

University of South Bohemia  
Faculty of Biological Sciences  
Department of Plant Physiology



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# **Annual Changes in Steady State Chlorophyll Fluorescence Yield ( $F_S$ ) of Two Evergreen Plants**

Václav Šlouf

Supervisor: Mgr. Julie Soukupová, Ph.D.

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### **Annotation**

This bachelor thesis refers to biophysics of photosynthesis. The aim was to contribute to the revealing of the information potential of the steady state chlorophyll fluorescence yield ( $F_S$ ), which can be detected by remote sensing technique. The theoretical part of my bachelor thesis deals with basic information about photosynthesis, fluorescence and remote sensing of vegetation. In the second part I describe the conducted experiments (measurements of chlorophyll fluorescence), present the following results and discuss them.

Prohlašuji, že svoji bakalářskou práci jsem vypracoval samostatně jen s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č.111/1998 Sb. v platném znění souhlasím se zveřejněním své bakalářské práce - v úpravě vzniklé vypuštěním vyznačených částí archivovaných Biologickou fakultou - elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách.

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Podpis:

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## LIST OF ABBREVIATIONS

A - CO<sub>2</sub> assimilation rate; ADP - adenosine diphosphate; ATP - adenosine triphosphate; Chl – chlorophyll; ChlF - chlorophyll fluorescence; Cyt *b<sub>f</sub>* - cytochrome *b<sub>f</sub>* complex; DAS - dark-adapted state; ESA - European Space Agency; F<sub>0</sub>/F<sub>0</sub>' - minimum chlorophyll fluorescence yield in dark-adapted/light-adapted state; fAPAR - fraction of solar radiation that green leaves in the canopy absorb; Fd – ferredoxin; FLD - Fraunhofer Line Discrimination; FLEX - Fluorescence Explorer mission; F<sub>M</sub>/F<sub>M</sub>' - maximum chlorophyll fluorescence yield in dark-adapted/light-adapted state; F<sub>P</sub> - maximum chlorophyll fluorescence yield measured when the actinic radiation is switched on; F<sub>S</sub> - steady state chlorophyll fluorescence yield in light-adapted state; F<sub>V</sub>/F<sub>V</sub>' - maximum variable chlorophyll fluorescence yield in dark-adapted/light-adapted state; LAI - leaf area index; LAS - light-adapted state; LUE – light use efficiency; NADPH/ NADP<sup>+</sup> - nicotinamide adenine dinucleotide phosphate, reduced/oxidized form; NDVI - normalized difference vegetation index; P680/P700 - reaction centre in photosystem II/photosystem I; P680\*/P700\* - excited reaction centre in photosystem II/photosystem I; P680<sup>+</sup> - oxidized reaction centre in photosystem II; PAS - pulse amplitude modulation; PC – plastocyanine; PFD - photon flux density; Ph – pheophytin; P<sub>i</sub> - inorganic phosphate; pmf - proton motive force; PPFD - photosynthetic photon flux density; PQ - plastoquinones; PQH<sub>2</sub> – plastoquinol; PRI - photochemical (plant, physiological) reflectance index; PSI - photosystem I; PSII - photosystem II; Q<sub>A</sub> - primary quinone electron acceptor of photosystem II; Q<sub>B</sub> - secondary quinone electron acceptor of photosystem II; R - reflectance coefficient; RC - reaction centre; Rubisco - ribulose biphosphate carboxylase/oxygenase; S<sub>0</sub> - ground state of a chlorophyll molecule; S<sub>1</sub> - excited state one of a chlorophyll molecule; S<sub>2</sub> - excited state two of a chlorophyll molecule; Y<sub>Z</sub> - amino acid tyrosine in the way of electrons from water to the reaction centre in photosystem II; ΦII - effective quantum yield of photochemical energy conversion in photosystem II; Φ<sub>P0</sub> - maximum quantum yield of photochemistry of photosystem II

# 1. MOTIVATION

Current efforts for remote sensing of vegetation include also chlorophyll fluorescence (ChlF) emission as a signal correlating with photosynthesis. In order to extend the approach to monitor and to map photosynthetic activity of vegetation at large scales, ESA (European Space Agency) in the framework of the Earth Explorer Opportunity Missions program has proposed an experimental mission FLEX (Fluorescence Explorer, [www.esa.int/esaLP/SEM2VSBE8YE\\_index\\_0.html](http://www.esa.int/esaLP/SEM2VSBE8YE_index_0.html)). One of the objectives of the planned FLEX mission is to map globally the actual vegetation photosynthesis. For this purpose, sun induced vegetation fluorescence will be monitored, and additional reflectance and temperature measurements will provide complementary information for the fluorescence signal interpretation.

However, the data-to-information conversion is not trivial primarily because the sun induced steady-state fluorescence signal that will be measured by FLEX depends on number of internal and external factors. Thus, my bachelor thesis contributes to the effort to assess the information potential of ChlF signal when regularly monitored in field. I studied the annual variation of steady state ChlF yield in light-adapted state ( $F_s$ ) of two evergreen plant species: *Picea omorika* and *Rhododendron x hybridum*. In parallel, CO<sub>2</sub> assimilation rate and measurement of the effective quantum yield of photochemical energy conversion in PSII ( $\Phi_{II}$ ) were conducted. Furthermore, reflectance indexes measurements using prototypes from P.S.Instruments, Ltd. (Brno, [www.psi.cz](http://www.psi.cz)) were performed to test their usefulness.

## 2. INTRODUCTION

### 2.1. Photosynthesis

#### 2.1.1. Basics

Photosynthesis is one of the most important processes on the Earth. Nearly all forms of life including humans depend on photosynthesis for direct or indirect energy supply. The global relevance of photosynthesis is also in sustaining the composition of the Earth atmosphere. In this process, solar energy is trapped, converted into chemical energy, and stored in the bonds of carbohydrates. Simplified complete reaction of photosynthesis can be written as follows:



where:  $\{\text{CH}_2\text{O}\}$  is a chemical formula of a structural unit of sugars, and  $\Delta G$  is dissipated energy.

In photosynthetic eukaryotes, photosynthesis takes place in chloroplasts (Fig.1). Chloroplasts are organelles separated from the cytosol by a double membrane (an outer and an inner membrane). The fluid compartment inside the chloroplast is called the stroma. In the stroma there is a system of membranes, called thylakoids that can be integrated into piles called grana. A region, which is enclosed by thylakoids, is known as the lumen.

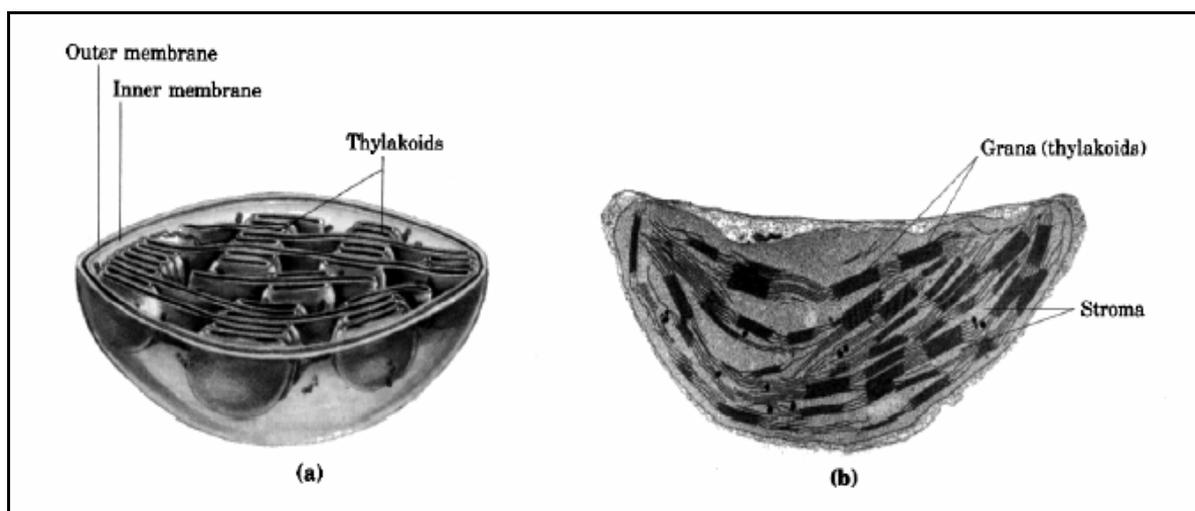


Fig. 1: The structure of a chloroplast (*courtesy of authors of the textbook on Plant Physiology: Šetlík, Seidlová, Šantůček*).

The whole process of photosynthesis consists of two sets of reactions: light reactions taking place within the thylakoid membranes and dark reactions proceeding within the stroma.

During light reactions, the energy of photons is trapped by chlorophyll molecules, which are bound in the thylakoid membranes, and converted into the energy of chemical bonds of two primary metabolites: ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate, reduced form).

Light is not directly involved in dark reactions (carbon reduction reactions). Here, the high-energy carriers (ATP and NADPH) are used in the reactions of *Calvin cycle* (Calvin and Benson 1948) where their chemical energy is used to fix carbon dioxide, and to synthesize glucose and other complex organic compounds.

### 2.1.2. Light Reactions

The main function of light reactions is to transfer electrons across the membrane and subsequently to generate proton ( $H^+$ ) gradient that is further used for synthesis of ATP by the enzyme ATP-synthase.

The pathway of electrons across the membrane during light reactions of photosynthesis can be best explained by means of the “Z”-scheme, showing the electron transfer system in terms of redox potentials (Fig. 2). It displays the pathway of the electron transport from water to  $NADP^+$  (nicotinamide adenine dinucleotide phosphate, oxidized form) leading to the reduction of  $NADP^+$  to NADPH.

