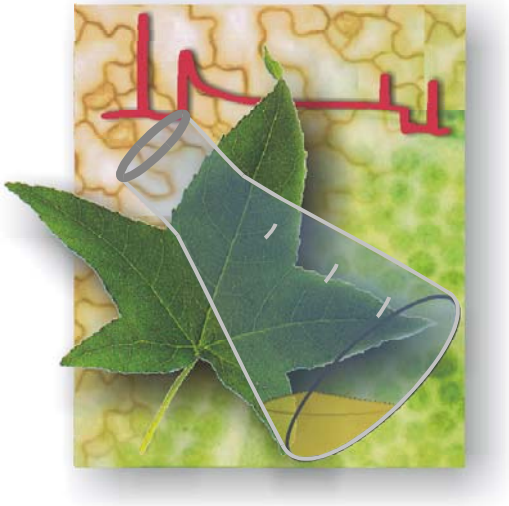
PHOTOSYNTHESIS AND STRESS

Biophysical and Biochemical Methods in Photosynthesis
Research Central-European Conference

Brno, Sept. 15 – 16, 2005



Book of Abstracts

Organized by
Masaryk University, Faculty of Science, Brno, Czech Republic
under the auspices of Dean of the Faculty of Science and
The Region of South Moravia

In co-operation with
TOCOEN, s.r.o., Brno
Czech Society of Experimental Plant Biology, Prague

ISBN 80-210-3762-8

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Welcome address

Dear colleagues,

It is my honour and pleasure to welcome you to the PHOTOSYNTHESIS AND STRESS conference at Brno, Czech Republic.

The conference continues the tradition of international photosynthetically-oriented meetings held in České Budějovice and Nové Hrady that were a platforms for exchange of knowledge in the last two decades. As with previous meetings, the PHOTOSYNTHESIS AND STRESS conference covers all major fields of photosynthesis research. In Brno, there will be a balanced mixture of updates in photosynthesis, invited lectures, oral presentations in sections, and numerous presentations in poster sessions. We are particularly pleased that the conference meets a broad international attendence, as well as an attraction for professionally-oriented companies and exhibitiors. Last but not least, we are pleased that numerous young scientists are coming to participate at the conference, which is very promising for future photosynthesis reasearch.

The Organizing and Scientific Committees have made up a program of topics ranging from molecular biology to ecosystem level in upscaling studies of photosynthesis. Moreover, the emphasis is given also to new and emerging methods in photosynthesis research, especially those of biochemistry and biophysics.

The Organizing Committe has arranged a social and culture programe in the evening for the participants and their accompanying persons. Finally, we would like to thank our sponsors for their contributions. The sponsors are presented in the last section of the Book of Abstracts.

Thank you for coming to Brno and attending the PHOTOSYNTHESIS AND STRESS.

Miloš Barták
chairman

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About the Organizers

The main organizer of the PHOTOSYNTHESIS AND STRESS conference is the Department of Plant Physiology and Anatomy, Masaryk University, Brno. The Department was founded after the world war I by prof. Vladimír Úlehla, a leading plant biologist of that time, as a part of a newly-founded the University in Brno. Throughout rich history of the Department of Plant Physiology and Anatomy, research activities has been oriented into several topics. In the 70-ies, the studies of interspecific plant relations and mineral nutrition in plants were of the most importance. In the 80-ies, differentiation and plant growth in the early stages of development was introduced to the Department using the approach of tissue cultures. In the 90-ies, ecophysiological studies focused on plant photosynthesis and growth under varying nitrogen supply, heavily acidified environment, elevated CO_2 concentration and temperature enlarged the scope of the scientific activities. In the late 90-ies, the studies of the effects of anorganic and organic toxic compounds on plant physiology were implemented as well as the study of mycorrhiza in plants, orchids in particular. Recently, the Department staff forms four laboratories focused on (i) photosynthesis under radiation, temperature, water and nutrient stress, (ii) the effects of polycyclic aromatic hydrocarbons on plant growth, (3) regulation of plant morphogenesis as regulated by phytohormones and growth regulators, and (iv) vesiculo-arbuscular mycorrhiza. Field experiments are caried out mainly in conifers, sap flow measurements in particular and photosynthetic studies in Antarctic lichens.

Among the co-organizing institutions, the Faculty of Science (personal auspices of dean of the faculty, Masaryk University, Brno), Czech Society of Experimental Plant Biology (Prague), and TOCOEN (Brno, research institution oriented to plant-environment interaction) must be mentioned. These institutions contributed to the organization of the PHOTOSYNTHESIS AND STRESS conference.

Brno and South Moravia

Brno with its population of 400,000 is the second largest city in the Czech Republic and the capital metropolis of South Moravia. It is situated at the crossroads of traditional trade routes and on the ancient cultural axes connecting Berlin-Prague-Vienna-Budapest and Krakow-Vienna-Graz-Triest. Brno is geographically a part of the Danube region and has historical ties with Vienna.

The first records of Brno date back to 1091. The royal rights were granted to the City more than 750 years ago. After the city was successfully defended against the Swedes during the Thirty Years' War, Brno became the capital of Moravia. The era of the Napoleonic Wars is marked in Brno's history by the Battle of Three Emperors, fought near Slavkov (Austerlitz) in 1805. In the 19th century, Brno ranked among the most industrial cities of the former Austro-Hungarian Empire. It was the first city to be connected with Vienna by railway (1839) and telegraph lines (1847). Brno's new theatre was the first in Central Europe to be illuminated by Edison's electric bulbs (1881).

Among the celebrities, who lived and worked in Brno, J. G. Mendel (founder of the heredity theory, 1865), Viktor Kaplan (water turbine inventor, 1914), Leoš Janáček (music composer) are those the most important. In Brno, Adolf Loos and Mies van der Rohe constructed their functionalist buildings and Milan Kundera wrote his first novels here.

Brno is a centre of culture and education. There are 6 universities with more than 35,000 students. There are numerous research institutes related to the key sectors of industry. Brno is one of the oldest industrial centres in Central Europe, currently dominated by the textile, mechanical and electrical engineering and metalworking industries. Brno is also a city of art. There are 10 permanent theatre companies in Brno. The city has a stylish concert hall and a dense network of museums, galleries, libraries.

Brno is also a traditional centre of international trade. The Brno International Trade Fair and Exhibition Centre, with about 50 specialized, mostly international trade fairs and exhibitions held on its premises every year, is closely linked to the Brno World Trade Centre.

Brno is famous for its nice environments. Thanks to its location between the forested Czech-Moravian highlands in the north and the fertile lowlands and vineyards in the south, Brno is surrounded by natural areas providing recreation and relaxation for its inhabitants.

Numerous sporting grounds, sports and entertainment centres, a motor-racing track, two airports for sport aviation, a golf club and yachting club, together with the nearby woods and Brno reservoir, all contribute to the city's pleasant, creative and cultural atmosphere. Within 50 km of the city, there are many attractive sites for tourists – castles and monuments, untouched landscapes and natural landmarks.

For more information see http://www.brno.cz/main/index-en.htm

Structure and function of PS II and thylakoid membrane components

Regulation of Chlorophyllase from *Fraximus excelsior*

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Introduction: Clorophyllase (EC 3.1.1.14, Chlase), the earliest identified plant enzyme [1] is thought to be the first enzyme in chlorophyll degradation pathway. It catalyses the hydrolysis of chlorophyll (Chl) to chlorophyllide (Chlide) and phytol. It was found that chlase in higher plants catalyses not only the hydrolysis, but also estrification and transestrification of Chlides or Chl, respectively [2]. The effects of exogenous growth regulators on hydrolytic loss of Chl in higher plants are quite well documented. E.g., ethylene has been shown to induce a several fold increase in Chlase activity, while giberilins and cytokinins delay the natural loss of Chl, counteracting the ethylene-induced increase of Chlase activity [3]. However the mechanisms regulating Chl hydrolysis by Chlase at biochemical level remain unknown. In this communication we report our studies on regulatory effects of chlorophyll hydrolysis products on chlorophyllase activity.

Material and Methods: Chlorophyllase was prepared via acetone powder methods from leaves of *Fraxinus excelsior* according to [2]. The essay of enzymatic reaction was done by single-phase reaction in 1% Triton X-100 and two-phase catalysis method (acetone powder in 70% aqueous acetone) in the presence of exogenous substrate. The reactions were monitored spectrophotometrically using the phase separation method [2].

Results and discussion: The effect of chlorophyllide (0.25 mM) and geraniol (analog of phytol) on Chlase activity were studied by carrying out the enzymatic hydrolysis of Chl a or Bchl (0.1 mM). The results show that only geraniol (0.8 mM) decreases Chlase activity in the single-phase catalytic method. Under the same experimental conditions the rates of reactions conducted in the presence/absence of Chlide, remain nearly identical. Ethanol (alcohol with short hydrocarbon chain) and decane (longer chain hydrocarbon), both added at 0.8 mM to the reaction mixture, do not show effects on Chlase activity.
These results suggest that only alcohols with long hydrocarbon chain affect activity of Chlase reconstituted in to Triton X-100 micelles. These molecules either influence the equilibrium state of the reactions catalyzed by Chlase [4] or bind to a different effectory site from the substrate (= chlorophyll binding site), for example to the so called “estrification active site” [5].

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Acknowledgements: Supported by KBN grant No 6 PO4B 00321.

Role of the PsbI subunit in the structure and function of the Photosystem II complex of the cyanobacterium *Synechocystis*

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Introduction: Photosystem II (PSII) is a thylakoid protein complex of oxygenic phototrophs performing light-induced electron transfer leading to the formation of molecular oxygen. It consists of four large membrane protein subunits D1, D2, CP47 and CP43, and a number of small polypeptides. The PsbI protein containing only 38 amino-acid residues belongs to the smallest PSII subunits. It has been identified in both the PSII core and PSII reaction center complexes. According to the latest PSII model created by [1] it is in close contact with D1. The functional and structural role of PsbI is not clear.

Material and Methods: Various strains of the cyanobacterium *Synechocystis* PCC 6803 were grown in BG 11 medium in shaken conical flasks at 29 ºC. For characterization of growth the strains were grown in microtiter plates and their OD_{750nm} was measured using a microplate reader. Photochemical activities of PSII were assessed as the rate of oxygen evolution in the presence of artificial electron acceptors using Clark electrode or as the variable fluorescence yield using modulated fluorimeter PAM. The decay of variable fluorescence reflecting reoxidation of the Q_A acceptor of PSII was measured by PSI fluorimeter. Cyanobacterial membranes were prepared by breaking the cells with glass beads followed by differential centrifugation. Isolated membranes were analyzed by 2D PAGE consisting of a blue-native electrophoresis in the first dimension and SDS-PAGE in the second dimension. Standard protein analyses were performed by SDS-PAGE using the same gel as for 2D analysis. Separated proteins were either stained by Coomassie Blue or transferred onto PVDF membrane and immunodetected using antibodies specific for the D1, D2 and PsbI proteins.

Results and Discussion: We constructed the *psbI*-deletion mutant ($PsbI^-$) with 6-105 bp of the original *psbI* gene replaced by a zeocin cassette. Although an elimination of *psbI* gene didn't influence growth of the $PsbI^-$ mutant under autotrophic conditions, absence of the PsbI protein resulted in a decrease of photochemical activities of PSII measured as the rate of oxygen evolution or variable fluorescence yield. Decay of variable fluorescence in the presence and absence of DCMU was not affected by this mutation indicating no apparent effect of PsbI on the acceptor side of PSII. The radioactive pulse-chase experiment showed an accelerated D1 turnover in the $PsbI^-$ mutant and the 2D analysis of membrane protein complexes of the mutant indicated destabilization of CP43 binding in PSII. We also deleted the *psbI* gene in the strain lacking CP47 that assembles only the reaction center (RC) complex containing D1, D2, cyt. b_{559} and PsbI. The resulting double mutant preserved the ability to form this RC complex showing that the PsbI protein isn't essential for the assembly of RC. We also constructed a new mutant PsbI-His/$PsbI^-$ lacking the original *psbI* gene and containing its new copy artificially extended with 6 histidines on the N-terminus inserted under the psbA2 promotor. This strain was used for isolation of the PSII core complex using the chelating chromatography column with immobilized Ni^{2+} ions. Analysis of its protein composition confirmed the presence of psbI-His with slightly decreased electrophoretic mobility when compared with the original PsbI.

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Acknowledgements: Supported by Academy of Sciences, Institutional Research Concept , No. AV0Z50200510

The FtsH protease homologue slr0228 plays a crucial role in the removal of photosystem II proteins from thylakoids of the cyanobacterium *Synechocystis*

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Introduction: Photosystem II (PSII) is a highly dynamic membrane complex of oxygenic phototrophs responsible for oxygen evolution. Due to its unique photochemical properties it exhibits a frequent light-induced inactivation that is repair via the selective replacement of its key integral subunit, the D1 protein. Recently, it has been shown that a homologue of the bacterial protease FtsH (slr0228) affects the selective D1 turnover in the cyanobacterium *Synechocystis* PCC 6803 [1]. In this study, we investigated a potential role of this protease in the removal of other PSII proteins.

Material and Methods: Various strains of the cyanobacterium *Synechocystis* PCC 6803 were grown in BG 11 medium supplemented with 5 mM glucose in shaken conical flasks at 29 ºC. Inactivation of *ftsH* (slr0228) was performed by transforming with plasmid constructs in which *slr0228* was interrupted at a SmaI site, 253-bp downstream of the initiation codon, by either kanamycin or erythromycin resistance cassette. Total cyanobacterial membranes were prepared by breaking the cells with glass beads at 4 °C followed by differential centrifugation. Isolated membranes were analyzed by 2D PAGE consisting of a blue-native electrophoresis (BN-PAGE, 5-14 % linear gradient gel) in the first dimension and SDS-PAGE (12-20 % linear gradient gel containing 7 M urea) in the second dimension. Standard protein analysis was performed by SDS-PAGE using the same gel as for 2D analysis. Separated proteins were either stained by Coomassie Blue or transferred onto PVDF membrane and immunodetected using antibodies specific for the D1, D2, CP43 and CP47 proteins.

Results and Discussion: Inactivation of the *slr0228* gene also stabilized the D1 protein in the CP43-less mutant (ΔCP43) with the previously reported very fast D1 turnover. In addition, 2D analysis of thylakoid membrane complexes in this ΔCP43ΔFtsH strain showed largely increased level of PSII core complex lacking CP43, a PSII assembly intermediate. When the gene encoding FtsH protease was removed from the strain lacking D1, the overall level of the unassembled D2 and CP47 markedly increased reaching the level of these PSII assembled proteins in the wild type. Radioactive pulse-chase experiments showed that this was due to reduced rates of their degradation. In contrast, level of free unassembled CP43 proteins remained nearly unchanged but in the case of this protein both, the synthesis and degradation was inhibited. The level of the unassembled CP47 also largely increased after inactivation of the *slr0228* gene in the strain lacking D2 due to the deleted *psbEFLJ* operon. Again, amount of free unassembled CP43 remained nearly unchanged. Overall our results show that FtsH (slr0228) plays an important role in controlling the level of PSII assembly intermediates and unassembled subunits in the thylakoid membrane and is not restricted to selective D1 turnover.

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Acknowledgements: Supported by GA ČR, grant No. 203/04/0800

Diffusion of reaction centres and light-harvesting complexes in the thylakoid membranes of cyanobacteria

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Introduction: We use laser-scanning confocal microscopy and Fluorescence Recovery after Photobleaching (FRAP) to observe the diffusion of reaction centres and light-harvesting complexes in the thylakoid membranes of cyanobacteria *in vivo*. *Synechococcus* 7942 is an excellent model organism for studies of this type, but qualitative measurements are also possible on *Synechocystis* 6803. The method shows us which membrane protein complexes are free to diffuse, and which are not. We have been investigating the physiological roles of the mobility of light-harvesting complexes and reaction centres.

Material and Methods: FRAP measurements were carried out on mutant and wild-type cells of *Synechococcus* 7942 and *Synechocystis* 6803 using a laser scanning confocal microscope (Nikon PCM2000). Fluorescence emission was detected at >665 nm. Phycobilisomes were observed by exciting at 633 nm, and Photosystem II (PS II) and IsiA by exciting at 457 nm.

Results and Discussion: Phycobilisomes are highly mobile, and are not stably coupled to reaction centres. Phycobilisome mobility is required for at least two mechanisms that regulate the function of the light-harvesting apparatus. PS II is normally completely immobile, and this seems to be required for efficient photosynthesis under low light. Some PSII becomes free to diffuse after treatment with intense red light. We suggest that this may be required for rapid PSII repair after photoinhibition.

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Chlorophyll *a* fluorescence quenching: main components, their definitions and quantification

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Introduction: The term 'chlorophyll *a* (Chl) fluorescence quenching' refers to all photochemical and nonphotochemical processes that lower Chl fluorescence yield (FY) below its maximum (F_M level). In fact, contribution of two principal components - the photochemical (q_P) and nonphotochemical (q_N) quenching of variable Chl FY (F_V) to the total quenching can be resolved by a saturation pulse method [1]. For this purpose, fluorimeters working on a principle of the pulse amplitude modulation of Chl FY are used [1]. The q_N-relaxation kinetics exhibits an exponential character and can be observed during transition of a photosynthetic apparatus from light-adapted to dark-adapted state [2]. In contrast to q_P, q_N consists of (at least) three components: (*i*) the "energy-dependent" quenching (q_E), (*ii*) the quenching related to state 1 – state 2 transitions (q_T), and (*iii*) the photoinhibitory quenching (q_I). These three main components differ significantly in their half-times of relaxation ($\tau_{1/2}$) and can be thereby distinguished [3]. In this contribution, the definition and quantification of the q_N components are theoretically clarified and experimentally tested.

Method: If an actinic light is switched off at the steady-state of photosynthesis, the maximum variable Chl FY (F_V") starts to relax exponentially back to the F_V level. Difference between F_V and F_V" is proportional to q_N. Analysis of the q_N-relaxation kinetics allows to find numerical values of the three main components of q_N. For this purpose, a method of the non-linear regression fitting of an experimental data with a multi-exponential function is used.

Results and Discussion: Using the three assumptions: (*i*) the dark relaxation kinetics of q_N as well as of all its components has an exponential character, (*ii*) the superposition principle is valid, and (*iii*) the same reference level (F_V) applied to all q_N-components, the definition formulae for q_E, q_T and q_I were found. The theory results in a rather simple equations allowing to compute the values of all q_N-components (q_E, q_T, q_I) and half-times of relaxation of corresponding quenching processes, as well. It is demonstrated that the equation of $q_N = q_E + q_T + q_I$ is really valid. Application of the theoretical formula to measurements on sunflower and tobacco leaves *in vivo* is also presented.

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Acknowledgement: This work was supported by the Research Contract No. AV0Z50510513.

Dark-recovery of the Chl *a* fluorescence rise (OJIP) after light adaptation of pea leaves: identification of the process responsible for the intermediate phase qT

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Introduction: Reversion of non-photochemical quenching can be studied by following the maximum amplitude of the Chl *a* fluorescence induced by saturating pulses (Fm'(t)) as a function of the time after lights off. Three phases have been distinguished: qE (fast process related to the relaxation of the ΔpH), qT (process with a halftime of 5-10 min) and qI (slow process lasting hours). Of these phases the qT is the most enigmatic. It is often associated with state transitions but clear experimental evidence to support this is scarce. We have investigated the nature of the qT to determine if it is associated with a change in the fluorescence yield of closed reaction centers (non-photochemical quenching) or with an incomplete reduction of Q_A at the F_m-level (photochemical quenching).

Material and Methods: For the measurements mature leaves of 2 to 3-week-old pea plants were used. Leaves were brought to steady state by 15 min of 540 μmol photons m^{-2} s^{-1}. The subsequent dark-adaptation kinetics of the fluorescence rise and the 820 nm transmission kinetics were studied in the 1-1200 s range by giving either single or multiple pulses of 1800 μmol photons m^{-2} s^{-1} red light. In the last case they were spaced 200 s apart. For this purpose a PEA Senior instrument (Hansatech, UK) was used. In parallel a HandyPEA instrument with a special high intensity head (Hansatech, UK) was used to study the fluorescence rise in response to up to 11500 μmol photons m^{-2} s^{-1}.

Results and Discussion: Following the F_m' as a function of the time after lights off, a recovery-phase with a halftime of 5-10 min was measured. This phase coincided with a reappearance of the IP-phase of the fluorescence rise absent in the light adapted state. For the 820 nm transmission data it was observed that in the 20-200 ms range the maximum level was not achieved (no full reduction of plastocyanin, P700 and ferredoxin). We have provided evidence for the concept that the IP-phase depends on transiently inactive ferredoxin-$NADP^+$-reductase (FNR) [1]. In this context our data indicate that the qT-phase represents the inactivation of FNR in darkness. Since the intensity of our pulses was rather low we also studied the fluorescence rise up to 11500 μmol photons m^{-2} s^{-1}. Although the IP-phase as a fraction of the variable fluorescence at high light intensities was less, it did not disappear. It indicates that the presence of active FNR may also suppress the F_m'-level if light intensities much higher than 1800 μmol photons m^{-2} s^{-1} are used. It therefore raises the question if it is possible to close all reaction centers in light-adapted leaves.

