w} \\ z_{2w} \\ \cdot \\ \cdot \\ \cdot \\ z_{qw} \end{pmatrix} = f_{1w} \begin{pmatrix} a_{11} \\ a_{21} \\ \cdot \\ \cdot \\ \cdot \\ a_{q1} \end{pmatrix} + f_{2w} \begin{pmatrix} a_{12} \\ a_{22} \\ \cdot \\ \cdot \\ \cdot \\ a_{q2} \end{pmatrix} + \ldots + f_{mw} \begin{pmatrix} a_{1m} \\ a_{2m} \\ \cdot \\ \cdot \\ \cdot \\ a_{qm} \end{pmatrix} + \begin{pmatrix} d_1 u_{1w} \\ d_2 u_{2w} \\ \cdot \\ \cdot \\ \cdot \\ d_q u_{qw} \end{pmatrix} \tag{4}$$

where the $m$ column vectors

$$\vec{a}_k = \begin{pmatrix} a_{1k} \\ a_{2k} \\ \cdot \\ \cdot \\ \cdot \\ a_{qk} \end{pmatrix} \qquad (k = 1 \ldots m) \tag{5}$$

contain the daily course of the corresponding factor $k$.

The total variance at spectrum $t$ is split into a fraction that is accounted for by the factor model, and an unexplained fraction given by the uniqueness $d_t^2$. This unexplained variance is reflected in observed variation (noise) in repeated measurements of single wavelengths. The coefficients $u_{tw}$ represent the contribution of wavelength $w$ to the uniqueness at spectrum $t$.

The direct solution of $A$ is obtained by principal factor analysis and the factor scores $F$ are obtained by using regression estimates as described in *Havinga,* 1998. The factor analysis has been performed by MathSoft S-PLUS 4.5.

The National Remote Sensing Programme 1990-2000, (NRSP-2) is implemented under the responsibility of the Netherlands Remote Sensing Board (BCRS) and coordinated by the Ministry of Transport and Public Works.

The objectives of the NRSP-2 are: to secure the long-term integration of the operationel use of remote sensing through temporary stimulation in the user-sectors of government and industry, to strengthen the development of remote sensing applications and the expansion of the national infrastructure.

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