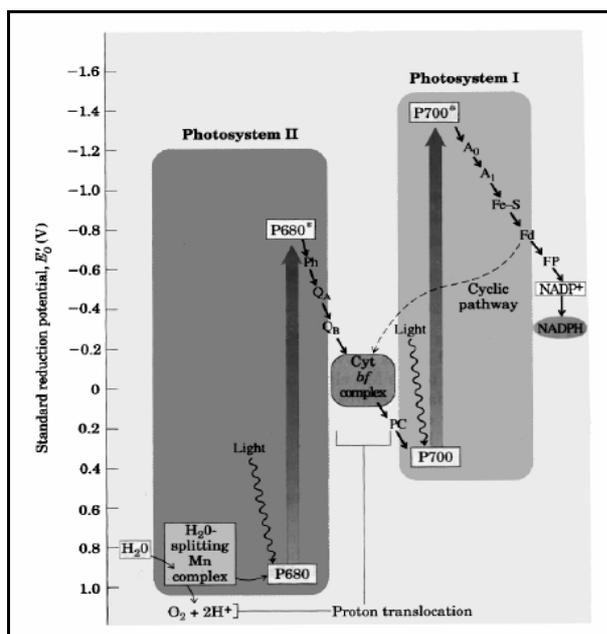


Fig. 2: The so-called “Z” scheme of light reactions of photosynthesis. It shows the pathway of electrons from water molecules to  $NADP^+$  in terms of redox potentials. Displayed components as well as the cyclic electron pathway are described in the text (from Lehninger et al. 1993).

Almost all reactions contributing to the light reactions of photosynthesis are carried out by four major protein complexes placed within the thylakoid membrane: photosystem II (PS II), cytochrome *b<sub>6</sub>f* complex (Cyt *b<sub>6</sub>f* complex), photosystem I (PSI) and ATP-synthase (Fig. 3). Plastoquinones (PQ) and plastocyanine (PC) function as mobile carriers to transport electrons between photosystems.

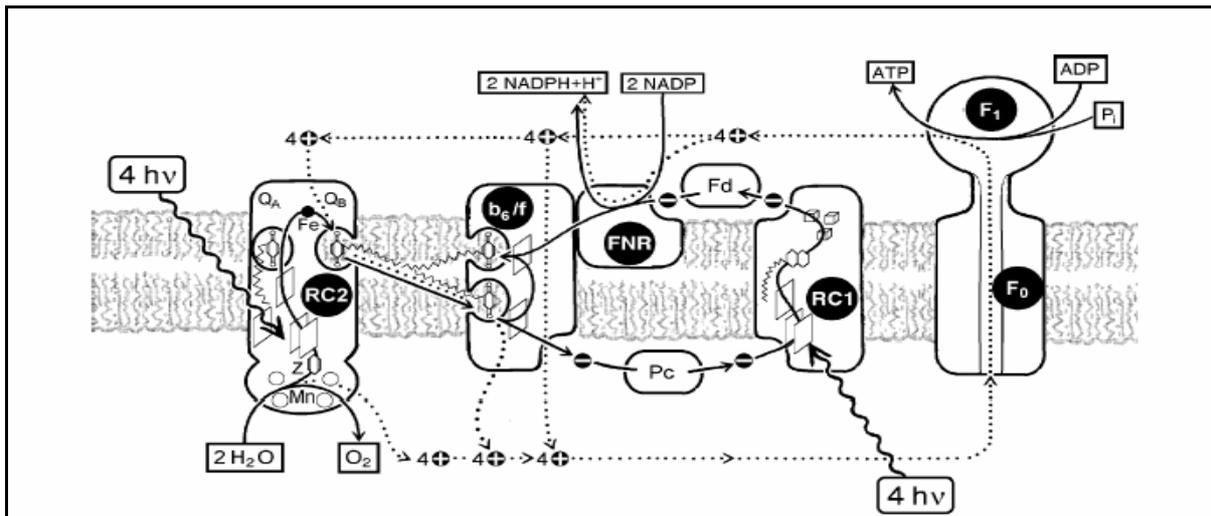


Fig. 3: The scheme of reactions, electron and proton transporters of the oxygenic photosynthesis in the thylakoid membrane (courtesy of authors of the textbook on Plant Physiology: Šetlík, Seidlová, Šantůček).

#### 2.1.2.1. The First Step

PS II is a complex of proteins, in which the light reactions start. Some of the proteins carry prosthetic groups like chlorophyll *a* molecules (Chl *a*) in the reaction centre (RC) of PSII (Fig. 3). Photosynthesis starts with the trapping of the light energy by photoreceptors. Chl *a* molecule is excited either directly by the absorbed photons or, most often, by the excitation energy transferred from surrounding pigment molecules (Chls and carotenoids) associated within antennae. Chl *a* in the RC of PSII is excited by the photon with a wavelength of 680 nm, so the RC in PS II is labelled as P680.

After excitation of P680 (P680\*), the electron is passed to acceptors mentioned below. The missing electron in P680<sup>+</sup> (oxidized P680) is substituted via the oxidation of a water molecule. An amino acid tyrosine (called Y<sub>z</sub> in this case) and a cluster of four manganese ions are intermediates in the way of electrons from water to P680<sup>+</sup>.

### 2.1.2.2. Electron Transfer

The electron is transferred from P680\* to pheophytin (Ph) and then to Q<sub>A</sub> (primary quinone electron acceptor) and to Q<sub>B</sub> (secondary quinone electron acceptor) that are also located in PSII. Once Q<sub>B</sub> receives two electrons and subsequently two protons (from now it is labelled PQH<sub>2</sub> (plastoquinone)), it leaves PSII for another membrane-bound complex Cyt *b<sub>6</sub>f*. Here, the protons are released into the lumen and electrons are further transferred via a mobile copper protein PC, which shuttles between Cyt *b<sub>6</sub>f* complex and PSI. In addition, so-called *Q-cycle* running round Cyt *b<sub>6</sub>f* provides extra protons into the lumen.

PSI consists of proteins, polypeptides and photosynthetic pigments. The core of PSI consists of the RC P700 (reaction centre in PSI). P700 is excited by the “red photon” ( $\lambda = 700$  nm) after receiving the electron from PC producing P700\* (excited P700).

The electron is passed from PSI via some intermediates to Fe-S complex (Rieske protein), and then to ferredoxin (Fd). Finally, the electron is used by ferredoxin-NADP<sup>+</sup>-reductase to reduce NADP<sup>+</sup> to NADPH. Instead of the already described linear electron transport, there also exists cyclic electron transport around PSI that transfers electrons from Fd to PQ. In this process, more protons are transported into the lumen.

Light reactions also provide energy for ATP formation. ATP is produced by the enzyme ATP-synthase from ADP (adenosine diphosphate) and inorganic phosphate (P<sub>i</sub>) by means of the proton motive force (pmf) according to the chemiosmotic theory (Mitchell 1961). There are two components of pmf: electrical potential and concentration gradient of protons (H<sup>+</sup>). The higher concentration of H<sup>+</sup> in the lumen and the transmembrane potential are maintained through several processes: oxidation of water (oxygen is released, electrons are transferred to P680<sup>+</sup> and protons stay in the lumen); electron transfer across the membrane (Q<sub>B</sub> transfers also protons that are released into the lumen in the process of oxidation of PQH<sub>2</sub>; *Q-cycle*; NADP<sup>+</sup> picks up one proton from the stroma when it is reduced by two electrons).

### 2.1.3. Dark Reactions

Reactions associated with CO<sub>2</sub> reduction to carbohydrates are referred to as dark reactions of photosynthesis, since light is not directly involved. Nevertheless, products of light reactions (ATP and NADPH) are used here.

The most common way of CO<sub>2</sub> fixation in photosynthetic organisms is the C<sub>3</sub> photosynthetic carbon reduction cycle, also called *Calvin cycle* (Calvin and Benson 1948) (Fig. 4).

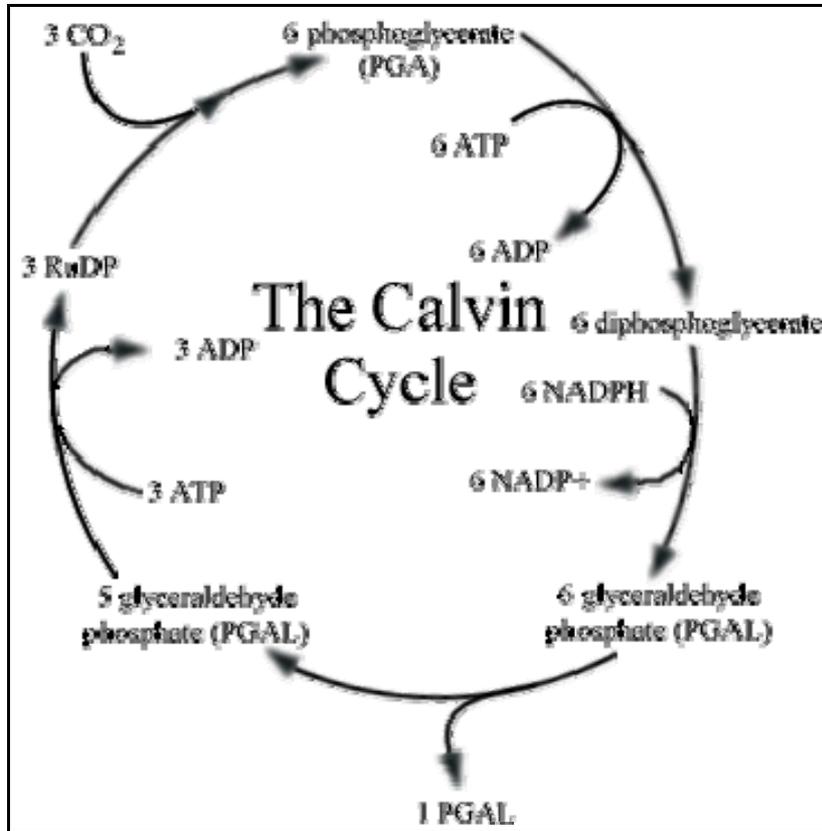


Fig. 4: The Calvin cycle ([http://library.thinkquest.org/27819/media/calvin\\_cycle.gif](http://library.thinkquest.org/27819/media/calvin_cycle.gif)).