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The role of the small plastome-encoded transmembrane subunits PetL, PsaI and PsaJ in the assembly, stability and function of the cytochrome-bf-complex and photosystem I

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Introduction: The function of several plastome-encoded small transmembrane subunits of both cytochrome-bf complex (cyt-bf) and photosystem I (PSI) is still unknown. From X-ray crystal structures of these complexes, it is obvious that these subunits are not involved in the intra-complex electron transport chains, but may rather function in complex assembly or stability. To characterize the function of three of these small subunits, we constructed knock-out mutants for the PSI subunits PsaI and PsaJ and the cyt-bf subunit PetL.
The PsaJ subunit, localized close to the plastocyanin binding site, might stabilize PSI, whereas the PsaI subunit was suggested to be involved in binding of the LHC proteins [3]. The PetL subunit of the cytochrome-bf-complex is localized at the periphery of the cyt-bf dimer and was suggested to be essential for cyt-bf assembly and stability [1].

Material and Methods: Knock-out mutants of the different plastome-encoded genes were constructed in *Nicotiana tabacum* cv. Petit Havana by chloroplast transformation and replacement of the wild type (WT) allele through homologous recombination with a vector containing the flanking regions and the *aadA*-gene as selection marker. All transformant lines were selected with spectinomycin until a homoplasmatic state was established [2].
Leaf assimilation capacity under non-photorespiratory conditions (5% CO_2) was determined using a Hansatech leaf disc electrode. Quantification of the photosynthetic complexes, redox equilibration of the high potential chain and activity measurements were performed as described in [4].

Results and Discussion: Homoplasmic transgenic lines of all knock-out transformants did not display any visible phenotype, indicating that none of these subunits is essential for the assembly and function of the complexes of the photosynthetic electron transport chain.
The assimilation capacity of young source leaves of the Δ*petL* mutant and of WT was equivalent, but with increasing leaf age, an earlier and more pronounced decline in leaf assimilation capacity was observed in the mutant, relative to WT, where leaf assimilation capacity declined only in senescent leaves. This decline is related partially to loss of cyt-bf, suggesting that the cyt-bf in transformants is less stable than in WTP plants, and that significant *de novo* synthesis of cyt-bf does not occur in mature and old leaves. Furthermore, the thylakoid energetization in Δ*petL* plants was clearly reduced.
In case of the PSI subunits, knock-out of *psaJ* resulted in a significantly increased (+20%) assimilation capacity of young leaves, whereas leaf assimilation capacity of Δ*psaI* plants was slightly reduced. In parallel to the increased assimilation capacity of Δ*psaJ* plants, also their chlorophyll-a/b-ratio was elevated, suggesting an increased number of reaction centers per electron transport chain. Δ*psaI* plants displayed a strong reduction in the chlorophyll-a/b-ratio, indicating an increased antenna content per reaction center. The latter data are in agreement with the postulated function of PsaI in antenna binding and exciton transfer to PSI. Further characterization of these mutants is under way.

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Can the experimentally measured initial fluorescence level be used as an indicator of the redox-state of the plastoquinone-pool?

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Introduction: In the dark the redox state of the plastoquinone (PQ) pool can be manipulated by modifying the activity of components of the chlororespiratory electron transport pathway. One possibility is to inhibit the terminal plastid oxidase that can re-oxidize PQ-molecules. This can be achieved by anaerobiosis. Under these conditions the PQ-pool becomes reduced and the J-step of the OJIP fluorescence transient significantly increases. The increase of the J-step is accompanied by an increase of the initial fluorescence level (O-level) which is widely used as an indicator of the redox state of the PQ-pool. The aim of our study is to define the relationship between the redox state of the PQ-pool and this O-level.

Material and Methods: Mature leaves of 2 to 3-week-old pea plants were used that were dark-adapted for 12 h before measurements. Anaerobic treatments were carried out in darkness, by flushing nitrogen gas to pea leaves placed in a plastic bag. The duration of the treatment was varied between 3 and 15 minutes to achieve different PQ-pool redox states. Chlorophyll a fluorescence was measured by a HandyPEA instrument (Hansatech, UK). The light intensity was 3000 μmol photons $m^{-2}s^{-1}$.

Results and Discussion: In the case of the ~15 min anaerobiosis, the J-level equaled to FM, suggesting full PQ-pool reduction [1, 2]. This was accompanied by a 54% increase of the O-level. The redox state of the PQ-pool was characterized by the J-level and the area above the fluorescence transient. Neither of these parameters gave a linear relationship with the O-level; instead, a hyperbolic function was found. We suggest that this may be due to the connectivity of photosystem II units. Another goal is to determine the equilibrium constant between the PQ-pool and Q_A.

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Monitoring of the electron transport during the fluorescence induction in whole cells of purple bacteria

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Introduction: Light-induced changes of the redox state of components of the electron transport chain were monitored in whole cells of the purple bacterium *Rhodobacter sphaeroides* by means of kinetic difference absorption spectroscopy. In addition, parallel measurements of kinetics of fluorescence yield were performed to obtain information on the energy transformation in the photosynthetic apparatus.

Material and Methods: Experiments were performed on photoheterotrophically grown cells of *Rhodobacter sphaeroides*, strain Y. A novel laboratory-built multichannel kinetic spectrophotometer was used for the measurements. The instrument is equipped with pulsed measuring light (Xe-flash lamp, pulse length 1.5 μs) and photodiode array detectors. Spectra recorded during single measuring flash are 200 nm wide with resolution 6.2 nm/point. The available spectral range is 400 – 1100 nm.

Results and Discussion: Kinetics of redox changes of primary donor (around 600 nm and 870 nm), cytochromes (420 nm) and the electrochromic bandshift of carotenoids (around 500 nm) and bacteriochlorophyll (800 nm and 850 nm) were measured [1]. In addition, a light-induced increase of the light scattering was observed. This effect can be eliminated by addition of gramicidin. We have found out that it is possible to satisfactorily fit the time course of the fluorescence yield by the kinetics of absorbance changes using a relatively simple mathematical model. Our results indicate important positive effect of the electric field detected by the carotenoid electrochromism on the fluorescence yield. Present work partially contradicts earlier findings about the electric field effect on the processes of light energy transformation in the purple bacteria [2]. Interestingly, the results correspond to recent studies obtained on plants [3].

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Reoxidation of photosystem II acceptor side at subzero temperature

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This presentation is dedicated to Pavel Šiffel who tragically passed away.

Material and Methods: Chlorophyll fluorescence decay kinetics after single- and multiple-turnover flash illumination in intact Scots pine (*Pinus sylvestris* L.) needles collected during the summer and winter were measured by the double-modulated fluorometer FL-200/PS with leaf clip accessories LFU031 (Photon Systems Instruments, Brno, Czech Rep.) in temperature range from minus 40 to plus 4 °C.

Results and Discussion: Primary photosystem II acceptor Q_A reoxidation and diffusion of plastoquinones in thylakoid membrane at subzero temperatures was studied by chlorophyll fluorescence methods. Temperature dependence of the kinetic parameters of Q_A reoxidation (solved from exponential (or hyperbolic) deconvolution of fluorescence decay kinetics) was used for construction of Arrhenius plots. Our calculated values of Arrhenius activation energy are in good agreement with the free energies of activation, known from the thermoluminescence glow curves.

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Acknowledgements: Supported by GA ČR, grant No. 206/03/1107; and partly by MŠMT, grant No. MSM6007665808, and AV ČR, grant No. AV0Z50510513.

Electron microscopy in structural studies of photosystem I from the red algae *Cyanidium caldarium*

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Introduction: Oxygenic photosynthesis is a process in which plants, algae and cyanobacteria use light energy to drive synthesis of organic compounds and produce all molecular oxygen, necessary for aerobic life on Earth. The light-harvesting and energy-transducing functions of oxygenic photosynthesis are localized in specialized photosynthetic membranes, thylakoids, and carried out by several types of protein complexes: Photosystem II (PS II), Photosystem I (PS I), cytochrome *b*6/*f* and ATP synthase.
Various techniques of electron microscopy such as ultrathin sectioning, freeze-fracturing, freeze-etching, negative staining and (cryo-)electron crystallography of two-dimensional crystals have been employed, since now, to gain much of the structural information of photosynthetic complexes.
In our work we used technique of negative staining and particle averidging to study the structure of PS I complexes izolated from the red algae *Cyanidium caldarium*.

Material and Methods: *Cyanidium caldarium* was cultivated at 42 ^{o}C at low light (20 μmol photons m^{-2} s^{-1}) and hight light (200 μmol photons m^{-2} s^{-1}). Cells were broken with glass beads and thylakoid membranes were isolated al low temperature (4 ^{o}C) under dim light conditions by centrifugation. Thylakoid membranes were solubilized with 1.5 % dodecyl maltoside at chlorophyll concentrations of 1 $mg.mL^{-1}$ of chlorophyll *a* for 15 min. The solubilized material was loaded onto a 0-0.55 M continuous sucrose gradient and centrifuged at 150 000g for 14 h. After centrifugation the band with the most of PS I was loaded onto a DEAE sepharose CL-6B anion-exchange column. The complexes was eluted from column with concentration gradient of 0-300 mM NaCl. Freshly prepared PS I complexes were used for electron microscopy. The specimen was placed on glow-discharge carbon-coated copper grids and negatively stained [1]. Micrographs were digitized for further image analyses with 'Spider and web' software.

Results and Discussion: Four fractions were resolved on sucrose density gradient after centrifugation of solubilized thylakoid membranes. The SDS PAGE and spectroscopic data showed that the most of the PS I complexes were in the third fraction. This fraction was loaded onto DEAE sepharose CL-6B anion-exchange column. The pure PS I was eluted from column at a concentration of 25 mM NaCl as showed SDS PAGE and spectroscopic measurements. PS I complexes were negatively stained with 2% uranyl acetate and visualized by electron microscopy. The single particle analysis of micrographs is in progress.

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Is violaxanthin de-epoxidase the enzyme regulated by pH and ionic strength-dependent aggregation?

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Introduction: Violaxanthin de-epoxidase (VDE) is an enzyme of the xanthophyll cycle and it catalyses de-epoxidation of violaxanthin (Vx) to zeaxanthin via anteraxanthin. VDE in chloroplast occurs in two forms: bound to thylakoid membrane (pH ~ 5) and unbound (pH about 7 and above). Parallel to binding state, an exposition of inhibitor sensitive disulphide bridge is changed. The enzyme behaviour is attributed to different properties of three domains: lipocalin domain, charged domain and cysteine-rich domain (for a review see [1]). Affiliation to lipocalins makes important studies of pH-dependent behaviour, which often regulates protein function inducing aggregation and ligand binding, for example see [2]. In this work we show that pH and ionic strength-dependent conformational changes of VDE result in different aggregation state of protein.

Material and Methods: VDE crude extract was isolated from 7-day old wheat plants by methods described in [3]. Higher purity was obtained by gel filtration on Sepharose 6B (Pharmacia, Fine Chemicals AB, Sweden) and anion exchange chromatography on MonoQ HR 5/5 (Amersham, Uppsala, Sweden). Native molecular mass determination was done on TSK G3000SW (7.5 x 600 nm) column (Tosoh Bioscience, Tokyo, Japan). Electrophoresis was performed in SDS-PAGE system. Circular dichroism spectra were recorded using Jasco 710 Spectropolarimeter. VDE activity was measured in monogalactosyldiacylglicerol-Vx *in vitro* assay with acorbate. After reaction time pigments were extracted and analysed on HPLC system (reverse phase Nucleosil 100 C18 with acetonitrile:methanol:water 72:8:1 v:v:v as mobile phase). Vx was isolated from daffodils. Monogalactosyldiacylglicerol was obtained from Lipid Products, GB and ascorbate from Merck, Germany. Acetonitrile and methanol came from LabScan Ltd, Ireland.

Results and Discussion: Purification of VDE crude extract using gel filtration and subsequent chromatography of highest purity fractions from that step on anion exchange column allowed us to identify for the first time wheat VDE as 38 kDa protein, which well corresponds to theoretical mass calculated from wheat VDE cDNA (39.9 kDa). Native molecular mass determination showed presence of 47 kDa protein and also peaks of higher molecular weight. SDS-PAGE examination showed presence of just 38 kDa protein band in fractions corresponding to all those peaks, what suggests aggregation. Destruction of high-level oligomers occurred if pH was lowered and/or ionic strength was increased. VDE activity was associated to monomer and lower level oligomer fractions only. Higher oligomer complexes are more stable and the parts of VDE molecule, which are engaged in aggregation seems to be important for enzymatic activity. Disaggregation by higher ionic strength implies that the charged domain plays a key role in this process. pH lowering induced changes in secondary structure of VDE molecule, what we observed in circular dichroism experiments. Such behaviour could be a part of physiological mechanism of activity regulation - presence of VDE in aggregated form in lumen in dark or shade conditions (higher pH) and dissagregation, followed by membrane connections after ilumination (lowering of pH).

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High protein density in grana thylakoids is indispensable for efficient energy transfer between PSII and its antenna

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Introduction: Light-harvesting by PSII in grana membranes requires an efficient intermolecular electron transfer between several LHCII and PSII complexes. The protein density in this subcompartement is very high (70 – 80 % area occupation). An advantage of a dense packed membrane is a higher probability for absorbing light quanta. However there are potential drawbacks associated with a high protein packing in biomembranes, i.e. diffusion processes and intermolecular energy migration between light harvesting complexes can be impaired. It is an open question how the native membranes avoid these problems. Here we analyze the impact of protein density on the excitonic coupling between LHCII and PSII in grana membranes with an increased lipid / protein stoichiometry.

Material and Methods: A preparation protocol was developed to incorporate lipids in grana membranes (BBY) by fusion with liposomes of a native lipid mixture. The incorporation was monitored by density gradient centrifugation and electron-microscopy of freeze-fractured fusion products. The membranes were characterized by 77 K fluorescence spectroscopy, absorption spectroscopy and chlorophyll *a* fluorescence induction spectroscopy.

Results and Discussion: We estimate that the relative membrane area occupied by lipids increased from 20 % (control grana) up to 70 % in the fusion products. In fused membranes a drastic decrease in the quantum yield of PSII photochemistry (from 0,85 to about 0,3) is observed, paralleled with a detachment of LHCII from PSII. The detachment is indicated by an increase in the Fo level (eight times higher than the control value), a blue-shift of the absorption maxima in the Qy region of chlorophyll *a* and the occurrence of a new emission band at 681 nm in the 77 K fluorescence spectra. Chlorophyll a fluorescence kinetics suggest a decreased connectivity of PSII alpha centers and a conversion of alpha centers to beta centers, while the size of the PSII antenna remains virtually constant.
In “titration” experiments an almost linear correlation between the detachment of LHCII and the protein density is apparent. This indicates weak interaction forces between LHCII and LHCII as well as between LHCII and PSII. Obviously a dense packing of LHCII and PSII in grana thylakoids is required to insure a high quantum yield of photochemistry of PSII.

Acknowledgements: S.H.and H.K. are supported by the DFG, K1818/2-1.

Spectroscopic characterization and modelling of ordered lamellar aggregates of LHCII, the main light-harvesting complex of photosystem II

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Introduction: Quite often we can encounter highly ordered molecular macroaggregates when studying biological substances, e.g. DNA condensates, chlorosomes, viruses or membrane structures. Our understanding concerning their self-assembly, molecular organization, structural dynamics and physiological functions is, however, still rudimentary. In particular, so is the correlation between the structure and the 'anomalous' spectroscopic features that are associated with the macroarray of chromophores but absent in the constituents [1]. Progress was hampered mainly by the lack of suitable model systems which would allow us to test existing theories on these, so-called psi-type aggregates [2] and carry out detailed model calculations. (Psi, *p*olimer and *s*alt *i*nduced). LHCII, the main chlorophyll *a*/*b* binding light-harvesting antenna complex of photosystem II (PSII), is ideally suited for studying macroassembly-specific spectroscopy: (i) isolated complexes of 25-27 kDa can self-assemble into lamellar macroaggregates with long range chiral order [3]; (ii) the structure of LHCII is known at near atomic resolution [4]; (iii) the high density of chromophores in the visible range makes LHCII ideal object for spectroscopy; (iv) reactions can be triggered by light, thus allowing the investigation of dynamic features; (v) the spectral features of lamellar aggregates of LHCII closely resemble those of granal thylakoid membranes, containing LHCII domains, and thus the results can be applied to native systems; (vi) the structural parameters can be varied in a broad range from suspensions of monomers and trimers to large arrays of loosely or tightly stacked lamellar aggregates (with domain sizes up to several hundred nm in diameter); (vii) the samples can easily be oriented in a magnetic field of about 1 T (macroaggregates) or by squeezing (trimers and monomers) in polyacrylamide gel. In this work, we have measured polarization spectroscopy parameters on oriented samples and interpreted them using the trimeric structure and the macroarray of dipoles inferred from crystallography.

Material and Methods: Linear and circular dichroism (LD, CD) were recorded, using a Jobin-Yvon CD6 dichrograph, on random and oriented suspensions of trimeric an aggregated forms of LHCII, as well as on intact thylakoid membranes, which were embedded in polyacrylamide gel.

Results and Discussion: We have found strong orientation dependence of the psi-type CD bands, which are characteristic of the long-range interactions between the chromophores in the LHCII-containing macrodomain. We compare these results with those of the trimers and intact chloroplasts. Based on this information we can distinguish between the long-range molecular order in trans-membrane and in lateral directions. Orientation dependency of the CD can be used for identifying the pigment molecules that are responsible for the generation of different CD bands in the trimer. Interpretation of spectroscopic data of psi-type aggregates, based on near atomic resolution data on LHCII crystals, can serve the basis for model calculations for complex, partly ordered multilamellar systems to an unprecedented precision.

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Influence of β-carotene and its derivative – carotane – on permeability of model membranes for glucose

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Introduction: To extend our knowledge how carotenoids influence model membranes we examined the permeability of large unilamellar vesicles containing β-carotene or carotane, its completely saturated derivative, for glucose – as an example of small, electrically neutral molecule. As model membranes we chose large unilamellar vesicles made of egg yolk phosphatidylcholine (EYPC) – the lipid often used in constructing models of thylakoid membranes.

Material and Methods: The method using a fluorescence label to measure permeability for glucose was introduced by Nilsson and Liljenberg*. Using the self-quenching properties of 6-carboxyfluoresceine, trapped in the aqueous space of the liposomes, the permeability for glucose was determined with a glucose gradient of 800 mM. To obtain model membranes we made EYPC unilameller liposomes using a vortexing technique to get multilamellar vesicles and then an extrusion technique with an extruder (*Avestin*, Canada) equipped with a polycarbonate filter with 200 nm pores. We incorporated β-carotene as 0.5 mol% or 2.5 mol% addition, or carotane as 1 mol% addition, during liposome preparation. The fluorescence measurements were performed at a Luminescence Spectrophotometer (*Perkin Elmer,* UK). Applying 495 nm excitation light we registered changes of 6-carboxyfluorescein fluorescence emission at 515 nm. Based on collected curves we calculated a permeability coefficient *P*.

Results and Discussion: The addition of glucose to the vesicle suspension increases the osmotic potential of the external solution. The effect is fast shrinkage of the vesicles when water is transported out. The increase of 6-carboxyfluorescein concentration inside the liposome causes a quenching of fluorescence. Glucose and water slowly penetrate through the membrane and 6-carboxyfluorescein concentration decreases what is visible as an increase of 6-carboxyfluorescein fluorescence. Our investigations show that the addition of β-carotene to EYPC vesicles decreases the membrane permeability for glucose what seems to be a result of heterogeneous distribution of rigid, rod-like molecules of the pigment in EYPC bilayer. This effect is strongly dependent on β-carotene concentration; addition of 2.5 mol% causes a decrease of permeability for about 40% when compared to blank vesicles. Addition of carotane, completely saturated derivative of β-carotene, showed only a slight effect on the permeability of examined bilayers. Flexible molecules of carotane are not such an obstacle for glucose penetration as the rigid molecules of β-carotene.

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Photosynthesis in twigs is characterized by deficient PSII activity but sufficient pools of PSI and intermediate electron transport carriers

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Introduction: Photosynthesis in twigs is believed to occur under extremely deep shade, high CO_2 and low O_2, i.e. under conditions not predisposing to photoinhibition. Yet, recent evidence [1] paradoxically indicated that twig PSII does suffer from chronic photoinhibition, while maximum electron transport rates under saturating light are only 5-15% of the corresponding leaf values. This communication is an attempt to characterize further the state of PSII and PSI in twigs and possibly elucidate the reasons of this extreme deficiency.

Material and Methods: A comparative approach was adopted, examining aspects of photosynthesis in leaves and corresponding twigs from three woody species, i.e. *Eleagnus angustifolius*, *Quercus coccifera* and *Pistacia lentiscus*. A prerequisite for species selection was the ability to remove twig periderm without damage to the underlying chlorenchyma and thus compare fluorescence and reflectance parameters under well defined light conditions. Fast fluorescence induction curves at both saturating and limiting actinic irradiances (Plant Efficiency Analyser, Hansatech), light-adapted PSII yields (MINI-PAM, Walz), reflectance changes at 820 nm (PAM-101 with appropriate reflectance attachment) and visible reflectance spectra (Unispec, PPSystems) were used to assess PSII, PSI and intermediate electron carriers pools and activities.

Results and Discussion: We confirmed low dark-adapted maximum PSII efficiencies and extremely low linear electron transport rates to PSI, which could be correlated to an observed high proportion of inactive, non-reducing PSII centers in twigs. However, pool sizes of plastoquinone and PSI were more than enough to support the observed linear electron transport activities. We assumed that the excess of PSI and intermediate carriers is indicative of an active engagement of cyclic electron flow in twigs, and this hypothesis was further strengthened by the observed rates of PSI oxidation/reduction rates in the absence of PSII activity under far-red light. We also assumed a high need for photoprotection of the light-absorbing, yet non-reducing PSII and we did find high ratios of photoprotective (carotenoids) to photoselective (chlorophylls) pigments in twigs. A model for twig photosynthesis accommodating our findings and the needs imposed by the twig internal micro-environment will be presented.

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Reversible room temperature photooxidation of the peripheral chlorophyll of Photosystem II reaction centre

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Introduction: The core of reaction centre of photosystem II (PSIIRC) contains four core chlorophylls, two pheophytins. Besides PSIIRC bears two peripheral chlorophylls, one or two β-carotenes and a cytochrome b_{559}. These additional cofactors are supposed to act as alternative electron donors to the oxidised special pair ($P680^+$) under conditions in which the primary electron transfer pathway from oxygen evolving complex to $P680^+$ is inhibited. Previously, the role and function of these alternative donors have been studied under cryogenic temperatures [1, 2]. However, no consensus has been made so far on a number of cofactors, their arrangement, the sequence of the electron transfer in the alternative pathway(s) and their bioenergetic importance. We present new data based on the room temperature optical absorbency studies using reaction centres lacking one of the peripheral chlorophylls.

Material and Methods: Five-chlorophyll photosystem II reaction centres (5ChlRC) were prepared according to [3]. Sample was diluted to a chlorophyll concentration of about 5 μg/ml. Silicomolybdate was added to the sample to a final concentration of 200 μM. The differential absorption spectra were measured using laboratory-built multichannel kinetic spectrophotometer during dark relaxation after treating the sample with actinic light.

Results and Discussion: Three spectral forms have been revealed by the global fit of sum of exponentials onto the dark relaxation kinetic. The first two forms we assign to the $P680^+$ species. The third one is characterised by maximum bleaching at 440 and 670 nm and relaxation time of about 200 seconds. Since the D1 peripheral chlorophyll is not present in 5ChlRC, we assign the absorption changes to the peripheral chlorophyll from D2 branch of the RC. The D2 peripheral chlorophyll therefore can be reversibly oxidised by $P680^+$ at room temperature either directly or via β-carotene.

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Relationship between composition of the chlorophyll protein complexes and thylakoid membranes structure

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Introduction: Nowadays dependance of chlorophyll protein complexes composition on thylakoid membranes structure attracts researchers' interest. Although LHCII complexes seem to play the main role in formation of appressed domains [1] it is not precisely established what causes differences in grana size in various higher plants.

Material and Methods: Size of appressed domains was examined on electrograms with the help of statistical analysis. These results correspond to the data obtained from confocal scanning laser microscop (CSLM). In contrast to intact chloroplasts isolated from *Phaseolus vulgaris* and *Lycopersicon hirsutum*, bigger appressed domains are observed in intact chloroplasts obtained from *Pisum sativum* and *Lycopersicon esculentum*. To get more information on the composition of chlorophyll protein complexes we combined NATIVE-PAGE electrophoresis, SDS-PAGE electrophoresis with immunoblotting and mass spectrometry (MS). With the help of these methods 10 bands attributed to chlorophyll protein complexes were described and characterized for chloroplasts of *P. sativum*, *L. hirsutum*, *Ph. vulgaris* and *L. esculentum*.

Results and Discussion: Presented results showing diversity in polipeptydes content of chlorophyll protein complexes could explain differences in grana size.

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Acknowledgements: This work was partly supported by a grant from the Polish Ministry of Scientific Research and Information Technology (MniI) No 3 PO4C 109 23 (MG) and partly by grant of MniI BW/2005 (IR).

Anisotropic properties of chloroplasts and lamellar aggregates of LHCII investigated by differential polarization laser scanning microscope and harmonic generation microscopy

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Introduction: Chloroplasts and lamellar aggregates of isolated light harvesting complexes (LHCII) contain highly organized macrodomains, molecular macroaaggregates with long-range order of the chromophores. Polarization spectroscopic techniques provide important information on this type of complex systems, and yield a number of parameters, which cannot be obtained with other tools. In leaves or in inhomogeneous preparations, the anisotropic parameters can only be determined microscopically, with the aid of differential polarization (DP) microscope. Second and third harmonic generation microscopy provide information on ordered macroarray of non-centrosymmetric systems (*e.g.* chiral macroaggregates), and on macrostructures with high non-linear susceptibility (*e.g.* multilamellar and/or pigmented membrane systems), respectively for SHG and THG.

Materials and Methods: With the use of DP-LSM, constructed in our laboratory, we can obtain 3D information on the anisotropic organization of various biological samples. By measuring CD or FDCD (circular dichroism or fluorescence detected CD) we obtain valuable information about the chirality of molecular macorarrays. Linear dichroism (LD and FDLD) and the anisotropy of fluorescence emission, as well as Linear birefringence (LB) also reveals the presence of anisotropically organized architectures. The DP-LSM unites the advantages of the confocal laser scanning microscope (LSM) and the modulated technique of modern dicrographs. For the generation and/or detection of polarized light, we use high frequency modulated light with a photoelastic modulator, and lock-in amplifier demodulation circuits. The polarization state generator unit and those for the analysis of the polarization of the transmitted, emitted or reflected beams are attached to a Zeiss LSM 410. Our DP-LSM is capable of measuring and mapping 8 additional DP parameters, which all provide valuable and largely independent information about the molecular architecture of anisotropically organized biological samples. Simultaneous imaging with THG, SHG and multiphoton fluorescence (MPF) was performed with a home built microscope, using a 27 MHz repetition rate mode-locked Ti:sapphire laser. The ~25 fs pulse duration laser was tuned to 820 nm. The laser beam was coupled to the microscope and directed to two rastering closed-loop galvanometric mirrors (GSI Lumonics, VM500 Series). The scanned beam was appropriately relayed to the 0.75 numerical aperture objective (Zeiss) via a 1:3 telescope for obtaining 2-dimensional images. For a 3-dimensional image, specimens are translated along the optical axis using a closed loop piezoelectric translation stage (Piezosystem Jena, PZ 400 series). MPF was collected with the same excitation objective and detected with a photomultiplier tube (PMT) (Hamamatsu model H5783). The single photon counting detection system features simultaneous detection from all three channels. This allowed us to obtain three images with different contrast mechanisms that can be directly compared and cross-correlated. For the measurements we used magnetically aligned chloroplasts and LHCII trapped in polyacrylamide gel in two preferential orientational (edge- and face-aligned) positions. We also imaged chloroplasts in protoplasts and leaves.

Results and Discussion: Granal chloroplasts exhibit very strong spatial heterogeneity with strong anisotropic features, revealed by LB, FDLD and CD imaging, as well as by SHG microscopy. These features are also carried by lamellar aggregates of LHC II. These microscopic features are potentially important for understanding of the optical properties of the multilamellar membrane system of granal chloroplasts, and might also be applicable for monitoring for reorganizations under environmental stress conditions.

Acknowledgements: This work was supported by grants from OTKA (T34188 and T42696) and NSERC, CFI and OIT, as well from the TeT Foundation.

Light and temperature effects on photosynthesis

Chlorophyll *a* fluorescence and photosynthetic pigment composition of three *Umbilicaria* lichen species in relation to light microenvironment

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Introduction: High irradiances may negatively affect the photosynthetic apparatus of the lichen photobiont via photoinhibition [1]. The main focus of this work was to determine whether an assemblage of lichens on a rock face exhibiting irradiance heterogeneity showed corresponding variability in photosynthetic parameters, including chlorophyll *a* fluorescence and assimilation pigment composition.

Material and Methods: A measurement of the chlorophyll fluorescence induction parameter – F_V/F_M as an undestuctive method was used for evaluation of the photosystem II (PS II) photochemical efficiency. Assimilation pigment composition was determined spectrophotometrically [2, 3].

Results and Discussion: This work detected differences in PS II photochemical efficiency within algal symbionts of the lithic lichens *Umbilicaria americana*, *U. mammulata* and *U. muehlenbergii* related to the effect of higher irradiances present among unmanipulated cliff microenvironments. Potential quantum yield of PS II, measured as F_V/F_M, decreased significantly with increased light intensities from shaded to full sun umbilicate populations. Observed lower recoveries within high - light microclimate samples, as compared to shaded microclimates, were consistent with permanent damage to photosystem II. Chlorophyll *a*, chlorophyll *b* and total carotenoids concentrations were less useful parameters for assessment of consistent differences among umbilicate thalli growing under different light regimes.

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Temperature effects on β-carotene in ionic solvents

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Introduction: The 15-*cis* (Z)-β-carotene has been shown as characteristic and universal in nature configuration for photoprotection function and was confirmed for all types of the photosynthetic reaction centers [3-6]. The all-*trans* (E) β-carotene molecules present in the photosynthetic antennae complexes are responsible for the singlet-singlet energy transfer between β-carotene and chlorophyll a [7].

Material and Methods: The β-carotene isomeric forms: *all*-trans and 15 *cis* were dissolved in ionic solvents and electronic absorption spectra were taken as a function of temperature in range (0 – 60 ^{0}C).

Results and Discussion: The ($1^1B_u^+$) energy value of synthetic β-carotene exhibits a linear dependence on temperature in both ionic and traditional organic nonpolar (n-hexane) and polar (methanol) solvents. This is valid for transition S_0-S_2 both (0-0) as well as (0-1).
The ionic solvents are very useful tools for modeling photosynthetic energy transfer system because of the viscosity mimicking well situation *in situ*. The all-*trans* and 15-*cis* β- carotene fluorescence yield in ionic solvent 1-methyl-3-octyloxymethylimidazolinum tetrafluoroborate are (1.96 ± 0.03) % and (2.53 ± 0.03) %, respectively. The fluorescence yield of β-carotene in this ionic solvent is around hundred times higher than in standard solvent n-hexane.

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Acknowledgements: This work was supported by the project DS 62-176/2005.

Chlorophyll fluorescence as a tool in field and lab photosynthesis research: From eco-physiology to molecular biology

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Introduction: Since about 20 years we are sampling chlorophyll fluorescence data in the field. The goal to be achieved was a data set well correlated with changes in the metabolic reactions of plants understood as a response to the growing conditions at the site. The first analyses of these data clearly showed, that PS II fluorescence will become a sensitive indicator of stress. Low temperatures combined with high light intensities ("photo-chilling") were found to be one of the most effective stress factors to influence the chlorophyll fluorescence signal. In contrast, moderate drought only creates small effects, whereas the impressive changes induced in strong sun light at noon frequently vanished in the course of a few minutes when exposed to dim light. On one hand, these findings need further interpretation e.g. using data from "Proteomics" and on the other hand implications at the canopy level have to be clarified.

Material and Methods: From the tree top of tall spruces needles were studied as well as narrow leaves from wheat or the tiny leaflets of clover. In the field portable time-resolving and modulated `Fluorimeters` were used together with portable infrared gas analyzers, diffusion porometers and pressure chambers. Climatic conditions and air pollution data were recorded on site or obtained from the next continuous monitoring station. In the lab recovery studies where performed to analyze differnt quenching mechanisms induced by varying patterns of stress factors. Data regarding the modification of the proteins and the xanthophylls cycle were predominantly collected from literature or in cooperation with competent research oragnisations.

Results and Discussion:
i. The molecular and cytological level: Quenching will be described in relation to the photosynthetic activity of PS II. The interaction between the zeaxanthin formation and the protonation of core proteins enable the PS II centers to compensate for many changes in the environment. These findings will be related to fluorescence data from recovery studies. Fluorescence data like remaining current photochemical capacity (RCPC); current photochemical capacity (CPC); current photochemical use (CPU) and "real" photonhibition (PI) will be related to changes in the PS II core.
ii. The anatomical and morphological level: Layer sharing in the mesophyll will be discussed as well as the difference between fluorescence data from the upper and lower side of leaves.
iii. The plant and canopy level: Results from i. and ii. will be related to plant physiology as modified by the site conditions.

References:
Poster at this conference: Judith I. Haumann, Rita B. Linke and Harald R. Bolhàr-Nordenkampf 2005: Application of chlorophyll fluorescence recovery studies to separate between adaptation to stress factors and damage by stress.

Acknowledgements: Support by grants from the Ministry of Science, Education and Culture, the Ministry of Agriculture and Forestry, the Austrian Science Foundation and the Academy of Sciences.

Thermo-optically driven reorganizations in light harvesting antennae

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Introduction: Earlier we have shown, mainly by polarization spectroscopic techniques, that the main chlorophyll *a*/*b* light harvesting complex, LHCII, of photosystem II form chirally organized macroaggregates with dimensions commensurate with the wavelength of the visible light. The high self-aggregation of LHCII and PSII particles (coated with LHCII), i.e. LHCII-containing macrodomains explain the lateral segregation (sorting) of the two photosystems betwen the granum and stroma membranes, a key attributum of granal thylakoids [1,2].
The chiral macrodomains have also been shown to possess a remarkable structural flexibility; most notably, have been shown to undergo light-induced reversible (and irreversible) structural reorganizations. These are largely independent of the photochemical activity of thylakoids, and are approximately linearly proportional to the light intensity above the saturation of photosynthesis – a potentially very important, unique feature with respect to protection of plants against excess excitation. Further, isolated lamellar aggregates of LHCII also possess the ability to undergo similar changes [3;4]. In LHCII, lipids have been shown to play a key role [5]. These structural changes are accompanied by fluorescence quenching transients [6], also suggesting their involvement in regulatory processes in excess light. As concerns the nature of the structural changes, we have shown that light induces (*i*) unstcaking of membranes, followed by (*ii*) a lateral desorganization of the macrodomains, and (*iii*) monomerization of the LHCII trimers [7,8,9].
These structural transitions are proposed to be driven by a novel, thermo-optic mechanism: fast thermal transients arising from dissipated excitation energy, which lead to elementary structural transitions in the close vicinity of the sites of dissipation, due to the presence of 'built-in' thermal instability in the (macro)assembly of complexes [7,8,9]. Recently, we have shown, by using ^{32}P NMR, that the changes in thylakoid membranes are accompanied (or triggered) by changes in the lipid phase [10]. Thermo-optically induced rearrangements of similar nature have also been detected in cyanobacteria, where energy migration from the phycobilisomes to the photosynthetic membranes can be regulated by heat or excess light [see the presentation of Zsiros et al.].
Results and Discussion: In the presentation I will outline how thermo-optically induced structural transitions lend a substantial *local* structural flexibility to molecular (macro)assemblies that otherwise possess large stability (and rigidity). The potential role of these structural changes in photoprotection and different light- and temperature-adaptation processess will also be discussed – by invoking recent literature data on enzymatic and light regulations of the light harvesting system [11-14] and some of our data [see the presentations by Várkonyi et al., Lambrev et al.].
Some of our very recent data will also be presented on the generation of the heat packages (in collaboration with L. Valkunas and coworkers), and the first direct, electron microscopic evidence on the occurrence and nature of the light-induced reversible reorganizations in lamellar aggregates of isolated LHCII (in collaboration with G. Hind and coworkers).

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Adaptive photosynthetic strategies of the evergreen Mediterranean species and their response to stress factors

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Introduction: Most temperature based models require a parameter to link temperature with plant functioning; photosynthesis may be an appropriate indicator for plant functional limitations imposed by environmental factors, particularly air temperature, which controls the magnitude of the photosynthetic response [1,2,3]. The intrinsic link between net photosynthesis and biomass production [4,5] suggests that photosynthesis and its response to drought is likely to play a major role in determining the ability of species to persist in the distribution area despite increasing drought stress. Global change effects on Mediterranean region are likely to produce warmer and drier conditions, water deficit, and more frequent and stronger drought periods [6]. Increased drought stress may be a discriminant of species distribution and abundance determining changes in vegetation in the long-time. The main objective of this study was to analyze differences in physiological traits among species co-occurring in the Mediterranean maquis. Plant survival in a given environment depends largely upon whether it can photosynthesize and keep its water loss lower than the supply; rates of these two gas-exchange processes may play a major role in determining the outcome of competition among species. The pronounced sensitivity of photosynthesis to heat can be used to detect early damage in plant tissue; we employed photosynthesis as a stress temperature indicator.

Material and Methods: The study was carried out in the Mediterranean maquis developing within the Castelporziano Estate (Rome). *Quercus. ilex*, *Phillyrea latifolia, Pistacia lentiscus, Arbutus unedo, Cistus. incanus, Rosmarinus officinalis, Erica arborea,* and *Erica multiflora* were monitored in the years 2000 - 2005. Photosynthetically active radiation (PAR), net photosynthesis (P_N), stomatal conductance (g_s), transpiration rate (E), sub-stomatal CO_2 concentration (C_i), and leaf temperature (T_l) were measured by an infrared gas analyzer Ciras-1 open system (PP System, UK). Gas exchange measurements were carried out in favourable periods (April-May), in drought periods (June-August), and in recovery periods (September-October). Instantaneous water use efficiency (WUE) and carboxylation efficiency (C_E) were calculated. The relationship between P_N and T_l was tested by regressing these variables, according to [1], using all data pooled throughout the study period.

Results and Discussion: The overall results of this research underline that the evergreen species of the Mediterranean maquis had different photosynthetic responses to drought stress: *R. officinalis*, *E. arborea* and *E. multiflora* had the lowest P_N rates in favourable periods, the highest decrease during drought but the highest recovery capacity at the first rainfall, when the drought stress lasts over a short period. *A. unedo* and *C. incanus* had the highest P_N rates during favourable periods, a high decrease during drought and a low recovery capacity. *Q. ilex*, *P. latifolia* and *P. lentiscus* had intermedial P_N rates in favourable periods, a low reduction during drought and a high recovery capacity. The upper leaf temperature threshold beyond which photosynthetic rates drop below half of its maximum value is the highest in *Q. ilex* and *P. latifolia*, i.e. they photosynthesize at sufficient rates at air temperatures highest than 37 °C. These functional traits associated to a large and deep root system result in a higher capacity of these species to adjust photosynthetic rates under severe drought, favouring biomass accumulation. On the contrary, *R. officinalis* seems to be particularly affected by a severe drought stress period, the low WUE and C_E, and the low tolerance to high air temperatures. It loses ca. 50% of its leaves at the end of a severe drought period. *Q. ilex* and *P. latifolia* might be at a competitive advantage relative to the other considered species in regard to increasing drought stress which is expected to occur in the Mediterranean Basin with the ongoing global climatic change.

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Chilling tolerance of photosynthesis in maize – From the physiology to the genetics

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Introduction: During the early growing season, growth of maize in temperate regions is often limited due to low temperature. Amongst others, deleterious effects of low temperature on the photosynthetic apparatus are of particular importance. Maize leaves developed under suboptimal temperature are characterised by a reduced photosynthetic capacity and efficiency associated with altered pigment composition and changes in the scavenging systems. However, these alterations result in an increased capacity to withstand more severe cold stress. Comparison between maize genotypes from different origins indicates a large genetic variation in chilling tolerance of photosynthesis within the species *Zea mays*. However, the exact mechanism for this variation remains unknown. In the past years, we have developed a method based on chlorophyll fluorescence measurements to efficiently discriminate maize with contrasting chilling tolerance of photosynthesis [1]. This approach has been applied to produce our own set of inbred lines. These new lines display a very different photosynthetic activity when grown at low temperature, and since they were selected solely on the basis of chilling tolerance of photosynthesis they are an unique material to identify genetic loci associated with chilling tolerance of photosynthesis by quantitative trait locus (QTL) analysis. Moreover, the QTL analysis is a powerful tool for studying the relationships between complex physiological traits and how they are affected by environmental conditions.