The *Calvin cycle* consists of three phases:

- 1) *Carboxylation* of the five-carbon CO<sub>2</sub> acceptor, ribulose-1,5,-bisphosphate (RuDP), and forming two molecules of the three-carbon intermediate, 3-phosphoglycerate (PGA). This reaction is catalysed by the enzyme ribulose bisphosphate carboxylase/oxygenase (Rubisco).
- 2) *Reduction* of 3-phosphoglycerate to a carbohydrate glyceraldehyde 3-phosphate (PGAL). Here, NADPH and ATP are required. The triose phosphate is the origin of starch and sucrose synthetic pathways.
- 3) *Regeneration* of the five-carbon acceptor, ribulose-1,5-bisphosphate, from glyceraldehyde 3-phosphate.

### 2.1.4. Measurement of the Rate of Photosynthesis

The core of the photosynthetic rate measurement consists in the monitoring of concentration changes of reactants (or products) in the overall reaction of photosynthesis /1/. Gas analysers are often used when the rate of photosynthesis is investigated.

## **2.2. Fluorescence**

### 2.2.1. Basics

The light energy captured by photosynthetic apparatus can be distributed into three competitive pathways: 1) photochemistry, 2) thermal dissipation and 3) ChlF emission (see also Fig. 8).

In leaves of healthy plants under steady state conditions, about 80% of the absorbed energy is transferred into photochemistry, ca. 15% is dissipated through thermal dissipation pathway, and ca. 3-5% is reemitted as ChlF (reviewed in e.g., Krause and Weis 1991, Govindjee 1995). Although ChlF represents a minor deactivation process in plants, it still gives the possibility for a non-invasive monitoring of the utilization of light energy absorbed by plants. Typically, when the rate of photosynthesis is high, ChlF is low and vice versa. Most of ChlF (more than 90%) originates from antennae of PS II at room temperature (e.g., Krause and Weis 1991, Govindjee 1995).

### 2.2.2. Chlorophyll Fluorescence Induction and Parameters

ChlF is not a static characteristic. When a dark-adapted leaf is illuminated by continuous light, the intensity of ChlF rises quickly to a peak level and then decreases to a steady-state value (Fig. 5). These characteristic variations have been called the fluorescence induction or the *Kautsky effect*, since Kautsky and Hirsch (1931) were the first, who described the time course of ChlF.

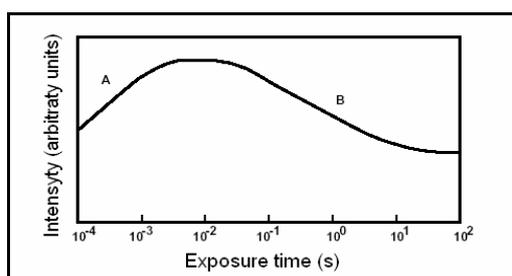


Fig. 5: The Kautsky effect. Characteristic variations of the fluorescence intensity are shown when a dark-adapted sample is illuminated by continuous light. The time axis is logarithmic ([http://en.wikipedia.org/wiki/Image:Kautsky\\_effect.PNG](http://en.wikipedia.org/wiki/Image:Kautsky_effect.PNG)).

In the dark-adapted state (DAS) of a plant, electron carriers in the acceptor side of PSII are reoxidised. When weak measuring light (photon flux density (PFD)  $< 0.1 \mu\text{mol}(\text{photons})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is applied, *minimum ChlF yield in DAS* ( $F_0$ ) can be measured (Fig. 6). *Maximum ChlF yield in DAS* ( $F_M$ ) is reached when saturating pulse (PFD up to  $10 \text{mmol}(\text{photons})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is applied. In such situation, all active RCs of PSII are closed because their electron acceptors  $Q_A$  are fully reduced. A difference between  $F_M$  and  $F_0$  is referred to as *maximum variable ChlF yield in DAS* ( $F_V$ ):

$$F_V = F_M - F_0 / 2/.$$

*Maximum quantum yield of PSII photochemistry* ( $\Phi_{P_0}$ ) requires values of  $F_M$  and  $F_0$  to be calculated. It is defined as follows:

$$\Phi_{P_0} = \frac{F_V}{F_M} = \frac{F_M - F_0}{F_M} = 1 - \frac{F_0}{F_M} / 3/.$$

This parameter is almost constant for many plant species under non-stressed conditions and amounts to  $0.832 \pm 0.004$ .

When the actinic radiation is turned on, changes in the fluorescence yield appear. Initially, *maximum ChlF yield measured when the actinic radiation is switched on* ( $F_P$ ) is achieved. Within few minutes, the leaf gradually passes from the DAS to the light adapted state (LAS). When processes of light and dark reactions of photosynthesis are equilibrated, the *steady state ChlF yield in LAS* ( $F_S$ ) is reached.

Similarly as in DAS, *maximum ChlF yield in LAS* ( $F_M'$ ) and *minimum ChlF yield in LAS* ( $F_0'$ ) can be measured.  $F_0'$  level is usually achieved using a short pulse of the weak far-red radiation. The equation of *maximum variable ChlF yield in LAS* ( $F_V'$ ) is then:

$$F_V' = F_M' - F_0' / 4/.$$

Frequently used ChlF parameter is the *effective quantum yield of photochemical energy conversion in PSII* ( $\Phi_{II}$ ):

$$\Phi_{II} = \frac{F_M' - F_S}{F_M'} \quad /5/.$$

When  $\Phi_{II}$  is multiplied by the light intensity, we receive the *relative rate of linear electron transport*. Under laboratory conditions, linear electron transport and CO<sub>2</sub> fixation correlate well (Genty et al. 1989, Maxwell and Johnson 2000). But under field conditions, the correlation may be broken. It can be caused by changes in the relative rates of CO<sub>2</sub> fixation and competing processes such as photorespiration.

Important detailed information about ChlF yields and parameters is described by Roháček (2002).

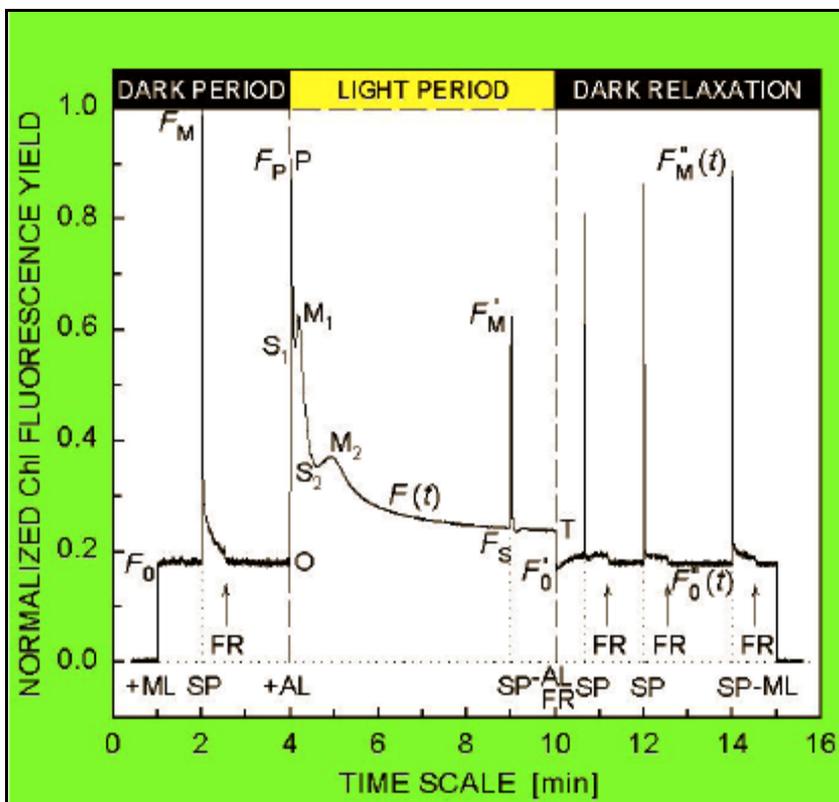


Fig. 6: ChlF induction curve (courtesy of K. Roháček).

### 2.2.3. Chlorophyll Fluorescence Measurement

The most frequently used fluorometers (devices measuring fluorescence) are based on PAM principle (Pulse Amplitude Modulation, Schreiber 1986). They enable measuring of ChlF even under full daylight.

Recently, two types of fluorometers are used: imaging and non-imaging ones.

*Non-imaging fluorometers* deal with an analysis of the ChlF signal collected or integrated from the whole sample area. Result of a measurement is an induction curve or/and a set of fluorescence parameters.

However, photosynthesis and consequently ChlF can be altered by various external and/or internal factors (e.g. pigment composition, senescence, abiotic and biotic stress factors) that vary over the surface of a leaf or plant. An imaging approach is required to reveal such heterogeneity. *Imaging fluorometers* allow the examination of individual pixels in the ChlF image with fluorescence transients. These transients can be analysed individually or grouped into objects, such as leaves, plants etc. Consequently, ChlF imaging systems can provide detailed information about spatial and kinetic heterogeneity of the fluorescence signal.

## 2.3. Chlorophyll Spectra

### 2.3.1. Absorption Spectrum of Chlorophyll *a*

Chl *a* is the major photosynthetically active pigment in plants. It absorbs blue and red light of the visible spectrum (Fig. 7). After absorption of a “red” photon, Chl *a* molecule is raised from the ground state ( $S_0$ ) into the excited state one ( $S_1$ ). Similarly, a “blue” photon raises Chl *a* into the excited state. But its energy is higher, so excitation into the excited state two ( $S_2$ ) occurs. However, the lifetime of  $S_2$  is very short, so it returns in about  $10^{-13}$  s to  $S_1$  while the energy is dissipated as heat (Fig. 8).

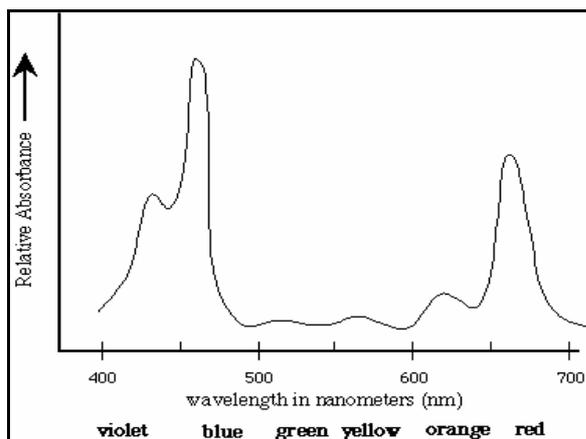


Fig. 7: Absorption spectrum of Chl *a*. There are two peaks of the absorbance - in the blue and red part of the visible spectrum ([www.bio.davidson.edu/Courses/Bio111/Bio111LabMan/lab1fig3.gif](http://www.bio.davidson.edu/Courses/Bio111/Bio111LabMan/lab1fig3.gif)).

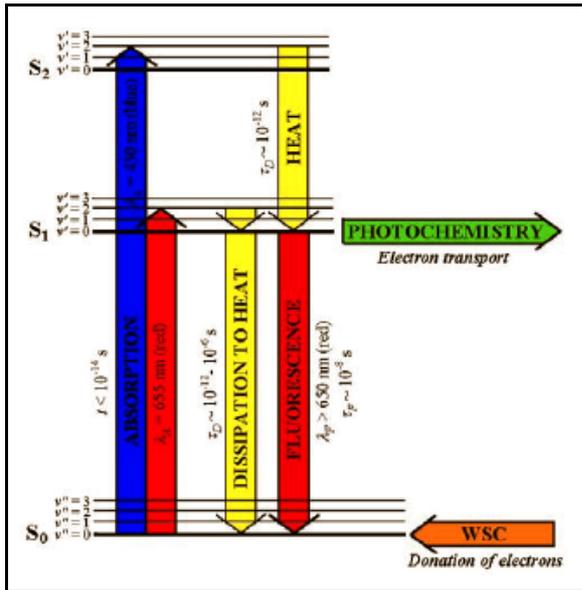


Fig. 8: The three-level scheme of the Chl molecule.  $S_0$  – ground state,  $S_1$  – excited state one,  $S_2$  – excited state two. Excitation (due to the absorption of a “red” or a “blue” photon) and deexcitation processes (photochemistry, dissipation as heat and Chl fluorescence) are shown (courtesy of K. Roháček).

### 2.3.2. Chlorophyll Fluorescence and Reflectance Spectra of a Leaf under Sunlight

The fluorescence spectra consist of three bands: 450 - 520 nm (blue-green), 685 nm (red), and 735 nm (far-red) (Fig. 9). The blue-green fluorescence emission originates from cell wall bound fluorophores, and it isn't linked to Chl or to photosynthesis. Red and far-red fluorescence are emitted from Chls that are probably the only constituents of the biosphere to fluorescence in those parts of the emission spectrum. Fig. 9 also shows the reflectance spectrum of a green leaf (for definition and more information see part 2.4.).

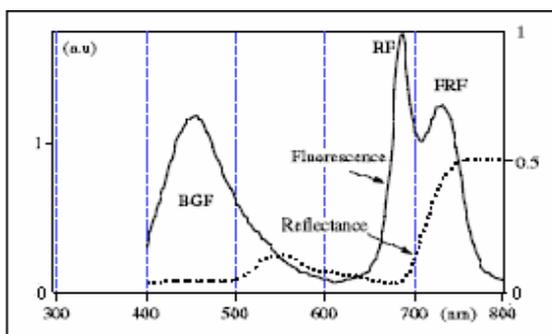


Fig. 9: Solid line shows the fluorescence spectrum of a leaf with three peaks: in the blue-green (BGF), red (RF) and far-red (FRF) part of the spectrum. Dashed line shows the reflectance spectrum of the same sample ([www.ese.u-psud.fr/ecophysio/biospectro/pdfs/MoyaI2004RemSensEnviron.pdf](http://www.ese.u-psud.fr/ecophysio/biospectro/pdfs/MoyaI2004RemSensEnviron.pdf)).

## 2.4. Remote Sensing of Vegetation

Sun radiance striking upon vegetation can be absorbed, reflected or transmitted. Up to now, most of the information that has been acquired by remote sensing has come from the analysis of reflectance data (Gitelson et al. 1996; Lichtenthaler et al. 1998; Kogan et al. 2003).

Reflectance coefficient (R) is defined here as the ratio between the energy flux reflected by the sample in a given solid angle and the energy flux reflected by a white lambertian surface (surface which reflects the energy equally into all directions) under the same conditions and the same solid angle. However, reflectance signal alone cannot report in sufficient detail and resolution on the photosynthetic activity and dynamics of vegetation. ChlF emission is frequently considered to be more direct signal of vegetation dynamics.

Recent approaches in remote sensing of vegetation are reviewed by Moya and Cerovic (2004) and Schaepman (2006).

### 2.4.1. Reflectance

Beside the whole vegetation reflectance spectra (Fig. 9), combination of selected wavelengths (optical vegetation indices) have been proposed with the aim to monitor biomass, phenology phases of plant canopy, nutrient status, general stress and photosynthetic efficiency (Gamon et al. 1995, Peñuelas and Inoue 2000). The most widely used indices follow:

Normalized difference vegetation index (NDVI; Rouse et al. 1973) is a widely used broadband canopy greenness indicator (correlates with chlorophyll content). It is also inter-correlated to the biomass variable, such as leaf area per unit of land area (leaf area index, LAI), leaf cover, and fraction of solar radiation that green leaves in the canopy absorb (fAPAR) (Gamon et al. 1995):

$$NDVI = \frac{R_{nir} - R_{red}}{R_{nir} + R_{red}} /6/,$$

where  $R_{nir}$  and  $R_{red}$  mean reflectance in the near infra-red and red parts of the solar spectrum.

Photochemical (plant, physiological) reflectance index (PRI; Gamon et al. 1995) is used to estimate the light use efficiency (LUE). PRI is closely connected to the processes of excess solar radiation dissipation by plants, i.e. xanthophyll cycle (epoxidation of violaxanthin via antheraxanthin to zeaxanthin), thylakoid membrane  $\Delta pH$  formation and chloroplast conformational changes (reviewed by Adams and Demmig-Adams 2004). This index is defined as follows:

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}} /7/,$$

where  $R_{xxx}$  means reflectance measured at particular wavelengths.

## 2.4.2. Fluorescence

The process of ChlF measuring in field differs from that in laboratory. Here, the monitored plants cannot be manipulated or dark-adapted. Recently, two approaches in *fluorosensing* (measurement of ChlF in remote sensing mode) are applied:

*Active fluorosensing* is based on using high power lasers to elicit fluorescence of vegetation. Its use in remote sensing mode is limited due to the eye-protection recommendations and standards, as well as due to technical limitation of instrumentation (reviewed by Moya and Cerovic 2004).

*Passive fluorosensing* does not use any additional light sources, since it measures sun-induced ChlF. Although the amount of ChlF emitted from vegetation represents only a small fraction of the reflected visible radiation, it's possible to detect the ChlF signal at certain wavelengths, where the solar spectrum (Fig. 10) is attenuated due to the absorption by elements contained in the solar and terrestrial atmosphere (Fraunhofer and oxygen lines). Two following oxygen absorption bands are suitable for remote sensing of ChlF: A band (760 nm) and B band (687 nm). Using FLD (Fraunhofer Line Discrimination) principle (Fig. 11) ChlF can be extracted from the reflectance spectra of vegetation (reviewed by Moya et al. 2004). Passive ChlF measurement is currently considered to be the most promising technique for global monitoring of vegetation photosynthetic activity at large spatial and long time scales. Furthermore, if passive measurement is conducted at wavelengths not affected by reabsorption, e.g. in the oxygen A band (760 nm), ChlF signal emitted also from lower layers of plant canopy can be obtained.

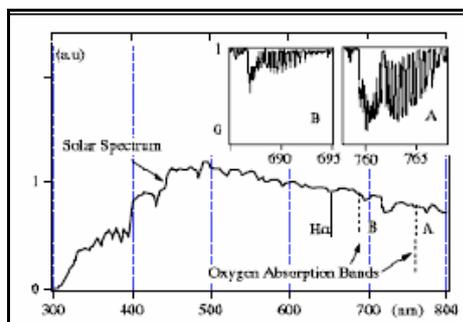


Fig. 10: Solar spectrum at sea level. Dashed lines: Oxygen absorption bands. Continuous line: Hydrogen absorption band. Inset: Moderately integrated profile of the oxygen absorption bands ([www.ese.upsud.fr/ecophysio/biospectro/pdfs/MoyaI2004RemSensEnviron.pdf](http://www.ese.upsud.fr/ecophysio/biospectro/pdfs/MoyaI2004RemSensEnviron.pdf)).