Material and Methods: The genetic basis of chilling tolerance of photosynthesis was studied by QTL analysis performed on a segregating maize (*Zea mays* L.) population in the $F_{2:3}$ generation grown both under controlled and field conditions [2, 3]. Under controlled conditions, maize seedlings were grown at optimal (25 °C) and suboptimal (15 °C) temperature. In the field, maize was sown early (mid of April) and late (mid of May) in Switzerland. Thus, plants sown early were exposed to chilling conditions, whereas plants sown later developed under conditions more favourable for growth. Carbon exchange rate, chlorophyll *a* fluorescence parameters (F_v/F_m, Φ_{PSII}, F_v'/F_m' and q_P), leaf greenness and shoot dry weight were determined at the three leaf stage. The method of composite interval mapping was deployed for mapping the QTLs by using QTL Cartographer v. 1.17b [4].

Results and Discussion: The main QTLs involved in the functioning of the photosynthetic apparatus at low temperature were stable across cold environments. Furthermore, analogies to another mapped population [5] exist when plants were raised under similar conditions and traits characterising the physiology of the photosynthetic apparatus were considered, indicating some common genetic basis for chilling-tolerance of photosynthesis across maize germplasms. Based on the QTL analysis, relationships between chlorophyll fluorescence parameters and leaf greenness were found frequently but not always. This indicates that the extent of the photosynthetic machinery and its functioning can be under different genetic control. The functioning of the photosynthetic apparatus did not noticeably affect the biomass accumulation in the field, but it did at suboptimal temperature under controlled growth conditions. Potential candidate gene, which are located close to the identified QTLs and which could explain the strong pleiotropic effects at these loci, were genes involved in the assembling of the photosynthetic apparatus or genes of enzymes playing an important role in carbon assimilation.

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Relation between leaf structure and chlorophyll fluorescence

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Introduction: Biophysical methods based on chlorophyll fluorescence are frequently used in plant physiology. They are important for studies of functional state of different types of photosynthetic samples (algae, chloroplasts, membrane or particle suspensions, gel segments, leaves, needles etc.). However, the fluorescence parameters (emission and excitation spectra, induction phenomena, kinetics) may reflect also the sample structure and composition [1-14]. Here we present a survey of main optical effects in leaves affecting the fluorescence parameters and several particular models involving the phenomena of fluorescence reabsorption, inner filtering and energy transfer.

Material and Methods: In order to get information on the shape and absorption of a chloroplast, suspensions of chloroplasts and thylakoid membranes were prepared from pea leaves. Pea seedlings (*Pisum sativum* L.) were cultivated hydroponically at 20 ^{o}C in perlite substrate with water at 16 h light/ 8h dark regime. Pea leaves were homogenized and centrifuged. The chloroplast number and dimensions were measured by a calibrated light microscope (Amplival pol. U., Carl Zeiss Jena, Germany) using Bürker's cell. The absorbance of the chloroplast suspensions or acetone extract was measured using a spectrophotometer Unicam UV 550 (Thermo Spectronic, England). The obtained data allowed to estimate the inner chloroplast pigment concentration of 0,049 ± 0,005 M and the spectrum of the mean molar absorption coefficient $\varepsilon(\lambda)$. The data were used for model calculations.

Results and Discussion: A short general overview of optical effects in a leaf in relation to chlorophyll fluorescence and types of theoretical models is given ([1-14] and others).

Several particular theoretical models are presented, discussed and supported by experimental data:

a) A comparison of homogeneous and heterogeneous models of leaf optical properties is given and demonstrated on the leaf reflectance spectra and fluorescence reabsorption effects ([12]).
b) A model based on a general theory of the effect of fluorescence reabsorption on the emission spectra revealing the effect of energy transfer in a chloroplast of different shape.
c) A model showing an influence of light gradient in a sample on the fast fluorescence transient (O-J-I-P curve). ([13]).
d) A model predicting the effect of chloroplast arrangement and movement in a cell on the fluorescence parameters of a leaf. ([2]).
e) A general model of the optical filtering effects (epidermis, carotenoids) on chlorophyll fluorescence.

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Acknowledgements: Supported by grant MSM 6198959215 of Ministry of Education of the Czech Republic

Update on Würzburg grapevine research

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Introduction: During the last years, a Bavarian-wide research network dealing with UV action on living matter was carried out. Within this frame, the Department of Botany II at the University of Würzburg measured and evaluated UV screening in Bavarian crop plants. Among the various species investigated, particular attention was paid to grapevine plants (*Vitis vinifera* L.). In my presentation, I present a short overview on the results obtained with grape leaves and berries during our research campaign.

Material and Methods: Two white grapevine cultivars (Silvaner and Bacchus) were investigated. Action of UV and visible radiation on plant material was investigated in outdoor exposure experiments under close-to-natural radiation conditions or with the UV-B (280-315 nm) or the entire UV (280-400 nm) screened out by filters. During these acclimation experiments, epidermal UV transmittance, concentration of UV absorbing phenolics, photochemical yield of PS II and CO_2 assimilation was measured.

Results and Discussion: Complete build-up of epidermal screening in grape leaves by accumulation of flavonoids and hydroxycinnamic acids occurred within a couple of days demonstrating the combined action of both phenolic groups in UV shielding, but also the highly dynamic behavior of UV screening [1, 2]. In berries from the cultivar Bacchus, however, hydroxycinnamic acids appeared to be unimportant for UV screening [3]. In leaves, UV inhibited PS II photochemical yield (measured as F_V/F_M) much less than CO_2 assimilation suggesting that the target of UV inhibition of photosynthesis is not PS II. However, modeling of outdoor data and results obtained with artificial UV radiation suggest that UV radiation quenches F_0 with the possible effect that F_V/F_M ratios report erroneous information on the state of PS II [4].

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Acknowledgements: This work was supported by grants from the state of Bavaria (BayForUV program).

Short-term dark chilling stress does not impair the photosynthetic parameters in the C4 Gramineae *Cynodon dactylon*

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Introduction: It is well known that low temperature is a major factor limiting the productivity and geographical distribution of many species [1]. Studing the effect of chilling in the dark on photosynthesis is very important since plants in natural habitats generally experience the lowest temperatures at night. However little is known about the effect of such stress, mainly in C4 grasses. The aim of the present study was to evaluate the photosynthetic response of the C4 grass *Cynodon dactylon* (NAD-ME subtype) to a short-term dark chilling stress.

Material and Methods: *Cynodon dactylon* var. Shangri-lá plants were hydroponically grown in a growth chamber, under controlled conditions The chilling stress was imposed by transferring the plants from the growth chamber (25º/18 ºC, day/night) to a refrigerator (5º±3ºC), for 1 or 2 consecutive nights, after which plants returned to the growth chamber. Gas-exchange measurements were performed at 25 ºC with the portable Infra Red Gas Analyser LCpro+ (ADC, Hoddesdon, UK) 1 to 5 hours after the start of the light period. After each assay, the leaf relative water content (RWC; determined according to [2]), the specific leaf area (SLA) and the ratios DW/FW and Area/FW were determined in the adjacent part of the leaves used for gas-exchange analysis.

Results and Discussion: *C. dactylon* RWC and the ratio Area/FW were not affected by the chilling stress imposed ($p>0.05$). However, an increase was observed in the ratio DW/FW (29.4%) and a decrease of SLA (21%) in 1 night chilled plants ($p<0.05$). A recover of these two parameters was observed in plants subjected to 2 nights of chilling. Despite this recover, it seems that there is a tendency to a DW increase after 2 nights of chilling, although not statistically significant ($p>0.05$). These results suggest an increase of DW in plants exposed to 1 night of chilling. This increase in DW could be related with a lower metabolisation of starch accumulated during the night, or with an increase in the protein content.

The net photosynthetic rate (A), the transpiration rate (E) and the stomatal conductance to water vapour (gs), showed no variation when expressed on an area basis, as well as the intercelular CO2 partial pressure (Ci) ($p>0.05$). However, it was observed a decrease of A (50% and 39.5%, 1 and 2 nights respectively), of gs (42.9% in both stresses) and of E (45.9% and 40.5%, 1 and 2 nights respectively) for the stress conditions when the parameters were expressed on a DW basis ($p<0.05$). Since this decrease was similar in both stresses and in all parameters, it seems that the decrease result from an increase of DW. In fact, the increase in DW after 1 night of chilling and its tendency to increase (although statistically not significant) after 2 nights of chilling may explain, at least in part, the decrease in photosynthesis and the other gas-exchange parameters.

We can suggest that *per si* the gas-exchange parameters of *C. dactylon* were not impaired by a short-term dark chilling stress, and that the increase of DW with 1 night of chilling could be a primary response of the plant to the stress. When compared with other two C4 grasses, *Paspalum dilatatum* (NADP-ME) and *Zoysia japonica* (PEPCK) [3], where it was observed a decrease of A, gs and E, either expressed by area or by DW, with 1 night of chilling and a recovery with 2 nights of chilling, it seems that *C. dactylon* plants are less sensitive to a short-term dark chilling stress.

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Acclimation of the forest lichen *Lobaria pulmonaria* to high light. Report from an experiment showing unexpected high growth rates

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Introduction: The forest lichen *Lobaria pulmonaria* does not occur in the most exposed sites. Normally it has the highest cover on the north side of trees, while thalli on the more exposed south side are smaller and have a brown, melanic colour [1,2]. The synthesis of this melanic pigment is dependent on UV-B irradiaton [3].

Material and Methods: In order to investigate the growth of the old forest lichen *Lobaria pulmonaria*, we transplanted thalli (n=600) in a dense young forest, an old forest, and an open clear-cut. Our study includes assessments of light climate (hemispherical photography) and thalli-specific variables such as chlorophyll content, chlorophyll fluorescence parameters, area and mass.

Results and Discussion *L. pulmonaria* showed an unexpected and plastic growth potential. Mean dry matter growth in 100 days varied from 8.3±0.3 % (young forest) to 21.1±0.7 % (clear-cut), with the old forest in between. Transplants acclimated to higher light by increasing thickness and by synthesis of brown melanic pigments. Acclimation of the photosynthetic apparatus was shown by increased nonphotochemical quenching and increased chlorophyll a/b ratios. Reduced maximal PSII yields (Fv/Fm) in the clear-cuts indicate that the thalli had been exposed to excess light. The natural lack of *L. pulmonaria* in open habitats, where potential growth is highest, is probably caused by the risk to be killed by high light during occasional long dry periods.

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Acclimation of leaf anatomical structure and photosynthetic electron transport to deep shade in four species of forest trees differing in ecological light requirements

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Introduction: The shade tolerance syndrome involves a number of physiological and morphological features expressed on the leaf level which lead to efficient utilization of low-flux light but make plants vulnerable to photooxidative stress when light flux density is high. We have previously studied the shade syndrome in seedlings of *Abies alba* from Karkonosze Mountains and found a remarkable ability of this shade specialist species to acclimate to diverse light conditions [1,2]. Here, we studied acclimation to deep shade in leaves of 3-4 year old plants of two highly shade tolerant (*Abies alba* and *Fagus sylvatica*) and two light demanding (*Acer pseudoplatanus* and *Picea abies*) tree species in an attempt to link their ecological light preferences to acclimatory responses of the photosynthetic apparatus.

Material and Methods: From April 2003 till July 2004 potted plants were grown outside either in full sunlight (controls) or under a shading cloth transmitting 5% of the light flux. In both seasons of the experiment, leaf anatomical structure was determined using paraffin embedded section. Light responses of PSII photochemistry (Φ_{PSII} and NPQ parameters) were investigated using the chlorophyll fluorescence technique (FMS2, Hansatech, UK). Subsequently shading cloth was removed and maximal efficiency of PSII (F_V/F_M) was measured over the following 30 days to monitor the onset and relaxation of photoinhibition. This latter treatment was designed to simulate an opening of the canopy, whether natural or man made.

Results and Discussion: Acclimation to the different light regimes was found at the anatomical level. The most striking response to shade was a reduction in the length of the outermost adaxial mesophyll cells (pallisade), as described for many species before. This anatomical response was strongest in the deciduous species *Fagus* and *Acer* and relatively small in the evergreen *Abies* and *Picea*. Within each of these species pairs, the more shade tolerant species displayed a greater extent of anatomical plasticity in response to the light regime. This observation suggests that the plasticity of leaf structure evolved as part of the shade acclimation strategy and is small in light demanding plants. This may reflect the fact that for shade tolerant trees extreme shade is only experienced early in life, before reaching the upper canopy or being exposed to a canopy gap.

Based on light response curves, the exposure to shade resulted in a greater susceptibility of Φ_{PSII} to light in at least one season of the experiment in each species. Exposure to shade resulted also in significantly reduced ability for the photoprotective energy quenching (NPQ). There was no obvious relationship of these acclimation responses with ecological ranking of species with respect to shade tolerance.

In the course of the experiment, dark-adapted photochemical efficiency F_V/F_M remained in the healthy range of >0.8. On exposure of shade acclimated plants to full sun, photoinhibition (drop in F_V/F_M) was recorded in all species, with the strongest decline in *Fagus*, followed by *Picea*, *Acer* and *Abies*. Again, there was no clear relationship to light requirements of the species. At the end of the 30 day period in full light, recovery of F_V/F_M to near-control level occurred in all species, with the most persistent photoinhibition, and incomplete recovery, seen in the shade-tolerant *Fagus*. These findings indicate that the acclimation abilities of photosynthetic electron transport only poorly correlate with ecological light preferences of tree species.

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Extent of photoinhibition and the rate of recovery in a lichen *Lasallia pustulata* depends on modus of irradiance and the capacity of photoprotective mechanisms

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Introduction: It has been demonstrated several times that photoinhibition of primary photosynthetic processes occurs in foliose lichens exposed to excess irradiation under laboratory [1] and/or field conditions [2]. In our laboratory, several experiments have been done on the spatiotemporal distribution of photoinhibitory responses over the thalli of foliose lichen species subjected to a short-term excess radiation under co-acting variety of factors, such as e.g. low temperature, osmotic stress. In the presented study, we focused on the response of *Lasallia pustulata*, a foliose lichen species possessing green alga *Trebouxia* sp. as a photosynthetizing partner, to different modes of irradiance varying in their duration and the repetition of high irradiance events.

Material and Methods: Thalli were optimally hydrated for 24 h and subjected to four different modes of photoinhibition: (*A*) short-term exposure (1800 μmol m^{-2} s^{-1} for 30 min), (*B*) continuous long-term exposure (600 μmol m^{-2} s^{-1} for 5 d), (*C*) long-term supplemented with a short-term exposure repetitively each 24 h, (*D*) repetitive short-term exposure each 24 h with recovery in dark. For each mode of photoinhibition, Fv/Fm, Φ_{II}, NPQ were monitored (PAM-2000, H. Walz) during treatment and recovery. At the time of Chl fluorescence measurements, samples were taken and actual amount of viola-, anthera-, and zeaxanthin (HPLC, Waters) determined.

Results and Discussion: Time courses of zeaxanthin formation and its conversion to violaxanthin and their relation to NPQ corresponded to our earlier observations [3], [4]. From the obtained results, it might be concluded that in hydrated *L. pustulata*, major component of NPQ induced by a strong short-term stress was related to qI. Regulatory part of NPQ played a dominant role in moderate and long-term stress. Under such conditions, the energy-dependent quenching (qE) related do de-epoxidation state of xanthophyll cycle pigments (DEPS), formation of zeaxanthin in particular, was of major importance. During recovery, DEPS was linearly related to NPQ indicating the involvement of the photoprotective mechanism into the resistance of *L. pustulata* to photoinhibition.

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Acknowledgements: The research was supported by the GAČR project 522/03/0754.

Acclimation of photosynthetic apparatus in two types of C4 plants to variable light conditions during growth.

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Introduction: The environmental factors as light intensity cause changes in fluorescence parameters, pigments content, efficiency of photosystems and in relative levels of thylakoid components in plants. In high light adapted C3 plants reduced level of PSII and PSI light harvesting complexes and higher content of photosystems core proteins are observed as well as higher a/b chlorophyll ratio. The overall effect of these changes is higher rate of photosynthesis during growth in high light [1].
Short exposure to high light leads to photoinhibition and changes in fluorescence parameters of leaves. Photoinhibition of photosystem II is also a process which induces damage and degradation of the PSII reaction center D1 protein [2].
It is known that photosynthesis in C4 plants involves mesophyll (M) and bundle sheath (BS) chloroplasts, which differ structurally and functionally [3] but up to date little information is available about acclimation strategies of two types of C4 chloroplasts at different light intensities.

Material and Methods: Plants – *Z. mays* and *P. maximum* were grown in a growth chamber under a 14 h photoperiod. PFFD was 50 (LL), 300 (HL) and 700 (HHL) μmol m^{-2} s^{-1}. Chloroplasts and thylakoids were isolated mechanically, protein content was analysed by SDS-PAGE and immunoblotting using specific antibodies. Activity of PSII was measured as a photoreduction of DCPIP at 595nm. Chlorophyll a fluorescence of the leaves was determined using fluorescence measuring system (Hansatech).

Results and Discussion: Amount of LHCII proteins decreased in response to increasing irradiance during growth and it was more evident in bundle sheath chloroplasts. LHCI proteins were also influenced by light but in a different manner. Fluorescence parameters such as a Φ_{PSII}, QP and NPQ differed slightly between studied plants.
The level of α subunit of ATP synthase was also influenced by light conditions, similarly in both C4 plants.
Moreover we studied light degradation of PSII core protein-D1 in two types of thylakoids of maize HL- and LL- plants. The degradation rate of D1 protein in BS thylakoids was faster than in mesophyll. In addition the D1 proteolysis occurred more quickly when plants were grown at high light conditions. Our results suggest that photosynthetic acclimation was achieved by changes on the level of light-harvesting and electron transport components in both C4 species grown at different light conditions. This process is more complex in C4 plants because of different strategies in M and BS chloroplasts.

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Acknowledgements: This work was supported by the Polish Committee for Scientific Research Grant 3 PO4C 043 25

Is the the xanthophyll cycle pool size regulated by excitation pressure?

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Introduction:. Besides being involved in nonphotochemical fluorescence quenching, zeaxanthin may also have importance as quencher of singlet oxygen and/or free radicals in the thylakoid membrane [1]. An increased pool size of the violaxanthin cycle pigments (violaxanthin + antheraxanthin + zeaxanthin, V+A+Z) results in a higher potential for zeaxanthin synthesis. V+A+Z acclimates to the photon flux density (PFD) a leaf has received during the last days. It has been shown in potato plants that excessive PFD serves as a signal for increased xanthophyll synthesis rather than PFD per se [2]. Excess absorbed light energy can lead to the overreduction of components of the photosynthetic electron transport chain. The reduction state may serve as signal for regulation of the the pool size. This was investigated by using potato plants with reduced ferredoxin content which showed an intrinsically increased reduction state of Q_A.

Material and Methods: 7 weeks old transgenic potato plants with Fd contents between 50-60 % of the wild type level [3] were grown in a growth chamber for six days at 21 °C and a PFD of ca. 200 µmol m^{-2} s^{-1}, before samples for carotenoid analysis were taken for the first time. After six further days at 9 °C and the same PFD leaf discs were sampled again. Both times we also measured in vivo chlorophyll fluorescence at the corresponding positions. Carotenoids were analysed by HPLC.

Results and Discussion: The cold treatment significantly increased $1\text{-}q_L$ of the transgenic and wild type plants. But although the transgenic plants had always an enhanced reduction of Q_A as compared to the WT, their xanthophyll cycle pool size was identical to the WT. We conclude that the signal responsible for the adjustment of the xanthophyll cycle pigments cannot be the reduction state of Q_A. If the signal originates from the excitation pressure, it must be located downstream the Fd. Experiments to test this hypothesis are in progress.

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Method for the estimation of effective concentration of polyols in green algal lichens at low temperature

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Introduction: Polyols (sugar alcohols) help lichens to maintain physiological activity at sub-zero temperatures. Among them, ribitol synthetised by symbiotic green algae in a lichen thallus represents one of the most important. Ribitol is main high-energy compound exported from a symbiotic producer (alga) to a consumer (fungus) and its natural concentration in a lichen thallus ranges 2-7 μg g^{-1} DM [1,2]. In fungal hyphae, ribitol is transformed into mannitol, which serves as energy source for maintenance and growth of fungal hyphae as well as production of fungal-specific compounds [3], such as *e.g.* pigments [4]. Limited number of experiments has been made so far, that focused on cryoprotective effects of ribitol on physiological processes of symbiotic algal cells [*e.g.* 5]. Here, some preliminary results are presented for two lichen species exposed to 3 stepwise increased concentrations of ribitol.