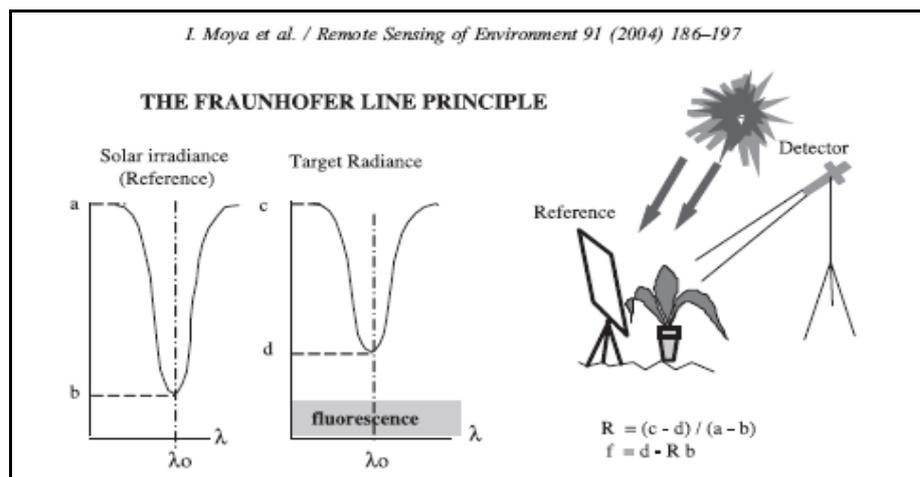


Fig. 11: The Fraunhofer line discrimination principle. The method is based on the partial in-filling of the absorption band by the sun-induced fluorescence emanating from the luminescent target ([www.ese.u-psud.fr/ecophysio/biospectro/pdfs/MoyaI2004RemSensEnviron.pdf](http://www.ese.u-psud.fr/ecophysio/biospectro/pdfs/MoyaI2004RemSensEnviron.pdf)).

Field measurements of ChlF are limited to parameters related to LAS of plants, i.e.  $F_S$  and  $F_M'$ . However, the only parameter that can be detected by *passive fluorosensing* is  $F_S$ . It is modulated by a convolution of photochemical and non-photochemical quenching processes that are also dependent on both external (e.g., light, temperature, humidity,  $CO_2$  and water availability, nutrients or various stressors) and internal factors (e.g., pigmentation, enzyme activation). However, several studies indicated that sun-induced ChlF (e.g., Carter et al. 1990, 1996; Freedman et al. 2002) or  $F_S$  induced by artificial irradiation (e.g., Dobrowski et al. 2005; Flexas et al. 2000, 2002) are linked to  $CO_2$  assimilation.

## 2.5. Photosynthesis in Evergreen Plants

Evergreen plants exhibit typical seasonal variations of photosynthetic activity (e.g., Ensminger et al. 2004; Öquist and Huner 2003). Photosynthesis declines significantly during autumn and it is completely recovered during spring. Especially, temperate and boreal evergreen plants are forced to face varying abiotic conditions during the year. The most difficult season to cope with is the winter season. Evergreen plants face mainly two problems during winter periods: cellular water freezing and consequent desiccation of living cells, and excess irradiance when photosynthesis is restricted or even inhibited by low temperatures. Since they retain their leaves/needles for several years, they developed acclimation processes that protect their photosynthetic apparatus. The complex adaptive mechanisms include changes in the thylakoid membrane, particularly in the composition of pigments, lipids, fatty

acids and chlorophyll-protein complexes (reviewed in Öquist and Huner 2003). In particular, one of the most important changes is a reduction of antenna size and antennae reorganization, partial loss of PSII RCs, and development of a sustained non-photochemical quenching of absorbed light as heat (Fig. 12). PSI is considered to be more resistant. Thus, number of PSI doesn't change significantly. PSI can act as a non-radiative quencher (energy is dissipated as heat) or can drive cyclic electron transport (in thawing periods), which supports pumping of protons into the lumen of chloroplasts and consequent ATP synthesis. Remaining PSII RCs can also act as non-radiative quenchers (e.g., Bukhov et al. 2001). PQ pool remains reduced thanks to the electron flow from remaining PSII RCs and cyclic electron transport. This reduction state also contributes to the prevention from photooxidative damage.

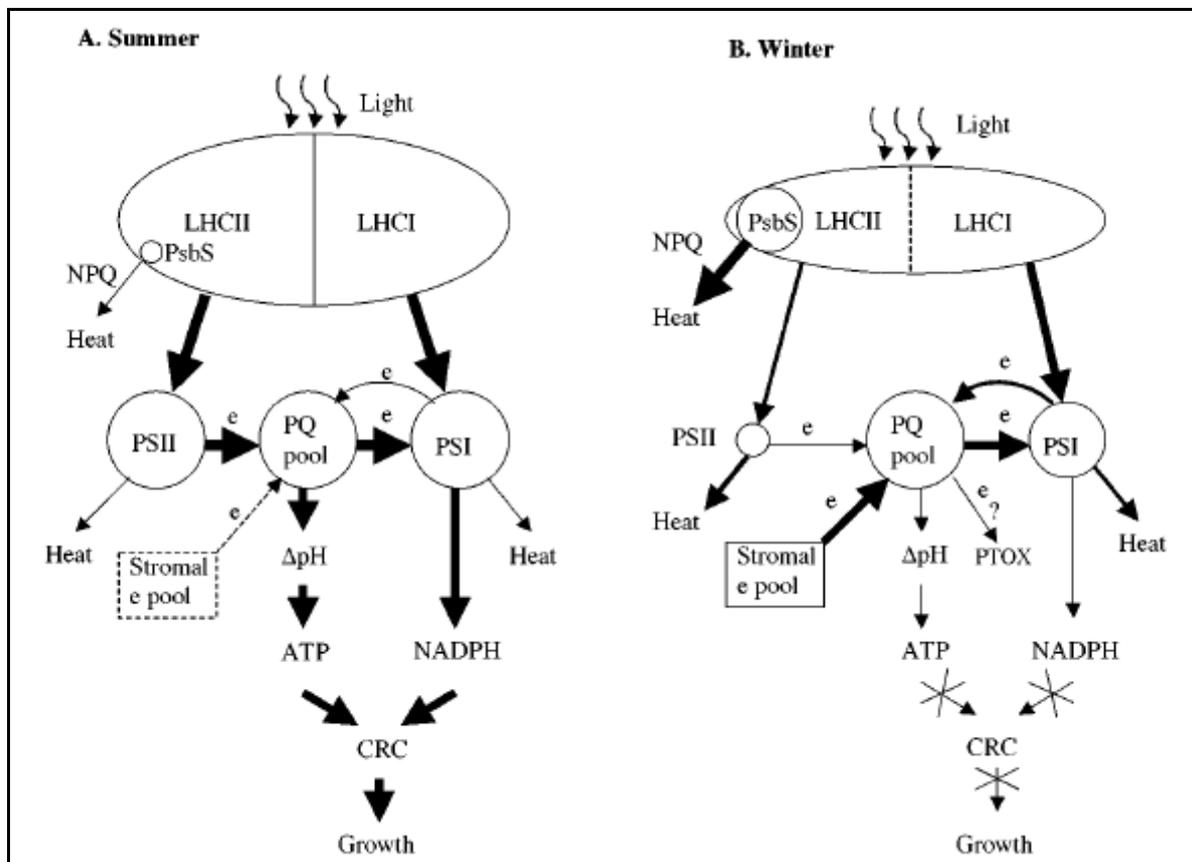


Fig. 12: A schematic overview illustrating the organization of the photosynthetic apparatus and the patterns of energy flow in evergreen Scotch pine during (A) summer and (B) winter. Most of the processes are described in the text (from Öquist and Huner 2003).

Cold acclimation also induces changes in the gene expression and in biochemical pathways (e.g., synthesis of anti-freeze proteins leading to the retardation of ice-crystal growth and consequent protection from the dehydration damage) that stand behind all above-mentioned adaptations.

At the end of winter period when temperatures rise, overwintering evergreens start to restore their photosynthetic apparatus. However, in the early spring period minimal photosynthetic efficiency was measured (Öquist and Huner 2003). Here, although average daily temperatures are higher, the combination of intense irradiation and freezing night temperatures leads to the decrease of photosynthetic efficiency.

### **3. MATERIALS AND METHODS**

#### **3.1. Locality and Plants**

Measurements were conducted in Nové Hradý (48° 47' 22.67"N, 14° 46' 42.19"E, 541 meters AMSL, SW of the Czech Republic) from February 2005 to April 2006 (partial measurements were not conducted during the whole period (for details see further): ChlF measurement of rhododendron from February 2005 to February 2006, NDVI and PRI measurements from October 2005 to April 2006). Here, I and my colleagues measured seasonal variations of ChlF emission under natural conditions of two evergreen overwintering plants: *Picea omorika* (*Pinaceae*; Fig. 13) and *Rhododendron x hybridum* (*Ericaceae*; Fig. 14). Plants grow in the park of the Institute of Systems Biology and Ecology ASCR, p.r.i. Both plants are approximately thirty years old. The measurements were performed on intact shoots/leaves. Four sun and four shade spruce shoots and six sun rhododendron leaves were monitored during the year. Furthermore, CO<sub>2</sub> fixation rate and reflectance indices (NDVI and PRI) were measured on rhododendron.

The temperature course was automatically monitored by data-logger in the meteorological station. Actual irradiance was measured using the light meter LI-250A (LI-COR Biosciences, USA; [www.licor.com](http://www.licor.com)) at each measuring time.



Fig. 13: *Picea omorika* growing in the park of the Institute of Systems Biology and Ecology ASCR in Nové Hradý.



Fig. 14: *Rhododendron x hybridum* growing in the park of the Institute of Systems Biology and Ecology ASCR in Nové Hradý.

### 3.2. Measurement of Chlorophyll Fluorescence Emission

ChlF imaging system FluorCam (P.S.Instruments, Ltd., Brno, Czech Republic, [www.psi.cz](http://www.psi.cz)) was used for measurements of ChlF emission from intact shoots/leaves. FluorCam measures sequences of ChlF images with user-defined irradiance protocols and timing of measurements, and allows measurement even under full sunlight (described by Nedbal et al. 2000). Here, I used Open version of FluorCam ([www.psi.cz/products/fluorcams/open.htm](http://www.psi.cz/products/fluorcams/open.htm)).

Open FluorCam consists of three functional components (Fig. 15): LED panels, a halogen lamp and a CCD camera. LED panels provide measuring flashes and continuous actinic light, halogen lamp (250 W) equipped with a shutter generates white saturating flashes. A CCD camera captures ChlF images at 12-bit resolution in 512 x 512 pixels taking maximally fifty images per second. The CCD camera, LED panels and the light source generating saturating flashes can be arranged at various angles and distances from the sample.

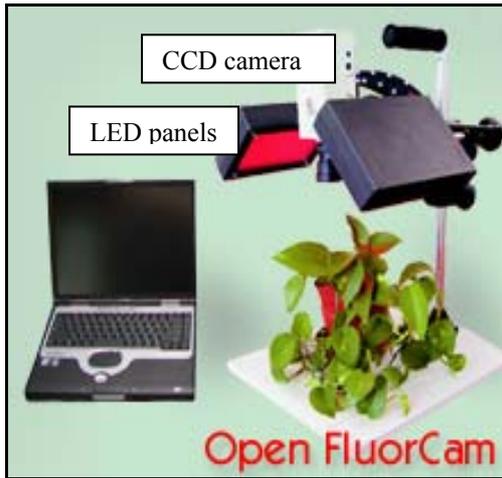


Fig. 15: Open FluorCam, P.S. Instruments, Ltd. ([www.psi.cz/products/fluorcams/open.htm](http://www.psi.cz/products/fluorcams/open.htm)).

The measuring protocol was as follows:  $F_S$  was measured using eight short ( $10\mu s$ ) weak non-actinic measuring flashes placed 800 ms apart. Then, saturating flash ( $1.6 s$ ,  $1500 \mu mol(\text{photons}).m^{-2}.s^{-1}$ ) generated by the halogen lamp was used to close all RCs and to measure  $F_M'$ .  $\Phi II$  was then calculated using the equation /5/.

The measurements were done on intact shoots/leaves under natural sunlight. Measured fluorescence signal was integrated over all shoots/leaves to simulate the signal that can be detected by remote sensing technique.

### 3.3. Gas Exchange Measurement

Actual  $CO_2$  assimilation rate under ambient light conditions, air temperature and humidity was measured using CIRAS I (Fig. 16; PP-Systems, Ltd., UK, [www.ppsystems.com](http://www.ppsystems.com)). Here, I conducted measurement on rhododendron leaves by means of the Broad leaf cuvette PLC6 (U) (Fig. 17). The leaves were kept inside the cuvette under a constant ambient  $CO_2$  concentration ( $375 \pm 5 \mu mol(CO_2).mol^{-1}$ ).  $CO_2$  consumption rate measurement was conducted in parallel with fluorescence measurement on the same leaves.

The principle of the measuring of  $CO_2$  concentration in the cuvette is based on the infrared gas analysis.  $CO_2$  has the peak of absorbance at the wavelength of  $4.26 \mu m$ .



Fig. 16: CIRAS I, PP-Systems, Ltd.  
([www.ppsystems.com](http://www.ppsystems.com)).



Fig. 17: Broad leaf cuvette PLC6 (U),  
PP-Systems, Ltd.  
([www.ppsystems.com](http://www.ppsystems.com)).

### 3.4. Measurement of Reflectance Indices

I measured two reflectance indices on rhododendron leaves: NDVI (see eq. /6/) and PRI (see eq. /7/). Prototypes from P.S.Instruments, Ltd. ([www.psi.cz](http://www.psi.cz)) were employed in order to test their usefulness. At the time of writing, commercial portable versions called PlantPen NDVI 300 & PRI 200 are available (Fig.18).



Fig. 18: PlantPen NDVI 300 & PRI 200, P.S.  
Instruments, Ltd. ([www.psi.cz/new/issue5.htm](http://www.psi.cz/new/issue5.htm)).

## 4. RESULTS

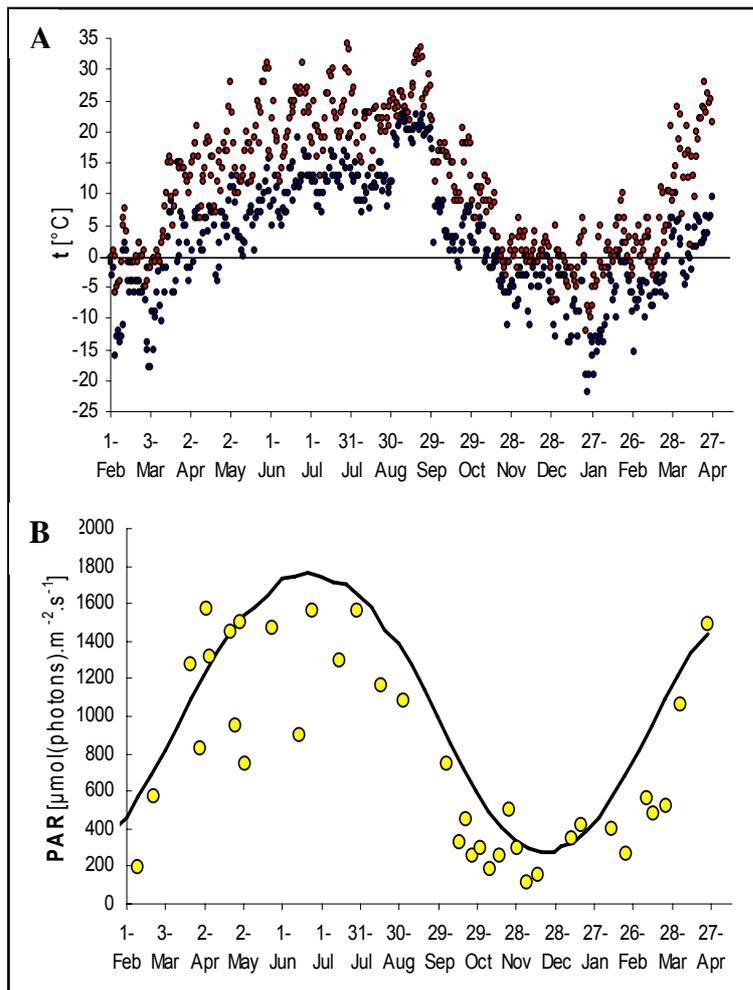


Fig. 19:

Panel A shows the temperature evolution during the measuring campaign, i.e. from February 2005 to April 2006. Blue dots: minimum daily temperatures. Red dots: Maximum daily temperatures.

Panel B displays the irradiance evolution during the same period. Yellow dots: Actual irradiance measured in the vicinity of monitored plants. Black curve: The annual course of potential daily maxima of PPFd for Nové Hradý location under clear sky conditions.

$F_s$  and  $\text{CO}_2$  assimilation rate (A) were measured from February 2005 till April 2006 (except for rhododendron, see Materials and Methods) under field conditions. Fig. 19 (panel A) shows how daily minimum (blue dots) and maximum (red dots) temperatures evolved during the measuring period. One can see that the daily minimum temperatures were dominantly above  $0^{\circ}\text{C}$  from the 24<sup>th</sup> April 2005 to the 18<sup>th</sup> October 2005 and from the 13<sup>th</sup> April 2006 to the end of the measuring campaign; and they were mostly below  $0^{\circ}\text{C}$  from the beginning of the measuring campaign to the 23<sup>rd</sup> April 2005 and from the 19<sup>th</sup> October 2005 to the 12<sup>th</sup> April 2006. Panel B presents the evolution of the actual irradiance that was measured in open place perpendicularly to the sky in the vicinity of monitored plants during the same period (yellow dots), and the annual course of potential daily maxima PPFd (photosynthetic photon flux density) for Nové Hradý location under clear sky conditions (black curve) estimated according to Jones (1992) calculated by PhotosynAssistant programme (Dundee Scientific, UK). This result was kindly provided by Mgr. Otmar Urban,

Ph.D. (Workgroup of Ecophysiology of Photosynthesis, Laboratory of Plants Ecological Physiology, ISBE, Brno).

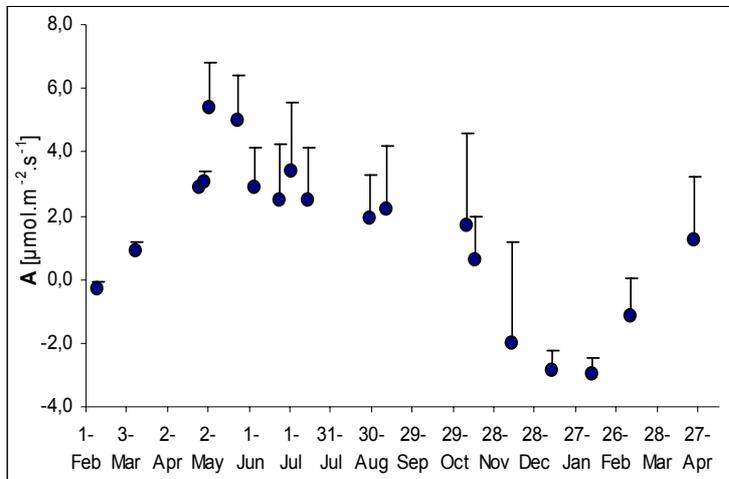


Fig. 20: The evolution of  $\text{CO}_2$  assimilation rate (A) of rhododendron during the measuring campaign.