Material and Methods: Thalli of foliose lichen species *Umbilicaria hirsuta*, *Lasallia pustulata* were collected from granitic rocks in the valley of the Chvojnice river in a close vicinity of Ketkovice, 35 km W of Brno. From fresh thalli, circular segments (1.5 cm^2) were cut. Two hours before expostion to low temperature, the segments were treated with 3 different concentrations of ribitol under room temperature. Then, the segments with individual ribitol concentrations were put at temperature gradient cultivator and exposed to four temperatures (-15, -5, +5, +22 ^oC) for 3 d at low light. Before exposition, and then in 24 h interval, chlorophyll (Chl) fluorescence parameters of the segments were measured using a kinetic fluorometric CCD camera (HFC-010, PSI, Czech Republic). For each segment, potential quantum yield of photochemical processes in photosystem II (F_V/F_M), effective quantum yield of PS II (Φ_{II}), and Chl fluorescence quenching parametres were evaluated. The effect of ribitol on the Chl fluorescence parameters was expressed as a change against control (demineralized water, no addition of ribitol).

Results and Discussion: Preliminary results showed that effect of polyols on PS II functioning is apparent in low temperature only when high concentrations are used. The question arises what is the effective concentration of polyols in thalli under natural conditions. Few improvements of the above described methods and some additional analyses are required to answed the question and evaluate the role of polyols in low temperature resistance in lichen thalli under the field conditions.

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Acknowledgements: The research was supported by the GAČR project 206/03/P140.

Application of chlorophyll fluorescence recovery studies to separate between adaptation to stress factors and damage by stress.

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Introduction: In order to achieve information about the current photochemical use (CPU) in leaves of various plant species, signals of chlorophyll fluorescence were taken to interpret the responses to different environmental stress factors (*e.g.*: drought, light, temperature, ozone). Predawn Fv/Fm values (Fv/Fm_{pd}) were used to quantify the maximum available photochemical capacity at the beginning of the day (100%).

Material and Methods: Measurements of chlorophyll fluorescence without and after dark adaptation were performed on upper and lower leaf surfaces of attached leaves with a time resolving instrument (PEA, *Hansatech,* UK). Fv/Fm measurements of leaves in light adapted state (without dark adaptation) were done under ambient light conditions. These values of Fv/Fm represent the remaining current photochemical capacity (RCPC). After 20 minutes of dark adaptation the current photochemical capacity (CPC) was measured. Fv/Fm values are known to correspond with the quantum yield (QY) of photosystem II (PSII; [1]), which correlates roughly with the QY of oxygen evolution but only vaguely with QY of CO_2 fixation, mainly because of processes like photorespiration. Current photochemical use (CPU) is calculated as the difference between dark and light adapted Fv/Fm values expressed in percent of the predawn value. The remaining difference between Fv/Fm values from dark adapted and predawn measured leaves can be ascribed to the amount of non functional reaction centers (PhotoInhibition; PI).

Results and Discussion: Using several examples a new way of data arrangement will be introduced, making visible short term regulatory phenomena in the photosynthetic apparatus of leaves. The increase of photochemical capacity during dark adaptation can be ascribed on one hand to the relaxation of photochemical quenching (q_P) during the first few minutes and on the other hand on fast relaxing components of non-photochemical quenching (q_{E1}, [2]). Non-functional reaction centers (q_I) show up by comparison of Fv/Fm values to predawn values. Norway spruce needles from study sites in the Austrian alps differently polluted by ozone exhibited much higher photoinhibition at a site with higher ozone load, whereas the portion of downward regulated reaction centers, which was detectable after one hour of low light recovery, decreased dramatically. “Photochilling” conditions (high light, low temperature) in early spring led to enormous amounts of photoinhibited centers in spruce needles, especially pronounced on the upper needle surface. In contrast, severe frost events gave rise to even higher proportion of non-functional centers irrespective of upper or lower needle surface.

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Short-term preheating activates low-light-induced violaxanthin deepoxidation in wheat leaves

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Introduction: It has been reported that 1-2 h heating of leaves at moderately elevated temperature (35 °C) in the dark increases availability of violaxanthin (V) to violaxanthin deepoxidase (VDE) through detachment of V from LHCII complexes and its solubilization in lipid phase of thylakoid membranes [1]. In such preheated leaves an enhanced increase in non-photochemical chlorophyll fluorescence quenching (NPQ) was observed during the measurement of slow fluorescence induction (at moderate exciting light – 200 μmol photons m^{-2} s^{-1}) at room temperature. It was shown that this rise in NPQ corresponds with an enhanced conversion of V to zeaxanthin (Z) [1]. In our work we focused on the preheating temperature (25 – 45 °C) and exciting light intensity profile of this effect.

Material and Methods: Detached 8-day-old primary leaves of wheat grown hydroponically (Knop solution) in perlit at the regime 16 h light (100 μmol photons m^{-2} s^{-1}, PAR)/ 8 h dark were used for measurements. Leaf segments were plunged in the water bath of different temperature (25 – 45 °C) and incubated for 5 min in complete darkness. NPQ parameter (F_M/F_M'-1) was measured with a PAM 2000 fluorometer (Walz, Effeltrich, Germany) 5 min after the onset of white actinic light of different intensity. The maximal fluorescence level F_M (F_M') was induced by a 1.4-s white light pulse (about 7000 μmol photons m^{-2} s^{-1}). Pigment separation was performed by a gradient reversed-phase HPLC/PDA. Eluted pigments were monitored by their absorption at 440 nm. The factors converting the measured area below the absorption peaks to the relative pigment content were determined by a calibration with pigment standards. The conversion state of the xanthophyll cycle pigments (DEPS) was calculated as ([Z]+[A])/([V]+[A]+[Z]); (A – antheraxanthin). Thermostability of V deepoxidation in preheated leaves was tested chemically by a vacuum infiltration with a buffer of pH 5 with 80 mM ascorbate according to [2].

Results and Discussion: We observed that even a very short-term preheating (5 min) in darkness is able to stimulate the NPQ increase and V deepoxidation during subsequent 5 min irradiation at moderate exciting light (100 μmol photons m^{-2} s^{-1}, irradiation growing conditions). The stimulation gradually increased with increasing preheating temperature up to 40 °C, for higher temperatures the stimulation gradually decreased. For leaves preheated to 40 °C for 5 min the V deepoxidation reached the level obtained for unheated leaves irradiated at 1000 μmol photons m^{-2} s^{-1} (DEPS ~ 40 %), however, the NPQ level measured for the preheated leaves was about a half of that measured for unheated leaves. This implies that a relation of NPQ to V deepoxidation for preheated leaves differs from that of unheated leaves.
Measurement of the irradiance profile of the stimulation in NPQ and DEPS obtained for leaves preheated to 40 °C showed that even a very low exciting light (about 10 μmol photons m^{-2} s^{-1}) leads to a dramatic V deepoxidation (DEPS ~ 30 %). Interestingly, this phenomenon is not accompanied by a dramatic increase in NPQ. Our data indicate that not lumen acidification but rather the V availability is a critical factor limiting V deepoxidation *in vivo*.

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Acknowledgements: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic, the grant MSM 6198959215.

Spectrophotometric determination of Rubisco content and activity – the daily course

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Introduction: The Rubisco enzyme plays a key role in photosynthesis. The knowledge about activity and content of Rubisco is important for understanding of the changes of photosynthetic activity including interpretation of stress impact. We report here method for measuring the amount of Rubisco enzyme. We carried out measurements of Rubisco activity and Rubisco content in laboratory conditions simulating summer day when usually depression of photosynthetic activity occurred. The main objective of this study was to establish influence of these microclimatic conditions on activity and/or amount of Rubisco.

Material and Methods: The measurements were carried out on current needles of 5 year old Norway spruces cultivated for two weeks under controlled microclimatic conditions in growth chamber HB 1014. The daily courses of irradiance, temperature and relative air humidity were set up according to conditions at Experimental ecological station (Bílý Kříž, Beskydy mountains) during clear day in July. For determination of Rubisco activity we used modified spectrophotometrical method [1].

Results and Discussion: The absorption spectrum of Rubisco standard diluted in solution (HEPES-KOH, $MgCl_2$, Na_2EDTA, pH = 8) had two absorption maxima, i.e. 270 nm and 340 nm. The same absorption spectrum had also Rubisco extract prepared from spruce needles. We prepared different concentration of Rubisco standard and plotted relation between absorbance (for wavelengths 270 and 340nm) and concentration of Rubisco standard. On the basis of linear fit of this relation we obtained linear equation for the determination of Rubisco content extracted from spruce needles. Rubisco activity qualitatively correlated with the diurnal course of irradiance, thus reached its maximum during midday and minima at the morning and afternoon, respectively. However, the Rubisco content was almost constant during the whole simulated day. Under the conditions simulating moderate summer day, the midday depression of photosynthesis was not observed on the Rubisco level.

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Acknowledgements: Supported by grant "CzechCarbo - studium cyklu uhlíku v terestrických ekosystémech ČR v souvislostech Evropského projektu CARBOEUROPE (VaV/640/18/03)"

The combined effects of enhanced UV-B radiation on growth, chlorophyll fluorescence and ultrastructure in early maturing barley (*Hordeum vulgare var. eam 799*) treated in the field

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Introduction: The effects of increased UV-B radiation are manifold, with marked decreases in yields of agricultural crop plants, damage to photosystem II (PSII) and photosystem I (PSI), disturbance in carboxylating enzyme, DNA damage, oxidative stress, and ultrastructural changes [1, 2].

Material and Methods: Chlorophyll fluorescence is a useful property for estimating photosynthetic capacity. At room temperature most chlorophyll emission results from PSII. Furthermore, the changes in the chlorophyll fluorescence reflect changes in the primary processes of photosynthesis, such as light absorption, excitation energy transfer and electron transfer from PSII to PSI. Fluorescence images were obtained after 10 min of dark adaptation. The maximum fluorescence (*F*m) was measured under saturating irradiance induced by actinic light. Variable fluorescence (*F*v) was determined as the difference between initial fluorescence (*F*o) and *F*m. In addition, *F*v/*F*m ratio was measured as an indicator of potential PSII activity. Chlorophyll fluorescence was measured weekly for two fully expanded leaves per pot using the PSI Fluorcam.

Results and Discussion: Changes in chlorophyll fluorescence and carboxylating enzyme activities confirm that non-stomatal limitations may have been responsible for reductions in photosynthesis. Leaves from high UV-B plants had increased minimal and maximal chlorophyll fluorescence and decreased half rise time from minimal to maximal fluorescence. We found no significant differences in maximum potential quantum yield of photosynthesis between UV-B treatments. Otherwise, UV-B radiation decreased total chlorophyll, total carotenoids, soluble proteins, soluble sugars, starch and UV-B absorbing compounds. The drop in total chlorophyll was due to proportional decreases in chlorophyll *a* and chlorophyll *b*, explaining that there was no significant difference in chlorophyll *a*/chlorophyll *b* ratio (data not shown). In addition, UV-B radiation had no influence on chlorophyll/carotenoid ratio, whereas the soluble sugar/starch ratio decreased in enhanced UV-B plants.

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Acknowledgements: Supported by Bio-Green 21 from RDA and RIS project

Fluorescence response of *Synechocystis* sp. PCC6803 mutant Δ rpa C to harmonically modulated light under different temperatures.

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Introduction: Photosynthetic organisms live in a dynamic environment where light usually fluctuates around a mean level that is slowly drifting during the solar day. We show that the far from equilibrium photosynthesis occurring in a rapidly fluctuating light differs vastly from the stationary-flux photosynthesis. Photosynthetic organisms in a static light can be characterized by a steady state quantum yield of chlorophyll fluorescence emission F'. In *Synechocystis* sp. PCC6803 and ΔrpaC mutant the steady state photosynthesis has not such variability as the measuring in dynamic harmonically modulated light. The method of harmonically modulated irradiance allow us to see reaction of the ΔrpaC mutant to the different light regimes in the large spectra of changing light conditions. Beside this the method describes possible variability of the reactions from in situ conditions. We see particularly the use of the method in study of light features dynamics response or study of the mutants or influenced photosystems.

Material and Methods: *Synechocystis* sp. PCC6803 and *Synechocystis* sp. ΔrpaC was cultivated at 25°C in BG11 medium. The double-modulation fluorometer [1] was custom modified by Photon Systems Instruments, Ltd., Brno, Czech Republic to provide harmonically modulated actinic light with modulation period, amplitude and with mean photon flux density defined by instrument protocol. The harmonically modulated actinic light and the 2 μs-long measuring flashes were generated by two sets of orange light emitting diods. The cell suspension was stirred during experiment and photosynthetic fluorescence response to harmonically modulated light was measured in increase temperature.

Results and Discussion: The fluorescence response to the harmonically modulated irradiance is highly non-linear exhibiting significant histeresis with multiple fluorescence levels corresponding to a single instantaneous level of the incident irradiance. We relate this dynamic picture to to a more common transient, the Kautsky effect: dark adapted cynobacteria respond to constant actinic light by a fluorescence transient that reflects largely by photochemical quenching due to changes in the redox state of the primary quinone acceptor Q_A and due to regulatory changes in the distribution of the excitation energy [2,3,4]. Different dynamic patterns emerges in different lengths of period and in irradiances in *Synechocystis* PCC6803 WT. Mostly, these patterns correspond to the higher harmonic oscillations and to the shift in the amplitude and phase. The comparison of WT with the mutant ΔrpaC show new and quite remarkable patterns talking about changes caused by the ΔrpaC gene deficiency. Mostly, this information corresponds to the different light harvesting efficiency in different light intensities. By this measurement we can confirm, proof and explain the known ideas about low light use efficiency with the studied mutant.

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Sequences of short lightflecks are more efficient in the photosynthetic induction of spruce trees

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Introduction: Plants growing in the forest understorey are subjected to both prolonged low diffuse background light and transient lightflecks, which provide high light intensities for a few seconds or minutes [1]. The photosynthetic response to fluctuating irradiances is dependent on the following main features of: 1) photosynthetic induction response to a rise in the irradiance, 2) ability to maintain photosynthetic induction under low-light conditions, which allows a plant to better exploit the next lightfleck, 3) stomatal response to light intensity, and 4) ability to extend the photosynthetic activity into the shade period immediately following a pulse of high light, *i.e.* post-illumination CO_2 fixation [2,3]. In this study we determined the impact of oscillating irradiance with different times of period on the activation and deactivation of CO_2 assimilation processes and light use efficiency during the lightflecks.

Material and methods: Dark adapted Norway spruce (*Picea abies* L. Karst) shoots (one-year-old) were periodically exposed to two levels of irradiances, 0 and 1200 μmol m^{-2} s^{-1} with four different times of period, *i.e.* T=30 minutes, T/2, T/3, and T/6 were used. The light intensity was varied using the LED light source LI-6400-02B (LI-COR, USA). The shoots were kept under the constant ambient CO_2 concentration (365 ± 5 mol mol^{-1}), air humidity (56 ± 4 %) and temperature (25 ± 1 °C) over the all measurements.
The time-courses of photosynthetic characteristics (CO_2 assimilation rate – A_N, stomatal conductance – G_S, intercellular CO_2 concentration – Ci) were automatically recorded by the open gas exchange system LI-6400 (LI-COR, USA) at 5-s intervals. Moreover, light response and CO_2 response curves were estimated under the steady-state conditions. Subsequently, additional parameters characterizing activation and deactivation of photosynthesis were calculated. *I.e.*, time required to reach 90% of maximal A_N (T_{90}), induction state after 60 s of exposure to saturating irradiance (IS_{60}), lightfleck use efficiency (LUE), photorespiration rate (R_L), and post-illumination CO_2 fixation.

Results and Discussion: Investigated spruce shoots were characterized by the maximal assimilation rate 7.30 ± 0.13 (mean ± standard deviation), dark respiration 0.90 ± 0.10 μmol(CO_2) m^{-2} s^{-1}, and apparent quantum yield 0.027 ± 0.003 mol(CO_2) mol^{-1}(photons). These parameters reflect the sun adapted character of the needles.
LUE, *i.e.* the ratio between actual carbon gain during the lightfleck and the carbon gain of a leaf with an instantaneous response to illumination, ranged from 33 (T/6) to 63 % (T) in the 1st period, whereas, there were no statistical differences from the 5th period (ca 86 %). During the short-term lightflecks, the lower total irradiation was required to reach 90% of maximal A_N: 0.67 ± 0.03 (mean ± standard deviation) for T/6, 1.0 ± 0.16 for T/2, 1 ± 0.12 for T/3, and 1.8 ± 0.1 (mol m^{-2}) for T.
This phenomenon may be explained by (1) the degree of photosynthetic deactivation after the dark period and (2) the rate of photorespiration induction during the lightfleck. Parameter IS_{60}, reflecting the activation stage after the dark period, was reaching 91 ± 1.6 % for T/2, T/3 and T/6, while, it was only 70 ± 3.4 % for T. Moreover, we clearly demonstrated that the lightfleck duration influences the photorespiration rate (R_L). The R_L values (mean ± standard deviation) estimated at the ends of the light periods were 2.5 ± 0.3 (T), 2.1 ± 0.6 (T/2), 2.0 ± 0.5 (T/3), and 1.3 ± 0.1 (T/6) μmol(CO_2) m^{-2} s^{-1}.

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Acknowledgement: The work forms a part of the research supported by grant 522/05/P515 (GA CR).

Changes in the lipid phase behavior in thylakoid membranes, modulated by temperature, light and pH, revealed by ^{31}P-NMR

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Introduction: Most biological membranes contain non-bilayer lipids. In the thylakoid membranes, the non-bilayer monogalactosyl diacylglycerol (MGDG) is the major lipid constituent (50% of the total lipid content). The high content of non-bilayer lipids determines the propensity of the whole lipid mixture to participate in different lipid phases. However, it is generally believed that non-bilayer lipids in biological membranes do not form non-lamellar phases, at least in sizeable quantities and for substantial time periods [1, 2]. Hence their function in the bilayer membranes is still unclear.

Material and Methods: Dark adapted spinach leaves were homogenized in a medium containing Tricine buffer (20 mM pH 7.5), 400 mM sorbitol, 5mM $MgCl_2$ and 5 mM KCl; the suspension was filtered through 4 layers of cheese cloth and centrifuged for 4 min at 4000 x g. The chloroplasts were osmotically shocked in a medium containing Tricine (20 mM, pH 7.5), 5 mM $MgCl_2$ and 5 mM KCl, and centrifuged for 5 min at 6000 x g. The pellet was finally resuspended in the same medium supplemented with 400 mM sorbitol. For low pH measurements the membranes were washed in this medium, the pH of which was adjusted by HCl to 4.6. ^{31}P-NMR spectra were recorded on an AMX300 wide-bore spectrometer (Bruker, Germany) tuned at the resonance frequency of the ^{31}P nucleus (121.500 MHz). 20 mm (o.d.) tubes were used containing 15 mL of thylakoid suspension. For the illumination of the sample the 514 nm line of an Argon ion laser, at a power of 1 W, was used.

Results and Discussion: In this work, we used phosphatidylglycerol (PG) as an intrinsic bulk lipid label for ^{31}P-NMR studies for the detection of different lipid phases, for the first time, in isolated intact thylakoid membranes. Our data show that untreated spinach thylakoid membranes, in addition to the bilayer, contain non-bilayer phases. The formation of these phases and the partition of PG molecules between bilayer and non-bilayer domains depend on the temperature, the pH of the medium, and is also modulated by light. We propose that intact thylakoids contain small membrane-associated lipid aggregates in the aqueous phases, which exhibit ^{31}P-NMR features characteristic for inverted hexagonal (H_{II}) structures; they might be associated with proteins. Upon extensive treatments, they probably assemble into larger aggregates, such as H_{II} tubuli, which become well discernible also with other techniques, such as small angle X-ray scattering and electron microscopy, and might dissociate themselves from the membranes.

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Aknowledgments: This work has been supported in part by the WNMRC, an EU FP5 grant (HPRI-CT-2001-00178 (2003-2004)) to H.v.A. and by the Hungarian Fund for Basic Research (OTKA T 34188) and by EU FP6 MC RTN (INTRO2) to G.G.