Fig. 20 displays the evolution of A of rhododendron leaves measured under ambient conditions. The course of A follows the temperature course, it increases during spring and decreases during autumn. Maximum value of A was observed on the 5<sup>th</sup> May 2005. There are quite constant values of A during summer and rapid decline in autumn when the minimum temperatures drop below  $0^\circ$ . Negative values of A were observed (from the 12<sup>th</sup> December 2005 to the 9<sup>th</sup> March 2006) due to restricted photosynthesis and continuous respiration in winter.

Fig. 21 shows the evolution of  $\Phi\text{II}$  in spruce shoots (panel A) and rhododendron leaves (panel B). One can observe that  $\Phi\text{II}$  evolution in rhododendron leaves as well as in spruce shoots followed similar course. Generally, during almost the whole measuring campaign, values of  $\Phi\text{II}$  of the shade spruce shoots were higher than sun exposed ones (data not shown).

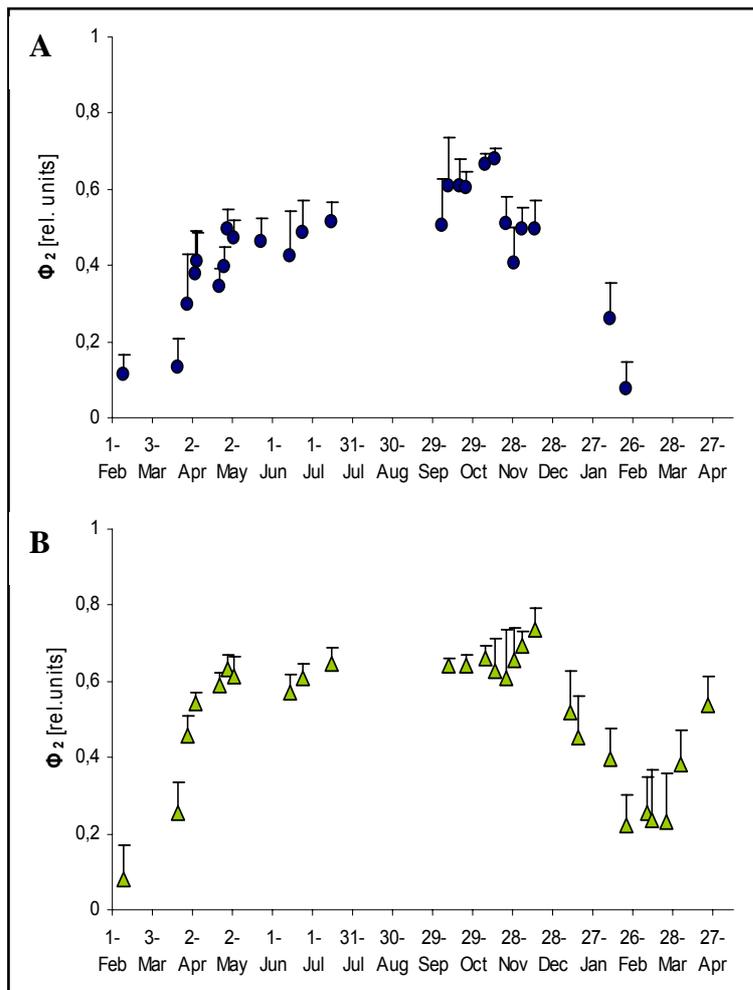


Fig. 21:  $\Phi_{II}$  evolution of rhododendron (panel A) and spruce (panel B). The measuring period for rhododendron was shortened (February 2005-February 2006).

This study was focused on the parameter  $F_S$ .  $F_S$  also evolved similarly to the course of  $\Phi_{II}$ . Generally,  $F_S$  was low and stable in winter and then it steeply increased in spring. During the vegetation season  $F_S$  was also quite stable and it rapidly decreased in autumn. The change of signal intensity occurred at the threshold time when night freeze temperatures frequently occurred combined with higher daily temperatures (second half of March and first half of April 2005; and similarly for spring 2006). We could say that the change of signal could indicate a transition state between the winter dormant state and the photosynthetically active state during vegetation season.

Here, I also investigated the correlation between  $F_S$  and the air temperature. According to Soukupová et al. (manuscript),  $F_S$  correlates well with variations of the minimum temperatures, especially with the mean minimum temperatures for the three days previous to the measurement. Thus, I also plotted  $F_S$  of rhododendron leaves (Fig. 23, Panel A), sun (Fig. 23, Panel B) and shade (Fig. 23, Panel C) spruce shoots measured during the whole campaign to the mean minimum temperatures for the three days previous to the measurements. One can

observe that variations of  $F_S$  are quite significant particularly within the temperature interval between  $-5\text{ }^\circ\text{C}$  and  $+5\text{ }^\circ\text{C}$ , i.e. mainly in the transition period during spring and autumn. Below  $-5\text{ }^\circ\text{C}$  and above  $+5\text{ }^\circ\text{C}$   $F_S$  is quite stable. These findings are in agreement with above-mentioned presumption that  $F_S$  is quite stable in summer and winter compared to periods approximately one month long in spring and autumn.

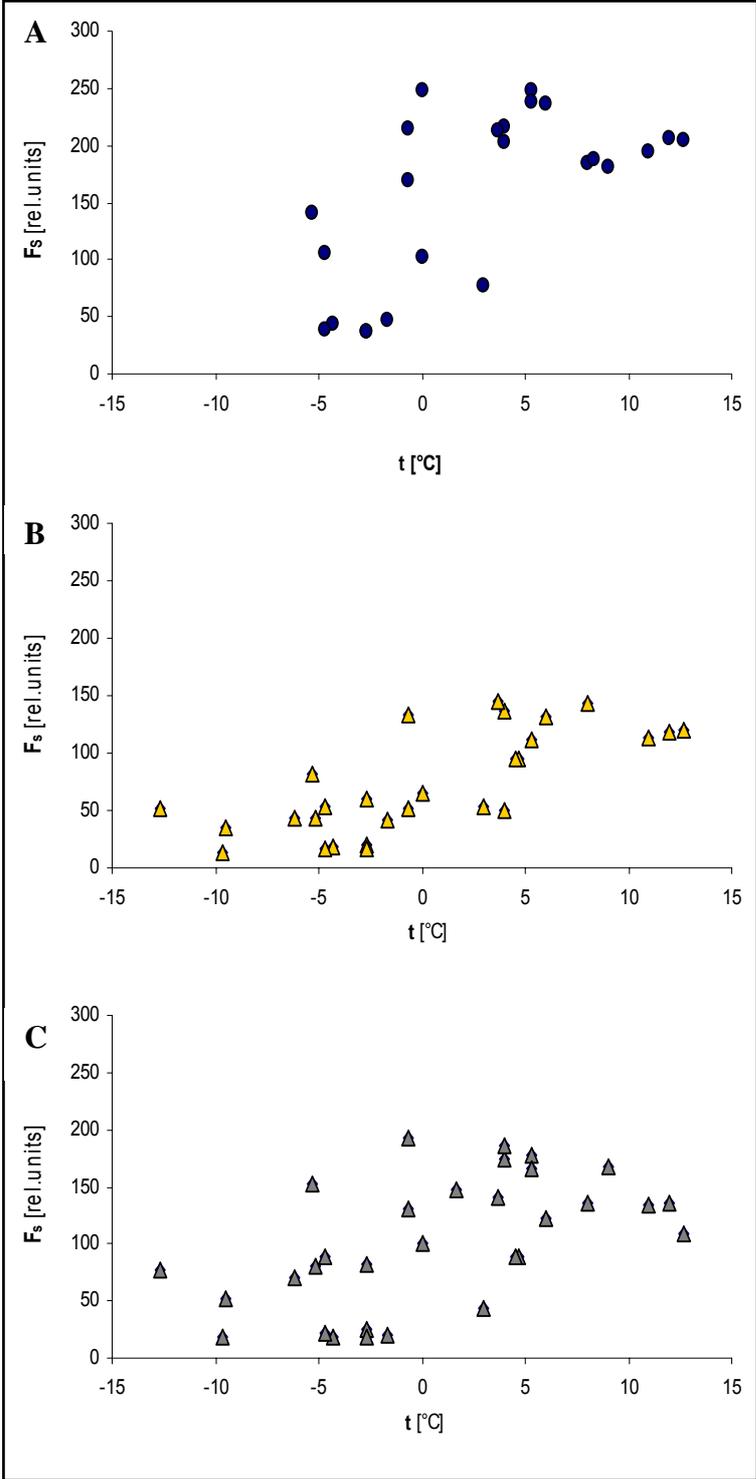


Fig. 23: The dependence of  $F_S$  on mean minimum daily temperature for the three days previous to the measurement. Panel A: rhododendron leaves. Panel B: sun spruce shoots. Panel C: shade spruce shoots.