Energetics of the trimer to monomer transition of the plant light-harvesting complex II

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Introduction: There is increasing evidence that the light-harvesting complex II (LHCII) plays an important role in the response of plants to changes in the light environment by regulating the structural properties of the thylakoid membrane and the dissipation of the excess light energy. It has been proposed that light can trigger structural changes in LHCII via thermo-optic effect, i.e. by thermal transients caused by dissipation of the absorbed light quanta [1, 2]. Recently it was shown that, in isolated LHCII preparations, the LHCII trimers can be disaggregated into monomers either by high-temperature or light treatment [3]. The light-induced monomerization of LHCII could be explained in the frame of a thermo-optic model although the exact site of dissipation and physicochemical nature of the induced structural change remained unknown. This study aims to further explore the mechanism of the light-induced trimer to monomer transition of LHCII by determining the quantum yield of monomerization, detected by circular dichroism (CD) spectroscopy and partially denaturating PAGE.
Material and Methods: LHCII trimers were isolated from dark-adapted spinach leaves by isoelectric focusing [4]. Lamelar aggregates of LHCII and thylakoid membranes were isolated as in [3]. For CD measurements, the LHCII suspension was diluted to 10 μg Chl/ml in 25 mM HEPES buffer, pH 8, containing 0.01% *n*-dodecyl-β-maltoside. The samples, in a cell of 1 cm optical pathlength, were incubated in a thermostated, magnetically stirred sample holder, which could also be illuminated by tungsten-halogen light source. CD spectra were registered, before and after the preillumination, using a CD6 dichrograph (Jobin Yvon, France). Partially denaturating "green" PAGE was performed as in [5].
Results and Discussion: It has been previously shown that the oligomeric state of LHCII is reflected in the CD spectra, which show marked differences in the excitonic band structure between 430 and 500 nm [3]. In order to define the changes more accurately, we compared the CD spectra of trimeric LHCII with the spectra of monomers obtained by heating or phospholipase A_2 treatment and separated by partially denaturating "green" PAGE. The spectra registered from bands on the gel corresponding to LHCII monomers as well as the spectra of phospholipase-treated LHCII lacked the (+) 483, (–) 473 excitonic CD band pair which was prominent in the spectra of trimeric LHCII. This band was used to estimate the relative amount of monomers in samples obtained either by light- or temperature-treatment. The quantum yield of monomerization was estimated as the fraction of LHCII trimers converted to monomers divided by the total number of quanta absorbed by one trimer. Comparing the quantum yield obtained by illuminating the samples at different temperatures it was found that the efficiency of conversion of the LHCII trimers increases with temperature in the region of 25-45°C. The results are fully consistent with the hypothesis that LHCII monomerization is driven by thermo-optic mechanism, and preliminary data on lipid-enriched lamellar aggregates of LHCII and on thylakoid membranes suggest that monomerization, albeit occurring with a low quantum efficiency, plays an important role in photoprotection of plants.
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Acknowledgement: This work was supported by INTRO2 (MRTN-CT-2003-505069) and OTKA (T34188).

Photosynthetic activity of bean plants at low temperature and high light intensity during short-term exposure to elevated CO_2 concentration

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Introduction: Plants in nature are often subjected to a combination of stress factors, for instance high or low temperature in combination with high light, which usually enhances the harmful effect of the stressors alone. On the other hand CO_2 is known by its potential ability to regulate various parts of the photosynthetic apparatus. The present study aims to trace the changes in photosynthetic activity in intact leaves of bean plants (*Phaseolus vulgaris* L.) exposed to different temperatures and light intensities in the presence of ambient or elevated CO_2.

Material and Methods: 19-days-old bean plants were exposed for 4 consecutive days (for 8 h per day) to 10 °C or 23 °C under 100 (LL) or 1000 μmol m^{-2} s^{-1} (HL) light intensity in the presence of elevated (1300 ppm) or ambient (350 ppm) CO_2 concentration. The A/C_i-curves were measured daily and basic physiological parameter and the parameters of the chlorophyll fluorescence were determined on the fourth day of the treatment on fully expanded first trifoliate leaves.

Results and Discussion: HL slightly reduced the CO_2-saturated photosynthetic rate (A_{max}) and carboxylation efficiency at 10 °C and increased them at 23 °C during the 4 days of treatment. HL-exposed plants showed higher values of the quantum yield of PSII photochemistry in the light (Φ_{PSII}) and the photochemical quenching of fluorescence (q_p) and accumulated more dry mass and soluble protein than LL plants. The investment in dry mass and soluble protein was lower at 10 °C than 23 °C and was intensified by the elevated CO_2 concentration only at HL. At 10 °C and LL the relative stimulation of A_{max} at 1300 ppm compared to 350 ppm [CO_2] decreased from a factor of 1.5 to less than 1.0 from first to forth day. In contrast, at HL the CO_2-induced stimulation increased during the experiment with a factor up to 2.5 at the end of the treatment. At 23 °C the stimulation factor of A_{max} was almost unchanged – about 1.5 and 1.0 for LL- and HL-exposed plants, respectively. The acclimation response of stomatal conductance to the elevated [CO_2] varied in parallel with photosynthesis. In low temperature exposed plants the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) increased in the course of the 4 days treatment, while at 23 °C it was almost unchanged. The significance of the stomatal limitation of photosynthesis at both temperatures was reduced on the fourth day in comparison to the first day of the treatment.

Temperature and light as a stress factors inducing the changes in pigment composition and photosynthetic performance in Baltic cyanobacterium *Phormidium amphibium*

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Introduction: The examined strain is a typical element of cyanobacterial mats growing on soft bottom in coastal, shallow part of the Puck Bay (Southern Baltic). The main aim of the work was the recognition of the photoacclimation mechanisms of the strain by the determination of the changes in chlorophyll, carotenoid and phycobilin, and the characteristic of photosynthesis by photosynthetic light response curves (P-I).

Material and Methods: The batch cultures were carried out on cyanobacterium, *Phormidium amphibium* (AL-13/994), isolated from the costal part of bay and maintained as unialgal culture in Culture Collection of Baltic Algae (CCBA), Institute of Oceanography in Gdynia [1]. The effect of light intensity PAR (5-125 μmol photons·m^{-2}·s^{-1}) was tested together with temperature (15-30 ºC) and interactions of these two factors were evaluated in factorial experiments. Two-way analysis of variance ANOVA and orthogonal polynomials to fit curvolinear regression methods (ksi prim) were applied [2]. Chromatographic analyses (HPLC) of chlorophyll *a* and carotenoids (ß-carotene, zeaxanthine and myxoxanthophyll) [3] and spectrophotometric analyses of phycobilin were used. The measurements of photosynthesis rate were carried out using volumetric microrespirometer [4].

Results and Discussion: The experiments with *P. amphibium* at various light intensities proved its strong adaptation ability to changing irradiance. Both chlorophyll *a* and the total level of phycobilin pigments were influenced by PAR as well as temperature, but stronger effect was noted in the case of irradiance. The content of chlorophyll *a* and phycobilins, expressed in pg per filament unit, dropped fourfold with increasing PAR in the tested range. Chromatographic analyses of pigments showed that the car/chl*a* ratio increased sevenfold, from 0.2 to 1.4, with an increase in light intensity. All carotenoid pigments increased significantly as compared to chlorophyll *a*, but the biggest changes (above tenfold) were observed for myxoxanthophyll. The data also indicated that irradiance and temperature had an effect on the concentration of *ß*-carotene and myxoxanthophyll in cyanobacterial filaments. *ß*-carotene concentration (pg/filament unit) dropped with an increase in light intensity and increased with an increase in temperature, while myxoxanthophyll concentration increased with an increase of light and dropped with an increase in temperature. However, zeaxanthine concentration was constant in the whole range of temperatures and light conditions (about 0.08 pg/filament unit).
On the base of P-I curves two mechanisms of photoadaptation in *P. amphibium* were recognized: a change in the number and size of photosynthetic units. These two mechanisms explain big changes in photosynthesis rate and its parameters (P_c, α, E_k, P_m and R_d) upon the influence of different light intensities and temperature. Especially great changes were observed in compensation point (P_c) values. The parameter changed about fifteenfold, from 5 to 75 μmol photons·m^{-2}·s^{-1}, in the range of the factors tested.

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Acknowledgements: Supported by KBN, grant No. 3PO4F02723.

Photosynthetic response of young soybean plants during heat stress

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Introduction: Photoprotection of photosynthetic apparatus is mainly carried out by thermal dissipation of excess excitation energy and by ability to transfer reductive power to acceptors different from CO_2. The proportion of absorbed photons that is thermally dissipated often gets a maximum before saturating irradiances are reached, so that photochemical pathway becomes crucial for photoprotection at saturating light intensities [1].

Material and Methods: The plants evaluated were grown in a greenhouse under different day/night (15-40 °C range) temperatures. They were watered three times a week. The plants were subjected to day/night heat treatment for 2 days while simultaneously withholding water, when they were 4 weeks old (just before the flowering stage). Subsequently, the plants were rewatered and the heat stress removed. Leaves, similar in age, from six independent plants of each plant type were sampled every day. Sampling took place the day before the implementation of stress, after 1 and 2 days of stress, as well as during the first day of recovery.

Results and Discussion: Photosynthesis, excitation energy dissipation and alternative electron sinks to carbon assimilation at different temperatures (15-40 °C range) were studied in plants of soybean (*Glycine max* (L.) Merrill) grown outdoors at spring time. Chlorophyll fluorescence and gas exchange measurements were performed in plants exposed to different temperatures for two hours at saturating irradiance (1000 μmol photons m^{-2} s^{-1}). At low temperatures (15 °C) a significant decrease of electron transport rate (ETR) was found and processes alternative to CO_2 fixation, as sinks of electrons, occurred but they were not affected by the temperature. However, at 15 °C a low and statistically significant photorespiratory rate was observed and the non-photochemical quenching (NPQ) was the highest. At higher temperature (30-35 °C) ETR saturated and hence non-assimilative processes, alternative to CO_2 fixation, sustained the photochemical activity. By increasing the temperature up to 30-35 °C NPQ values did not show appreciable differences compared to 25 °C. The obtained results indicate that in *G. max* plants the thermal dissipation represents the main dissipative process of the excess excitation energy at low temperatures. On the contrary, at high temperatures (30-35 °C) the main dissipation of the excess excitation energy is due to alternative pathways to CO_2 assimilation.

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Acknowledgements: Supported by Bio-Green 21 from RDA and RIS project.

Characterization of chlorophyll-containing protein complexes separated by improved native Deriphat-PAGE and its use for study of their thermal disintegration

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Introduction: At present, native electrophoresis (PAGE) is widely used for the separation of chlorophyll-containing protein complexes (PPCs) from thylakoid membranes of chloroplasts. Thylakoid membranes are fractionalized by non-ionic surfactants like decylmaltoside (DM) that preserves pigment-protein interactions in PPCs. The fractionalized PPCs are then partly charged by addition of a mild surfactant (e.g. Deriphat 160, N-lauryl-beta-iminodipropionate) and separated by polyacrylamide gel electrophoresis (e.g. Deriphat-PAGE) [1,2]. In this work we present an improved Deriphat-PAGE (using a purified Deriphat) that results in the separation of more native PPCs in comparison with that reported earlier by Thornber's group [1,2]. The separated green zones with PPCs were characterized by their molecular weights and 77 K chlorophyll fluorescence spectra. The improved PAGE was used for study of thermal disintegration of PPCs.

Material and Methods: Thylakoid membranes were isolated from untreated and preheated (70 °C, 5 min.) barley leaves (*Hordeum vulgare* L. cv. Akcent). Thylakoid membranes were isolated according to [1,2] and solubilized by 2% DM, to yield a final 25:1 (w/w) ratio of surfactant to chlorophyll. Deriphat-PAGE was performed according to [1,2] with slight modifications. Molecular masses of PPCs were determined by Ferguson plot [3]. Electrophoreograms were scanned by the home-made 2-D monochromatic gel densitometer [4,5]. Chlorophyll fluorescence spectra of green pieces of the gel containing PPCs were recorded at 77 K using a Fluorescence spectrofluorometer F-4500 (Hitachi, Tokyo, Japan).

Results and Discussion: Our electrophoreograms of samples prepared from untreated barley leaves contained 5 green zones that were characterised by 77 K chlorophyll fluorescence spectra and molecular masses. The results were compared with those obtained by Thornber's group with the same plant species [1,2,6]. We have identified zones containing supercomplexes of photosystems and LHCs, PSI (photosystem I), CCII (core complex of PSII), LHCo (LHC oligomers) and LHCm (LHC monomers). Electrophoreograms did not contain the separate zone CCI implying that all CCI remained associated with LHCI in the PSI zone. The absence of CCI together with very low amount of free pigments in our electrophoreograms led us to the conclusion that our Deriphat-PAGE with purified Deriphat is very mild and can be used to study the stress-induced disintegration of PPCs. We applied our Deriphat-PAGE to study changes in PPCs induced by heating of barley leaves (70 °C, 5 min). In electrophoreograms we obtained 3 green zones within the gel, high amount of free pigments and aggregates remaining on the top of the gel. Based on the molecular masses of PPCs in these zones and their chlorophyll fluorescence spectra we suggest that the first zone contains CCI whereas the second and third zone contain oligomeric and momoneric form of LHC, respectively. The supercomplexes, CCII and PSI zones completely disappeared. Our results confirm that the CCI is more thermostable than CCII.

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Acknowledgements: This work was supported by the grant of the Ministry of Education of the Czech Republic No. MSM 6198959215.

Photosynthetic response of young soybean plants during cold stress

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Introduction: Recently, chlorophyll fluorescence is widely used in analyzing the photosynthetic apparatus and understanding the mechanism of photosynthesis and the mechanism by which a range of environmental factors alter photosynthetic activity under both biotic or abiotic stresses. Fluorescence parameters have been applied in rapid identifying injury to leaves in the absence of visible symptoms and a detailed analysis of change in photosynthetic capacity. Therefore, chlorophyll fluorescence is also frequently used as a potential indicator of environmental stress and a screening method of tolerant plants. We investigated the FI in soybean leaves and isolated chloroplasts.

Material and Methods: After a 30-min dark period in ambient conditions in the laboratory, chlorophyll *a* fluorescence in soybean leaf was measured using a pulse-amplitude modulated fluorometer (Fluorcam, CZ). Measurements of minimal (F0) and maximal (Fm) fluorescence yields allowed determination of the optimal quantum yield (Fv/Fm), the ratio (Fm – F0)/Fm being used to calculate the maximal potential efficiency of PS II of dark adapted leaves. Leaves were then irradiated by actinic radiation (110 μmol m^{-2} s^{-1}), and saturation pulses of 3,500 μmol m^{-2} s^{-1} were also triggered repeatedly (every 20 s) during approximately 6 min. For assessment of light use efficiency, leaves harvested on illuminated plants were gradually exposed to higher irradiance (2 min at each intensity: 50 to 1,850 μmol m^{-2} s^{-1}). The operational PS II quantum yield (Y) and relative electron transport rate (RETR) were calculated using, respectively, the following formulas according to Genty et al. (1989): $Y = (F'm – Ft)/F'm$ and $RETR = Y. PAR$.

Results and Discussion: The growth, photosynthetic gas exchange and chlorophyll fluorescence were studied in soybean (*Glycine max* var. Daepoong) plants grown under salt stress. The stressed plants showed decreased plant weight, leaf area, plant height although they had similar leaf number. Chlorophyll content was significantly reduced in stressed plants after 2hr of cold condition as compared to control plants. Decreased net photosynthetic rate, transpiration and stomatal conductance, and a slightly higher intercellular CO_2 concentration were observed in stressed plants. For the chlorophyll fluorescence parameter Fv/Fm, Fv_0/Fm_0, ETR and qP, there were no significant differences between cold stressed and control plants, but qN showed a reduction in stressed plants.

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Acknowledgements: Supported by Gyeonggi Province Special Project Group and GRRC project.

The involvement of short-chain prenyllipids in superoxide production by Photosystem II

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Introduction: Light-induced production of superoxide ($O_2^{\bullet-}$) in PSII membrane particles was demonstrated by either an assay involving cytochrome c reduction in the presence of xanthine/xanthine oxidase [1] or EPR spin-trapping spectroscopy [2]. Prenyllipids are natural components of thylakoid membrane involved in 1) electron transport chain and proton translocation across the membrane and 2) scavengering of reactive oxygen species. It has been shown that short-chain prenyllipids (PQ-1, PQ-2) significantly stimulated photoreduction of low potential form of cytochrome b_{559} (LP cyt b_{559}) [3]. In the present study, an involvement of short-chain prenyllipids in $O_2^{\bullet-}$ production was studied by EPR spin-trapping spectroscopy.

Material and Methods: The spin-trapping was accomplished by EMPO, 5-(ethoxycarbonyl)-5-methyl-1-pyrroline N-oxide (Alexis Biochemicals). Spinach PSII membranes (150 μg Chl ml^{-1}) in glass capillary tube were illuminated with continuous white light (900 μmol m^{-2} s^{-1}, 5 min) in the presence of 25 mM EMPO, 100 μM desferal and 40 mM Mes (pH 6.5). EPR spectra were recorded using EPR spectrometer MiniScope MS100 (Magnettech GmbH, Germany).

Results and Discussion: Illumination of PSII membrane particles resulted in $O_2^{\bullet-}$ generation. The presence of short-chain prenyllipids (PQ-1, PQ-2) enhanced $O_2^{\bullet-}$ generation, whereas in the presence of long-chain prenyllipids (PQ-9) production of $O_2^{\bullet-}$ was rather unaffected. Based on these results it is suggested that short-chain prenyllipids stimulate autooxidation of LP cyt b_{559}. The possible mechanism involves electron flow from Pheo to LP cyt b_{559} [3].

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Acknowledgements: Supported by the grants of The Ministry of Education, Youth and Sports of the Czech Republic No. 1K04103 and MSM 6198959215.

Photosynthesis of spring barley and its acclimation to irradiance under natural conditions

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Introduction: A plant´s light environment commonly exhibit large changes in both intensity and spectral quality during the vegetation. For a cereal crop species such as rice, wheat and barley acclimation of photosynthesis to the light depend upon the intensity of incident sunlight and the attenuation of sunlight by the canopy. The extent of acclimation is depended on whether the acclimation ceiling has been reached. For the latter, the extent of acclimation of photosynthetic processes is also be determined by the canopy structure, the leaf age and the position of the leaf in the canopy [1, 2]. We evaluated photosynthetic activity of leaves under fluctuating light conditions.

Material and Methods: Barley plants (*Hordeum vulgare* L.) were grown in plastic pots with soil in the natural environment. The activity of photosynthetic processes was determined as net assimilation rate using an open gas-exchange system (Ciras-2PP-Systems, Norfolk, UK). Chlorophyll fluorescence (MiniPam, Walz, Germany) was used to measure the efficiency of PSII electron transport and the redox state of PSII during periods of full sunlight. Fluctuation of microclimatic factors was determined applying by Datalogger 1400 (LiCor, Nebraska, USA) and irradiance was measured as PPFD using by light sensors (LI-190USA). Light sensors were located in two layers in the canopy and one was over the canopy.

Result and Discussion: As expected, irradiance decreased considerably from the top to the bottom of the barley canopy. At midday, irradiance values at the lowest leaves of the canopy may be less than 100 μmol m^{-2} s^{-1} whereas the upper leaves are directly exposed to the high light conditions. These changes had major effects on the degree of saturation of photosynthesis and the extent of photoinhibition. Photosynthetic activity of lower leaves was determined by light penetration to lower parts of the canopy with great importance in acclimation of leaves to this irradiance values.

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Acknowledgements: Supported by grant VEGA 1/1350/04.

Cultivation of microalgae in a tubular photobioreactor based on solar concentrators: distribution of light

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Introduction: Photosynthetic organisms must adapt to unfavourable conditions (high irradiance, nutrient deficiency, salinity) in their environment to optimise and preserve the function of the photosynthetic apparatus. One of the photoadaptive mechanisms represent biochemical changes in the content and composition of cell pigments (chlorophylls, carotenoids). This mechanism is employed in microalgal biotechnology for production of carotenoids (carotenes and xanthophylls) [1]. Various strains of microalgae (prokaryotic cyanobacteria and eukaryotic algae) can be used as source of carotenoids, especially secondary carotenoids which synthesis is induced under high irradiance.

Material and Methods: Recently, a new experimental tool – tubular photobioreactor based of solar concentrators (linear Fresnel lenses) has been tested for cultivation of microalgal cultures at the Academic and University Centre in Nové Hrady in collaboration with the ENVI Ltd. Třeboň [1]. This instrument can be used to study the behaviour of microalgal cultures under very high irradiances.
The photobioreactor consists of four loop modules of about 100 litres placed in a greenhouse construction to grow microalgal mass cultures under defined conditions (O_2 concentration, pH, CO_2 supply, temperature, optical density, heat exchange and photochemical activity). The custom-made software Algotron v. 4.05 (based on LabView, NI) is used for process control and data acquisition.

Results and Discussion: The cultivation system consists of two types of irradiance modules: vertical, lower-irradiance unit (close to ambient conditions) and roof, higher-irradiance unit (up to 3.5-times higher than ambient intensity) which are used to compare the growth and behaviour of microalgal cultures at various light intensities and biomass densities.
The aim of these experiments has been to monitor a diel course of irradiance intensity and spectral composition concentrated by Linear Fresnel lenses and the light distribution on the surface and inside cultivation tubes.
As a model organism, we have used the cyanobacterium *Spirulina platensis* M2. Three concentration of biomass (about 0.5, 1 and 2 g L^{-1}) have been tested. In higher-irradiance modules we also follow the induction of carotenoids synthesis.