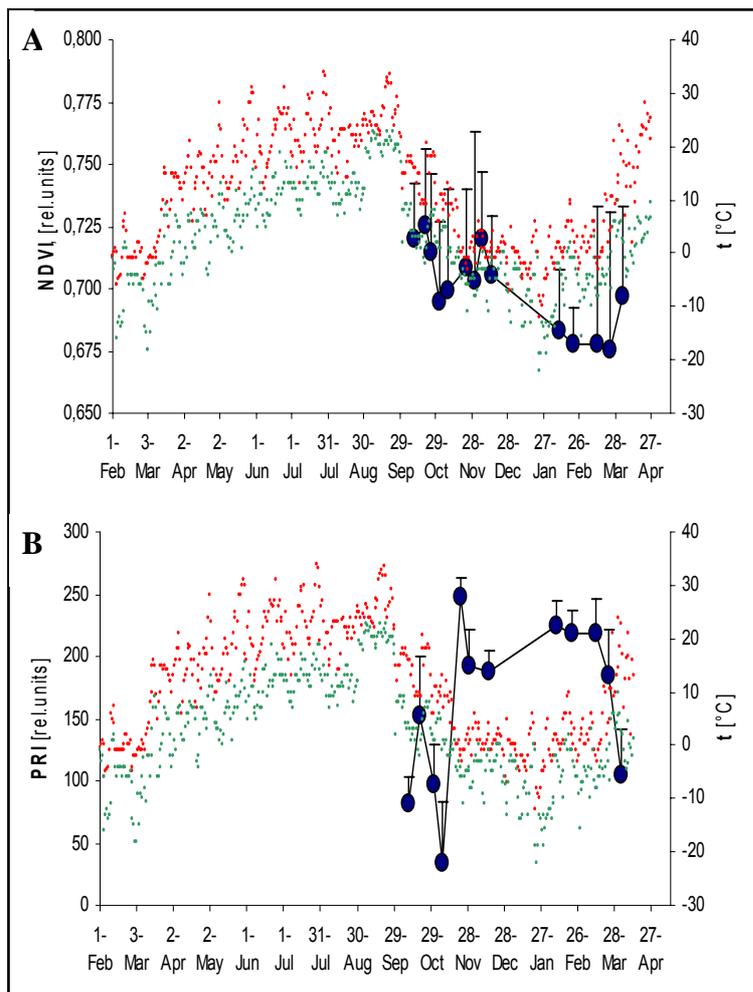


Fig. 24: The evolution of reflectance indices (big blue dots) NDVI (panel A) and PRI (panel B). Minimum (small blue dots) and maximum (small red dots) daily temperatures are also plotted in order to show the relationship of the indices to temperature. Measurements were conducted from October 2005 to April 2006.

The last task of this study was to test two prototypes of handy portable instruments for measurement of two reflectance indices, PRI /6/ and NDVI /7/ (P.S.Instruments, Ltd. Brno, [www.psi.cz](http://www.psi.cz)), and to investigate changes of reflectance indices during the year.

Since we had these prototypes only for limited time, Fig. 24 presents the variations of NDVI (panel A) and PRI (panel B) indices from the 11<sup>th</sup> October 2005 to the 4<sup>th</sup> April 2006. The measurements were conducted on rhododendron leaves. Moreover, because the prototypes did not calculate the indexes in their appropriate values, the attention should be paid only to their time course.

NDVI seems to be higher during vegetation season compared to winter. I observed its decrease during autumn and slight increase in spring. Since NDVI relates to the chlorophyll content (Rouse et al. 1973; Gamon et al. 1995), its variation during the measuring period is in agreement with Öquist and Huner (2003). They reviewed that during winter period partial loss of chlorophyll occurred in plant tissues.

Panel B shows the time evolution of PRI. Values of PRI were lower in late autumn and in spring compared to winter period. These findings are also in agreement with what we expected from literature: PRI correlates well with zeaxantin formation and with the non-photochemical quenching involvement (Adams and Demmig-Adams 2004), which are the processes extensively evolved in winter preventing the photooxidative damage of photosynthetic apparatus (Öquist and Huner 2003).

## 5. DISCUSSION

The aim of this study was to contribute to the revealing the information potential of  $F_S$ , which can be detected by remote sensing technique. It is proposed to be a suitable tool for long term monitoring of photosynthetic activity of the whole ecosystems (see planned FLEX mission, [www.esa.int/esaLP/SEM2VSBE8YE\\_index\\_0.html](http://www.esa.int/esaLP/SEM2VSBE8YE_index_0.html)). Here, we measured seasonal variation of  $F_S$  and  $\Phi_{II}$  in two evergreen plant species: *Rhododendron x hybridum* and *Picea omorika*. The measurements were done on shoots/leaves level. Measurements of A, PRI and NDVI were performed on rhododendron leaves.

I observed that  $F_S$  evolved similarly to A (Fig. 20) during the year. The seasonal variations of A in these two evergreen plants are in agreement with the already published data (Öquist and Huner 2003): there is a decline during autumn and a complete recovery during spring. According to Genty et al. (1989),  $\Phi_{II}$  should correlate well with A. Nevertheless, this correlation was broken as follows from the results of my experiments, e.g. due to high values of  $\Phi_{II}$  in early spring and late autumn (see Fig. 21) compared to low values of A during the same periods (see Fig. 20). One of the reasons could be that my experiments were conducted in field conditions, unlike those described by Genty et al. (1989) that were done under controlled laboratory conditions.

I observed that  $F_S$  in two evergreen plants was low and stable in winter, and then it rapidly increased in spring. During summer the  $F_S$  signal was high and stable, and its rapid decrease from the beginning of November was recorded. These changes may be explained according to Öquist and Huner (2003): in winter there are mechanisms of non-radiative energy dissipation, which are extensively involved in order to prevent photooxidative damage of photosynthetic apparatus and which maintain a low ChlF yield. Other factors, which might influence the level of  $F_S$  particularly in summer, are as follows: high irradiance and resultant intense excitation and, on the other hand, stronger photochemical quenching due to light reactions of photosynthesis. These mechanisms could be dynamically activated and deactivated during the transition period often combined with intermittent night freeze in spring and in autumn. Specifically in the spring period plants often experience the intense temperature/irradiance stress (Öquist and Huner 2003). It happens due to the coincidence of the high irradiance and concurrent below-zero night temperatures. Photosynthesis does not reach the total recovery until temperatures stay above 0 °C (Ensminger et al. 2004).

Based on the results, we propose that the remotely sensed  $F_S$  of vegetation could be used as a marker of “on”/“off” state of its photosynthetic apparatus: the value of  $F_S$  signal can indicate whether the photosynthetic apparatus is active or inactive.

Simultaneously to my measurements of ChlF in field, Silvie Svidenská performed lab-based experiments under controlled light conditions but under temperatures corresponding to those in field. Her experiments were conducted on shoots/leaves detached close to the labelled ones (i.e. measured ones) in field. She also found out that the conventional ChlF yields ( $F_0$ ,  $F_M$ ) and parameter  $F_V/F_M$  as well as  $F_S$  under actinic light ( $100 \mu\text{mol}(\text{photons})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) revealed the same seasonal pattern (published in bachelor thesis of Silvie Svidenská).

I observed that spruce sun shoots had generally lower ChlF signal than shade ones (data not shown). I would explain it by the difference in chlorophyll content. Sun shoots have enough light to drive photosynthesis, so the content of pigments is lower than in shade shoots.

Photosynthesis is largely influenced by two external parameters: irradiance and temperature. To obtain complete information about  $F_S$  and its relation to photosynthesis, dependence of  $F_S$  on these two crucial parameters should be studied. Soukupová and collaborators (manuscript) demonstrated that the main impulse for the activation of photosynthesis and increase of  $F_S$  during spring recovery period is temperature. They found the best correlation of  $F_S$  in spruce shoots with the mean minimum temperature for the three days previous to the measurement. It supports the hypothesis that temperature alone has a major impact on the timing of seasonal changes in photosynthetic efficiency of plants (reviewed by Öquist and Huner 2003). Moreover, Ensminger and collaborators (2004) figured out that total recovery of photosynthesis of Scots pine occurred when temperatures stayed long term above  $0^\circ\text{C}$ .

Here, I correlated changes of measured  $F_S$  signal in two evergreen plants during the whole year with temperature (Fig. 23). According to Soukupová et al. (manuscript), I have also chosen the mean minimum temperature for three days previous to the measurement. In contrast with the strong correlation in spruce shoots during the spring recovery (Soukupová et al., manuscript), the correlation of  $F_S$  when measured during the whole year to the minimum temperatures is not so obvious. I conclude that it is because my measurements were not restricted just to spring period. As a consequence, more processes influencing the  $F_S$  yield (including photoinhibition, reduction of chlorophyll content in winter, different involvement of photochemical and non-photochemical quenching during the year etc.) affected my findings.

The last aim of my bachelor thesis was testing the handy portable instruments measuring two reflectance indices: NDVI and PRI. Since NDVI correlates with chlorophyll content in plants (Gamon et al. 1995), it decreases in autumn and increases in spring (see Fig. 24, panel A). It is in accordance with the published data about the annual changes of chlorophyll content in leaves (reviewed by Öquist and Huner 2003).

PRI expressed an opposite pattern (Fig. 24, panel B). It is higher in winter compared to summer. PRI is closely connected to the processes of excess solar radiation dissipation by plants, and with the concentration of xanthophylls in leaves, which is higher in winter period (reviewed by Öquist and Huner 2003). Although the measuring period of NDVI and PRI was not long enough to follow their variations during the whole year, I can conclude that the evolution of NDVI and PRI followed the expected course.

## 6. CONCLUSION

The most important conclusions referring to the steady state ChlF yield in LAS ( $F_s$ ) are as follows:

Annual  $F_s$  evolution tracks seasonal changes of  $CO_2$  assimilation rate. Transition periods between photosynthetically active and inactive state in plants are accompanied by significant rise of  $F_s$  signal. Thus, the change of the  $F_s$  signal of the vegetation could be used as a marker of the “on”/”off” state of the photosynthetic activity in plants.

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