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Acknowledgements: This work is supported by the Czech Science Foundation, project 522/05/P276 and by the Ministry of Education, Youth and Sports, project MSM6007665808.

The effect of metal chelators on the production of hydroxyl radical in pea thylakoid membranes

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Introduction: Photosynthetic apparatus was shown to produce hydroxyl radical under light stress conditions [1,2]. This strong oxidant can directly damage all biomolecules and contributes significantly to oxidative stress under high light. The reaction pathway of its formation involves the univalent reduction of hydrogen peroxide by reduced metals. We have studied the effect of various metal chelators on the generation of hydroxyl radical in thylakoid membranes using the EPR spin trapping spectroscopy with 4-pyridyl-1-oxide-N-tert-butylnitrone (POBN)/ethanol as the spin trapping system. Many contradictory results were published that describe both pro-oxidant and anti-oxidant properties of metal chelators [3,4].

Material and Methods: Thylakoid membranes were isolated from pea (*Pisum sativum* L. convar. medullare). The reaction mixture containing thylakoid membranes, POBN, ethanol, metal chelators and in some cases exogenous metals was illuminated in a glass capillary with white light (900 μmol m^{-2} s^{-1}) for 10 min. EPR spectra were recorded immediately after the end of the illumination at room temperature using EPR spectrometer MiniScope MS100 (Magnettech GmbH, Germany).

Results and Discussion: We have measured the production of hydroxyl radical in illuminated thylakoid membranes in the presence of various concentrations of metal chelators. We have studied the effect of exogenously added iron on the action of these chelators. The obtained results are discussed in terms of the suitability of individual chelators for the use in photosynthetic research and of their ability to affect the free-metal catalyzed decomposition of hydrogen peroxide to hydroxyl radical.

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Acknowledgements: This work was supported by the grants of The Ministy of Education of the Czech Republic No.1K04103 and MSM 6198959215.

Kinetics of induction of non-radiative dissipation and de-epoxidation of the xanthophyll cycle pigments in the course of acclimation of Norway spruce to excess irradiance

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Introduction: For conifers the enhanced efficiency of non-radiative energy dissipation (NRD) within photosystem II (PS II) is supposed to represent a key process of acclimation to elevated irradiance under both laboratory and field conditions [1,2]. The needles of Norway spruce acclimated to high irradiance exhibit considerable acceleration of NRD induction and significantly greater efficiency of violaxanthin de-epoxidation (approximately 90%) than is usual for other plant species with corresponding xanthophyll cycle pool size and LHC II size (Chl *a*/*b* ratio) [2]. Nevertheless, knowledge of dynamics of these processes is insufficient. Therefore we focused on the relation between kinetics of NRD induction and de-epoxidation of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) during acclimation of Norway spruce to high irradiance.

Material and Methods: 5 year-old Norway spruce saplings (*Picea abies* [L.] Karst) were acclimated under controlled environment conditions inside growth chamber at the irradiance of 100 μmol m^{-2} s^{-1} for 17 days (LI plants). Then the irradiance was increased to 1000 μmol m^{-2} s^{-1} for the next 10 days (HI acclimation). The induction of chlorophyll *a* fluorescence and de-epoxidation state of xanthophyll cycle were estimated simultaneously after illumination of needles that were kept in darkness for at least one hour. Pigment composition was estimated by the gradient reversed-phase HPLC (TSP Analytical, USA). Chlorophyll *a* fluorescence at room temperature was measured using a PAM 101/103 fluorometer (H. Walz, Effeltrich, Germany).

Results and Discussion: Acclimation to HI resulted in the pronounced changes in the de-epoxidation state of the xanthophyll cycle: 1, V convertibility [(A+Z)/(V+A+Z)] increased from 70%, reached after short-term exposure of LI plants to HI, to more than 90% after 10d at HI; 2, population of Z+A persistent during dark period was enhanced from about 20% observed for LI plants up to 60% after 4d at HI. This shift of the xanthophyll cycle state was accompanied by increased capacity of NRD. The proportion of the absorbed light energy dissipated as heat (D) increased by 20% during 2d at HI. On the contrary, the maximum level of F_0 quenching (SV_0), corresponding to the NRD localized within LHC II, increased by 40% immediately after the 1st day at HI, and was approximately doubled (in comparison with LI plants) at the end of HI acclimation. Light-induced V de-epoxidation was surprisingly rapid even in the LI plants. About one third of V pool, converted during 20 min of exposure of dark adapted plants to HI, became de-epoxidized within 20s after onset of illumination. After 10d at HI this rapid phase of V de-epoxidation reached more than 55% of convertible V. However, the slower phase of V de-epoxidation was not affected during HI acclimation and the steady state de-epoxidation was reached after 10-20 min during the whole period of HI acclimation. Qualitatively similar effects were observed on the kinetics of D induction, the rapid phase of D induction was considerably accelerated during HI acclimation, but the steady state D level was reached approximately after 10 min for both LI and HI plants. SV_0 induction was pronouncedly more affected during HI acclimation. Whereas, for LI needles SV_0 was not detected after 20 s, after 3d at HI about 70% of the steady state level was reached within that time period. Moreover, the induction of maximum SV_0 was accelerated during HI acclimation. Although our results confirmed that rapid phase of V de-epoxidation was enhanced during HI acclimation, it does not seem to be the main reason of the increased rate of SV_0 induction. It was clearly demonstrated that induction of maximum SV_0 does not require the maximum degree of V de-epoxidation. We suggest, that probably the population of persistent Z in thylakoid membranes mediates the rapid rearrangement of PS II unit that reduce the efficiency of excitation energy transfer from LHC II complexes to the PS II core.

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Acknowledgements: We thank B. Piskořová, A. Žemlová and P. Krausová for excellent technical assistance. The work was supported by grant of Faculty of Science of Ostrava University (IGA 05/2005).

Dynamics of xanthophyll cycle and non-radiative dissipation of excitation energy for Norway spruce in response to excess acclimation irradiance

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Introduction: One of the typical responses in acclimation of the photosynthetic apparatus of higher plants to high irradiance is increase of the capacity for non-radiative dissipation of absorbed light energy that is associated with enhanced ability to de-epoxidize xanthophyll cycle pigments [1]. This acclimation response represents a key protective process for Norway spruce [2]. We studied dynamics of changes in the pigment composition and utilization of absorbed light energy in photosystem II during short-term acclimation (10 days) of Norway spruce to excess irradiance.

Material and Methods: 5 year-old Norway spruce saplings (*Picea abies* [L.] Karst) were acclimated under controlled environment conditions inside growth chamber at the irradiance of 100 μmol m^{-2} s^{-1} for 17 days. Then the irradiance was increased to 1000 μmol m^{-2} s^{-1} for the next 10 days. Pigment contents were estimated spectrophotometrically (UV/VIS 550, Unicam, England) and by the gradient reversed-phase HPLC (TSP Analytical, USA). Chlorophyll *a* fluorescence at room temperature was measured using a PAM 101/103 fluorometer (H. Walz, Effeltrich, Germany). Photosynthetic activity was determined as CO_2 assimilation rate using an open gas-exchange system (CIRAS-2, PP Systems, UK).

Results and Discussion: After 10 day-exposure of Norway spruce to high irradiance (1000 μmol m^{-2} s^{-1}; HI) photosynthetic activity was significantly reduced for the whole range of applied irradiances (50-1420 μmol m^{-2} s^{-1}). Light-saturated rate of CO_2 assimilation decreased by 44% as compared to plants acclimated to low irradiance (100 μmol m^{-2} s^{-1}; LI). Only slight changes were observed in the pigment contents. The content of total chlorophylls tended to decrease and the Chl *a/b* ratio remained unchanged during the experiment. Lutein and the pool of xanthophyll cycle pigments (violaxanthin + antheraxanthin + zeaxanthin; VAZ) expressed on Chl *a*+*b* basis showed the same increase (by about 30%). Neoxanthin content decreased (by 15%) and α+β-carotene content showed no change during acclimation to HI. Thus, the typical HI acclimation responses resulting in enhancement of photosynthetic capacity, reduction of the light-harvesting complexes and increase of photosystem II core complexes amounts were not induced. Instead, spruce responded immediately to HI stress at the level of xanthophyll cycle activity and non-radiative dissipation of excitation energy. The conversion state of xanthophyll cycle [(Z+A)/(V+A+Z)] under acclimation irradiance rapidly increased from 54% in LI-acclimated plants to 86% after three days under HI. In the following days (Z+A)/(V+A+Z) still increased, although more slowly and finally reached 92% after 10 days of acclimation to HI. This increase was associated with enhanced non-radiative dissipation estimated as $1-F_V'/F_M'$. In addition, the back conversion of Z+A to V in darkness was pronouncedly slowed-down during the first two days under HI. (Z+A)/(V+A+Z) determined after 7 h in darkness ($DEPS_{dark}$) strongly increased during acclimation to HI (from 19% in LI-aclimated spruce) and stabilized after four days under HI, when it amounted 61%. Both the photochemical efficiency of photosystem II in dark-adapted state (F_V/F_M) and non-photochemical fluorescence quenching (NPQ) under HI exhibited decrease after exposure of spruce plants to HI that was stopped after four days. We found strong negative correlation between these fluorescence parameters and $DEPS_{dark}$. These results demonstrate that the key protective strategy of Norway spruce under HI stress lies in enhanced V convertibility and persistent accumulation of Z+A in darkness associated with sustained quenching of maximal fluorescence level.

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Acknowledgements: We thank B. Piskořová and A. Žemlová for excellent technical assistance. The work was supported by the Ministry of Education of the Czech Republic (LN00A141) and Faculty of Science of Ostrava University (IGA 05/2005).

Control of Rubisco enzyme activity and its amount in Norway spruce needles during the day

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Introduction: Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase; EC 4.1.1.39) enzyme catalyses carboxylation of D-ribulose-1,5-bisphosphate (RuBP), the first step of the Calvin cycle, in competition with oxygenation of RuBP that leads to the photorespiratory pathway. We estimated diurnal changes of Rubisco initial and total activities as well as Rubisco amount under the different light conditions to find the mechanism of Rubisco activity regulation in Norway spruce needles.

Material and Methods: The experiment was done in 19th and 21st October 2004 on the full developed Norway spruce (*Picea abies* [L.] Karst) shoots. Needles were sampled at 6:00 (before the sunrise) and then every unpaired hour till 19:00. We estimated fresh weight and leaf area of sampled needles and then put them into the liquid nitrogen. Rubisco extraction for activity assay was done according to [1]. Rubisco initial and total activity was assayed spectrophotometrically by the continuous measurement of 3-phosphoglycerate-dependent NADH oxidation in a coupled enzyme system based on the method of [2], modified by [3]. Rubisco amount was determined by SDS-PAGE, according to [4].

Results and Discussion: With increasing irradiance both initial and total activities increased till 9:00 and than decreased till 13:00 (midday depression), while the irradiance was still increasing. The second maximum of activities was observed at 17:00. Rubisco amount decreased during the day from 3.10 g m^{-2} to 1.39 g m^{-2} (19th October) and from 3.62 g m^{-2} to 2.92 g m^{-2} (21st October). The difference between initial and total activities of Rubisco is a measure of the regulation by carbamylation, whereas changes in total Rubisco activity can be attributed to the regulation by: (1) Rubisco amount, (2) 2-carboxy-D-arabinol-1-phosphate (CA1P) and similar inhibitors [5]. Relatively low morning and evening values of total activities indicate that nocturnal inhibitor CA1P plays an important role in Norway spruce. However, the midday depression of total activity indicates that besides CA1P there are present some other inhibitors of Rubisco [6]. These are: (1) D-xylulose-1,5-bisphosphate (XuBP) and 3-keto-D-arabinitol-1,5-bisphosphate (3-KABP) [7] which are formed during the catalytic reaction and for that reason this inhibition is called catalytic inactivation, (2) some another, little characterized daily inhibitor [6]. In addition, the diminution of Rubisco amount during the day may indicate the repression of expression of photosynthetic genes.

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Acknowledgements: Supported by grant No. VaV/640/18/03 (Ministry of Environment Czech Republic).

Effect of phosphorylation on the temperature and light dependent structural changes of chloroplast thylakoid membranes

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Introduction: Thylakoid membranes of higher plants contain a protein kinase that can phosphorylate specific residues on the surface of the membrane bound light-harvesting complexes (LHCII). Phosphorylation of antenna proteins regulates the state transition [1]. Unphosphorylated LHCII delivers more energy to photosystem II (PSII) but upon phosphorylation it delivers more energy to photosystem I (PSI). In its non-phosphorylated form LHCII is found mainly associated with PSII (state I). The phosphorylated LHCII migrates to the unstacked region of the thylakoid, which is enriched in PSI (state II) [2].
It has recently been discovered that light plays a dual role in the process of regulation of the thylakoid protein phosphorylation. Via redox sensors it regulates the enzyme activation. In addition, it induces conformational changes in LHCII, which leads to the exposure of the N-terminal domain to protein kinase, and thus phosphorylation can be regulated at the substrate level [3]. The light-induced conformational changes of the non-phosphorylated LHCII alters the interaction between PSII and LHCII trimer. This causes the exposure of LHCII N-terminal domain to the protein kinase. Following phosphorylation the LHCII-PSII interaction becomes less stable which leads to their dissociation. However, higher light intensity inhibits the phosphorylation of LHCII. Hence, light-induced inhibition of protein phosphorylation does not correlate with the redox state of the thylakoid electron carrier components. Linear relation between the temperature of preillumination and accessibility of the phosphorylation site to the kinase indicates that this effect is related to the light-induced thermal effect within the complex, and thus consistent with the thermo-optic mechanism [4]. In this work, we investigated the effect of phosphorylation on the thermal and light stability of the macrodomains and LHCII trimers in intact leaves and isolated thylakoid membranes of pea, and found that phosophorylation significantly alters the structural flexibility of the membranes.

Material and Methods: Isolation of thylakoid membranes, preillumination, non-denaturing, “green” gel electrophoresis, circular dicroism spectroscopy and differential scanning calorimetry were performed according to procedures described earlier [5,6]. Phosphorylation of the membranes was triggered by duroquinol.

Results and Discussion: CD spectra of thylakoid membranes revealed that phosphorylation decreased the temperature stability of the so-called psi-type bands, associated with the macrodomain organization, i.e. the long range order of the complexes. This effect could also be observed in intact leaves, in which phosphorylation also apperaed to lower the light-stability of the macrodomains. Phosphorylation also induced a significant, approximately 6-10 °C shift, toward lower temperatures, in the thermal stability of LHCII trimers. These data strongly suggest that phosphorylation modulates the thermo-optic response of the LHCII in thylakoid membranes, and increases the structural flexibility of the membranes.

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Acknowledgements: Supported by OTKA, grant No. T/34188.

Determination of temperature and light optima for photosynthetic activity and growth of green algal lichen photobionts of the genus *Trebouxia*

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Introduction: Lichens are known for their ability to survive in extreme environments. This indicates a wide range of growing conditions for both partners forming a lichen thallus: fungus and alga (cyanobacteria). Isolated green algal lichen photobionts exhibit growth pattern characteristic by a very low growth rate. It was shown [1] that individual testing of optimal growth conditions for cultivated photobiont cultures is extremely demanding for number of treatments. Therefore, it is very useful and time-saving to combine environmental factors in a single experiment. Here, we applied the method of cultivation in crossed gradients of temperature and light [2] for the estimation of optimum growing conditions for symbiotic algal photobionts of genus *Trebouxia.*

Material and Methods: In the experiment, strains of *Trebouxia erici* and *T. irregularis* from a collection as well as *Trebouxia* sp. photobionts isolated from *Umbilicaria antarctica, Lasallia pustulata* and *Usnea antarctica* were used. The isolation of the photobiont from the lichens were done by a differential centrifugation method [3] supplemented with micro-filtration [4], both optimized for *Trebouxia* photobionts. Serological dishes containing the above-specified strains grown on 3N BBM medium were exposed to controlled light-temperature treatment for 35 d. The dishes were arranged at the crossed gradient cultivator of temperature and light. The cultivator consists of a 105 x 65 cm metal plate cooled and warmed simultaneously at the cold and hot side of the plate. Thus, a gradual temperature gradient was reached along the plate. For light, 4 different levels were used across the plate. The range of temperature was set 0 to 28 °C (cold to hot side). Light intensity levels were 20, 40, 60, and 80 μmol m^{-2} s^{-1} (low- to high-lit side). A serological dish had the eight-replicates in one treatment and one strain. The biomass growth of individual strains was measured using digital photographs and image analysis system (Lucia, CZ) at 2, 5, 9, 15, 20, 27, and 35 d of cultivation. The intensity of green colour and the area of algal colony were determined as main growth parameters. At the same time, the chlorophyll fluorescence induction (CFI) parameters (F_0, F_M, F_V/F_M, Φ_{II}, NPQ) were measured (FluorCam 700MF, PSI, CZ). Time- and treatment-dependent changes in chlorophyll fluorescence parameters served for the evaluation of photosynthetic activity of studied strains.

Results and Discussion: The parameters of biomass growth as well as the parameters of CFI showed that temperature and light intensity optima for growth and photosynthetic activity of *Trebouxia* photobiont strains lied between 16 – 18 °C and 20 – 40 μmol m^{-2} s^{-1}. Limitation of growth and primary production of studied strains became apparent at the temperatures under 0 °C and above 28 °C. At the tested light intensities (20 – 80 μmol m^{-2} s^{-1}), there were no significant limitation of growth and no signs of photoinhibition of photosynthesis were apparent from chlorophyll fluorescence data. At light intensities above 60 μmol m^{-2} s^{-1}, all studied strains exhibited slightly slower growth and reduced photosynthetic activity. However, these changes were not statistically significant. In the forthcoming study, the range of light intensity of 50-120 μmol m^{-2} s^{-1} will be studied so that the photoinhibitory light intensity might be evaluated for *Trebouxia* cultures.

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The effect of high light on zeaxanthine formation in the foliose lichen *Lasallia pustulata*

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Introduction: Photosynthetic organisms possess several photoprotective mechanisms. One of the most important is xanthophyll cycle, in which violaxanthine (V) is reversibly de-epoxidated via anteraxanthine (A) to zeaxanthine (Z) [1]. In our previous studies [2,4] we investigated the effect of short term high light (HL) exposure of lichen thalli on xanthophyll cycle pigments. The aim of this study was to compare the effect of different HL intensities and HL durations. During exposure to HL and recovery, we evaluated de-epoxidation state of xanthophyll cycle pigments (DEPS) in thalli of *Lasallia pustulata*.

Material and Methods: Four different HL treatments were used to induce HL stress. Thalli were: (1) exposed to the light of 1800 μmol m^{-2} s^{-1} for 30 min and recovered in a dim light; (2) exposed to the light of 600 μmol m^{-2} s^{-1} for 6 days; (3) exposed to the light of 600 μmol m^{-2} s^{-1} for 6 days and, simultaneously, to the light of 1800 μmol m^{-2} s^{-1} for 30 min every 24h; (4) kept in the dark for 6 days and simultaneously exposed to the light of 1800 μmol m^{-2} s^{-1} for 30 min every 24h. Samples of thalli were taken for xanthophyll cycle pigments analysis several times during each treatment. Analysis were done according to [3]. DEPS was calculated as (Z+A)/(Z+A+V).

Results and Discussion: Exposure to HL led to an increase of DEPS as an immediate response. DEPS of the thalli recovering in a dim light (1) declined to its original value within 10 hours. On the contrary, DEPS of the thalli exposed to continuous HL (2) slightly rised during the time of exposure. The thalli of a short-term repetitive HL exposure (treatment 3 and 4) did not show any additional change compared to (2). Our results indicate that Z formation depends more on the HL duration than HL intensity. Long-term continuous HL exposure seems to have more negative effect on Z formation in *Lasallia pustulata* than repetitively applied short-term HL doses.

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Induction of carotenoid production in the cyanobacterium *Spirulina platensis* under stress conditions

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Introduction: Chemical composition of microalgae biomass is not an intrinsic constant factor, but varies from strain to strain and from batch to batch, mainly depending on environmental conditions. Biomass of microalgae (eukaryotic algae and prokaryotic cyanobacteria) cultivated in mass cultures can enhance nutritional content of conventional food preparations and acts as probiotic agents that positively affect the health of humans and animals. There are a number of different modes in which microalgae are utilised as sources of biomass, especially carotenoids – most important group of pigments for biotechnological purposes. These pigments represent extremely powerful antioxidants with several mechanisms of action making them valuable in human health care e.g. cancer prevention, anti-ageing potential and boosting the immune system.

Material and Methods: The cyanobacterium *Spirulina platensis* is widely used in commercial applications as a source of carotenoids, *e.g.* [1].

Results and Discussion: The microalgal biomass enriched in carotenoids was produced in a novel type of high-irradiance solar photobioreactor with full control of cultivation conditions was tested [2]. Adverse cultivation conditions (high dissolved oxygen and/or suboptimal temperature under high irradiance) were applied to increase the carotenoids content in biomass. The cultivation experiments were performed at various biomass concentrations. The highest content of carotenoids – 41.4 mg per g DW was achieved at the initial culture biomass concentration of 0.7 g L^{-1} after 36 hours of treatment.

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Thermo-optically induced changes in the antenna system of *Cyanobacteria*

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Introduction: The main chlorophyll *a/b* light-harvesting complex of photosystem II, LHCII, has earlier been shown to be capable of undergoing light-induced reversible structural changes and chlorophyll *a* fluorescence quenching in a way resembling those observed in granal thylakoids when exposed to excess light. This unexpected structural flexibility has been assigned to originate from thermo-optic effect [1,2]. According to the thermo-optic mechanism, fast local thermal transients, arising from the dissipation of excess (photosynthetically not used) excitation energy, induce elementary structural changes due to the "built-in" thermal instabilities of the given structural units in the LHCII-containing molecular assemblies. Here we show that this type of ability of structural reorganizations is not confined to higher plant antenna systems. We show, by using room temperature and low temperature fluorescence spectroscopy, that energy migration from the cyanobacterial external antenna, phycobilisome (PBS), to the membrane can also be regulated thermo-optically.

Materials and Methods: *Synechocystis* PCC 6803 was grown photoautotrophycally in BG-11. Liquid cultures were maintained in a temperature- and light regulated box. The cells were grown at 25°C/35°C at an intensity of 70 μmol photons m^{-2} s^{-1}. *Thermosynechococcus elongates* BP1 Tokyo WT strain was grown photoautotrophically in BG-11 medium at 35°C/60°C. At 35 °C the cells were grown in Erlenmeyer flasks under continuous illumination of 40 μmol photons m^{-2} s^{-1}. At 60 °C the cultures were grown in test tube illuminated with incandescent lamps providing 40 μmol photons m^{-2} s^{-1} intensity and aerated by sterile air containing 1% CO_2. Light treatments were performed at different temperatures between 5 °C, and 60 °C with white or broad-band blue, green or red light of intensities between 500 and 7000 μmol photons m^{-2} s^{-1} and time periods of 30 min to 3 hours. Heat treated cells were incubated in a heat box at 40-90 °C for 5 min. Energy transfer between PBS and the membrane was monitored by using room temperature and liquid N_2 temperature fluorescence spectroscopy using a Fluorolog (Jobin Yvon-Spex) fluorimeter.

Results and Discussion: In cyanobacteria PBS serves as the major light-harvesting antenna. Light energy is absorbed by PBSs on the cytoplasmic surface of the thylakoid membrane, then transferred toward the photosynthetic reaction centers. We found that upon increasing the temperature from the growth temperature of the culture to high temperatures, but below the temperature of thermal denaturation, the chlorophyll fluorescence band (around 685 nm) sharply decreased at around 60 °C, without noticeable changes in the absorbance. The position of this transition temperature could be varied by changing the growth temperature of the cells. At the same time, the fluorescence emission associated with PBS also underwent minor spectral modifications. Virtually the same changes could be observed upon exposing the cells to high light at temperatures well bellow the transition temperature. For short and mild treatments, the changes were largely reversible in the dark. The light-induced changes also depended on the growth temperature of the cell culture, as well as on the the temperature, intensity and duration of the preillumination – in a manner that was in good agreement with the thermo-optic model.

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Nutrient availability and photosynthesis

Application of JIP test to study the photosynthetic apparatus reactions of maize seedlings growing under salt stress

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Introduction: Salt stress affects plant gas exchange at different structural levels [1]. However, when low and moderate salts concentrations are applied, gas exchange seems to be modified mainly due to osmotic stress through partially stomata closure. High salt concentrations provide to toxicity and structural irreversible changes [2]. The relationships between gas exchange measurements and chlorophyll *a* fluorescence undergo multidirectional moderations especially under stress conditions. This work is to investigate the reaction of photosynthetic apparatus at PSII and stomata levels of maize plants growing under salt stress conditions to get better understand of the specific effect of salt stress on each component. This was done by measuring gas exchange of plants and application of the theory of energy flux in thylakoid membranes and JIP test created by Geneva Bioenergetics laboratory [3].

Material and Methods: Salt stress of 0, 60, 90, 120 and 180 mmol dm^{-3} NaCl was applied to Maize seedlings (*Zea mays cv.* Nysa) growing in nutrient cultures (modified Hoagland) under greenhouse conditions. Analyzing the sequences of plants reactions to salt stress emphasis within short time course (2, 6, 24, 72, 120, and 168 h after salt application use) was performed. The activity of photosynthetic apparatus was examined through gas exchange using CIRAS-2 Photosynthesis Measurement System (PP Systems, UK) and chlorophyll *a* fluorescence measurements using HandyPEA Fluorimeter (Hansatech Instruments, UK).

Results and Discussion: Salt treatment significantly inhibited chlorophyll content and photosynthetic performance of leaves. Already after 2 hours first plant reactions to the highest salt concentrations were observed (area above chlorophyll fluorescence curve and PSII Performance Index PI dropped). This work suggests that measurement of chlorophyll *a* fluorescence is a very fast and valuable technique for obtaining fast qualitative information about light dependent photosynthetic phase. However, we recommend that in such kind of experiments both techniques (gas exchange and chlorophyll *a* fluorescence) should be used as complementary information sources to understand the adaptive and/or tolerance mechanisms of salt stress.

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The impact of constitutive overexpression of trans-zeatin O-glucosyltransferase gene in tobacco on photosynthetic pigments and gas exchange during water stress

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Introduction: In *Pssu-ipt* transgenic tobacco plants, increased content of endogenous cytokinins (CKs) was associated with marked morphological changes. In their responses to water stress it was impossible to differentiate direct effects of increased CK content and indirect effect of decreased root/shoot biomass ratio [1]. On the contrary, constitutive expression of *trans*-zeatin O-glucosyltransferase *(ZOG1)* gene from *Phaseolus vulgaris* in tobacco plants increased the total level of CKs, especially of their storage forms (O-glucosides) without affecting significantly the amount of the corresponding active free bases and in consequence their morphology. Thus transformed plants posses a pool of reserve, temporarily inactive CKs, which could be used under sresss conditions.

Material and Methods: During water stress and subsequent dehydration of 8-week old tobacco plants β-glucosidase activity was determined quantifying of product released from p-nitrophenylglucoside endogenous CK content was followed using ion-trap mass spectrometer LCQ (*Finnigan*, USA) equiped with electrospray interface. The contents of chlorophyll *a*, chlorophyll *b*, β-carotene, lutein, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin was determined by HPLC (*ECOM*, Prague, Czech Republic) and the degree of deepoxidation of xanthophyll cycle pigments was calculated. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_S) were determined on attached leaves using the commercial gas exchange system LCA-4.

Results and Discussion:
Both control and transgenic plants responded to water stress by stimulation of the activity of β-glukosidase, enzyme which cleaves CK O-glucosides yielding the active CKs. This stimulation was significantly higher in transformants. After cessation of watering stomata closed sooner in *35S::ZOG1* trasgenic plants than in control plants. In consequence g_s, E and P_N were lower but relative water content (RWC) reminded higher in transgenic than in control plants. However, the positive effect of *ZOG1* expression on RWC retention disappeared during severe water stress and after subsequent rehydration. CKs are often considered abscisic acid (ABA) antagonists in many processes [2]. Under mild water stress the abscisic acid (ABA, 100 μM) or 10 μM benzyladenine (BA) pre-treatment ameliorated the negative effect of subsequent water stress on gas exchange parameters to greater extent in transgenic tobacco, while under severe water stress and during rehydration ABA pre-treatment exhibited positive effect only in control tobacco plants. In transgenic and ABA-pretreated plants the total contents of chlorophyll and carotenoids show greater stability during water stress and subsequent rehydration. The contents of xanthophyll cycle pigments and their de-epoxidation state increased during water stress, especially in transgenic and ABA pre-treated plants and decreased after rehydration, but zeaxanthin/chlorophyll ratio was much higher in control plants. It seems that CKs can play positive role in plant stress response especially at early, relatively mild stages.

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Acknowledgements: Supported by GACR (No. 522/04/0710).

The impact of BAP and ABA on the photosynthesis during water stress

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Introduction: The study of energy balance of the photosynthesis by the method of fast fluorescence induction (FFI) consists of the evaluation of open/closed reactive centre rate, which determines distribution of energy to photosynthetically active structures [5]. FFI evaluates the changes within a fragment of a second, when characteristic O-J-I-P transient reveals. From these curves the impact of stress, similarly as the impact of biologically active chemicals on the photosynthesis, could be evaluated. The benefit of FFI are rapidity, repetitiveness, and reliable response to environmental variations [2]. Stress, equally as the treatment with pesticides ordinarily restrains F_0, F_M, F_V/F_M and extends Tfm duration, which has been studied in our field experiments.

Material and Methods: At the Department of Plant Production of the Czech Agricultural University at Prague, the growth of sugar beet (cv *Epos*) stressed by drought, the effect of plant growth regulator (PGR), and phytohormons on the adaptation to stress were studied. Irrigated and non-irrigated treatments were compared. The effect of cytokinin (BAP), abscisic acid (ABA) and PGR (Atonik) on the adaptation, was simulated by spraying with 1mM BAP,10mM BAP, 100 mM BAP, 10 mM BAP+100 mM ABA and Atonik. FFIs were measured by Plant Efficiency Analyser PEA Fy Hansatech LTD Norfolk, England, software Winpea 32 (45% light intensity from 6 diodes supplied by 13,6 V batery, duration 1 sec.). Chlorophyll fluorescence parameters F_0, F_M and F_V/F_M as Tfm duration, were registered as indispensable components for evaluation of energy balance of photosynthesis, $Q_{a,b}$ reducing/non-reducing reaction centres, and the yield of PS II [5].

Results and Discussion: Stress, induced by drought, restrains F_0, F_M and during two days prolongs Tfm duration. Increases of red/ox state, initial energy, and flux in biomembranes were observed together with extension of PS II yield in stressed treatments, compared to irrigated control, as agree with literature [7], [1]. Cytokinin (1mM BAP,10mM BAP) escalates F_0, F_M, reduces Tfm, and re-strains red/ox activities. BAP+ABA immediately after the application escalates F_0 and F_M, but later on they are restrained under parallel diminution Tfm duration, as the result of stress adaptation. ABA applied alone decreases F_0 as F_M, protracts Tfm duration, declines red/ox, initial energy, and flux in biomembranes. That is one of the reasons, why PS II yield slightly escalates. Atonik escalates F_0, slims F_M and reduces Tfm PS II yield slightly cuts down. The results correspond to physiological processes caused by droughtness, and to the interactions of plant hormones connected with water content, leaf water potential, stomatal conductance, transpiration rate and net photosynthesis rate, often as reported [4], [6].

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Acknowledgements: Supported by the grant MSM 60460709/01, Czech Republic.

Carbon isotope discrimination as indicator of water stress

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Introduction: Many techniques applied in photosynthesis research allow investigate *instantaneous* biotic and abiotic stresses. Chlorophyll fluorescence, oxygen and/or CO_2 exchange rate measurements or enzyme assays are examples of those. In contrary, stable isotope technique, monitoring fractionation of naturally occurring carbon and oxygen isotopes in plant biomass, have the capability of *integration* of an adverse effect. Here, we give examples of field and laboratory studies showing that carbon isotope discrimination yields valuable information on water shortage, high temperature stress, fluctuating atmospheric CO_2 or light regime. Leaf carboxylation capacity and stomatal control of gas exchange are two main constrains of photosynthesis. Both contribute to the carbon isotopic composition of plant body (see the abstract of Šetlík and Šantrůček here). Mechanism of their isotopic effect is well understood, however, distinguishing between limitations caused by carboxylation and diffusion is still difficult.

Water and high temperature stress: Three tree species, *Robinia*, *Quercus* and *Fraxinus*, growing in habitats with contrasting water availability and irradiance/leaf temperature, were investigated on abundance of heavy carbon isotope ($\delta^{13}C$) in dry mass of leaves, stem and fruits. Our results show significant differences in $\delta^{13}C$ between the habitats in all three species and all the plant organs. Dry mass of leaves, stem and fruits produced at water shortage and higher irradiance was enriched in ^{13}C when compared to dry mass of well-water-supplied plants. Therefore, photosynthetic CO_2 fixation in chloroplasts proceeded at lower time-averaged CO_2 concentration in water stressed plants.

Water stress simulated by abscisic acid (ABA): Sunflower (*Helianthus annuus*) plants were grown in hydropony pots with perlite and nutrient solution under controlled climate (16 hours photoperiod, irradiance 1100 μmol (photons) m^{-2} s^{-1}, day/night air temperature and relative humidity 26/16 ^oC and 50/75 % respectively, ambient CO_2 370 μmol mol^{-1}) for 6 weeks. ABA, added to the nutrient solution (10^{-5}M) 14 days prior to the harvest, simulated dry conditions by reducing stomatal conductance. Controls, which were not treated with ABA, were more depleted in ^{13}C than ABA fed plants. The difference between control and ABA plants was highest in young leaves built during the period of ABA treatment and diminished, but still occurred, in old leaves constructed before the ABA treatment. Maintenance costs can be assessed.

ABA, senescence and relocation of assimilates: Reduced irradiance (250 μmol (photons) m^{-2} s^{-1}) and elevated ambient CO_2 (750 μmol mol^{-1}) were applied in the experiment described above with the aim to reduce the CO_2 gradient from ambient air to mesophyll cells. As expected, the difference in $\delta^{13}C$ between ABA and control plants was lower in young leaves when compared to the high-light treatment. Interestingly, the difference in $\delta^{13}C$ between young and old leaves was smaller in ABA-treated plants than in controls in low-light treatment. It indicates that ABA enhance relocation of assimilates from old to young leaves under the lack of newly synthesised carbohydrates.

Acknowledgement:
We thank our colleagues M. Hronková, J. Květoň and M. Šimková for their assistance. The research was funded by Grant Agency of Academy of Sciences of the Czech Republic grant No. A6011410505.

Carbon isotope (^{13}C) composition of the leaf discs and cuticles

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Introduction: Stable carbon isotope (^{13}C) abundance, $\delta^{13}C$, in the ambient air CO_2 is approximately -8‰ when compared to VPDB standard. The abundance in the leaf biomass of C3 plants is lower than that in VPDB by –20‰ to –30‰. This depletion, arising on the way of the CO_2 from its source in the atmosphere to carbon incorporated in biomass, is known as isotopic discrimination Δ. The discrimination against ^{13}C in atmosphere can be expressed as $\Delta^{13}C$ ‰=a+(b-a)(c_i/c_a), where a is the discrimination against $^{13}CO_2$ during diffusion through the stomata (~ -4.4‰), b is the net discrimination due to carboxylation (~ -27‰), c_i is intercellular and c_a is ambient CO_2 concentration [1]. Because the discriminations that occur due to diffusion and carboxylation are constants, $\Delta^{13}C$ of the produced sugars depends mainly on the c_i/c_a ratio [2]. This relation can be used to calculate c_i concentration at the sites of carboxylation. As the leaf cuticle originates from adjacent mesophyll cell layer, we can calculate, from the measured isotopic composition of the cuticle, the internal CO_2 concentration in these cells. In the case of hypostomatous leaves, it is possible to calculate the gradient of CO_2 from lower to upper side of the leaf. We hypothesise that the gradient is proportional to diffusive limitations imposed by the mesophyll and/or to the photosynthetic capacity of mesophyll cells.

Material and Methods: 10 leaf discs were punched out from each of 35 plant species sampled and were weighted to obtain fresh weight. 7 discs were hydrated with distilled water under vacuum and weighted again. This weight was used to calculate the leaf thickness and the difference between fresh and hydrated weight was used to calculate volume fraction of intercellular air spaces, f_{ias}, within the leaf. 3 discs were dried out at 70 ºC to constant weight. Leaf mass per area, LMA, was calculated from known disc area and weight. Cuticles were isolated by immersing leaf disc in a mixture of 2% (v:v) cellulase (Celluclast, Novo Nordisk, Denmark), pectinase (Trenolin Super DF, Erbsloh, Germany) dissolved in citric buffer (pH=4.0). The stomata density was counted on both adaxial and abaxial cuticles. $\delta^{13}C$, $\delta^{15}N$, carbon and nitrogen content of homogenised dried leaf disc and $\delta^{13}C$ of isolated cuticles were measured on the elementar analyser NC 2100 (ThermoQuest Italia S.p.A.) coupled with mass spectrometer DeltaXLplus (ThermoFinnigan, Bremen, Germany).

Results and Discussion: Analysis of the entire leaf discs show that $\delta^{13}C$ is positively correlated to leaf thickness and negatively to fraction of intercellular air spaces. The differences among species are caused mainly by long-term photosynthetic capacity. Adaxial astomatous cuticle had usually less negative $\delta^{13}C$ than the abaxial stomatous cuticle isolated from the same leaf. When converted to $\Delta^{13}C$ and expressed in terms of c_i (see Methods for the formula), CO_2 concentration in adaxial intercellular space was less than that in the abaxial space by 20-40 μmol (CO_2) mol^{-1}.

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Acknowledgement:
We thank our colleague M. Šimková for her assistance The research was funded by Grant Agency of Academy of Sciences of the Czech Republic grant No. A6011410505.

Salinity, osmotic and water stress

Differential maintenance of photosynthetic capacity under abiotic stresses in trehalose- and LEA protein-producing transgenic plants

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Introduction: Recently, we have shown that transgenic plants producing trehalose or a hot pepper LEA protein exhibit remarkably enhanced tolerance against desiccation, high salinity and high temperature. In this study, we compared their protective effect on maintaining photosynthetic capacity under those abiotic stresses.

Material and Methods:
Administration of abiotic stresses: Stress treatment was given to nontransformants and transgenic tobacco plants normally grown for 4 weeks. Water stress was given by immersing the roots into 10% (w/v) PEG solution. Heat stress was given by incubating plants in a darkened growth chamber maintained at 45 °C. Salt stress was given by supplementing 250 mM NaCl twice a week at the time of water supply.
Measurement of Chl fluorescence & O_2 evolution: O_2 evolution and Chl fluorescence were measured simultaneously using leaf discs of 3.5 cm-diameter at 25 °C in a Hansatech (Kings Lynn, UK) LD2 leaf disc chamber with a Walz PAM Chl fluorometer (Effeltrich, Germany) and a Clark type electrode connected to Hansatech O_2 electrode control box.

Results and Discussion:
1. Upon dehydration, LEA protein-producing transgenic plants maintained higher rate in Pmax of O_2 evolution in relation to *CaLEA6* expression level than nontransformants while all trehalose-producing plants exhibited no visible improvement in Pmax.
   Chl fluorescence parameters of *Fo* and *Fv/Fm* were not affected by dehydration, but decrease in *qP* and increase in *NPQ* were lessened in all transgenic plants.
2. Both transgenic plants maintained higher Pmax as well as more favorable Chl fluorescence parameters (*Fo* & *Fv/Fm*) after heat stress in the dark.
3. After sustained NaCl-treatment, Pmax decreased similarly in nontransformants and trehalose-producing plants, but maintained higher in LEA protein-producing plants.
   Chl fluorescence parameters (*Fo*,*Fv/Fm*, *qP* & *NPQ*) were not much affected in overall, but lesser affected in LEA protein-producing plants.

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Acknowledgements: supported by BK21 program.

Chlorophyll fluorescence induction in *Lactuca sativa* leaves during salt stress

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Introduction: Recently, chlorophyll fluorescence is widely used in analyzing the photosynthetic apparatus and understanding the mechanism of photosynthesis and the mechanism by which a range of environmental factors alter photosynthetic activity under both biotic or abiotic stresses. Fluorescence parameters have been applied in rapid identifying injury to leaves in the absence of visible symptoms and a detailed analysis of change in photosynthetic capacity. Therefore, chlorophyll fluorescence is also frequently used as a potential indicator of environmental stress and a screening method of tolerant plants. We investigated the fluorescence induction (FI) in *Lactuca sativa* leaves and isolated chloroplasts.

Material and Methods: Lettuce plants were grown in the pot. Young seedlings were cultured under salt stress at 20 °C at the regime 16h light/8h dark. FI was measured using Fluorcam (PSI, CZ). Intensity of red exciting light was about 6000 μmol photons m^{-2} s^{-1}.

Results and Discussion: The growth, photosynthetic gas exchange and chlorophyll fluorescence were studied in lettuce (*Lactuca sativa* L.) plants grown under salt stress. The stressed plants showed decreased plant weight, leaf area, plant height although they had similar leaf number. Chlorophyll content was significantly reduced in stressed plants after 2 weeks of stress infection as compared to control plants. Decreased net photosynthetic rate, transpiration and stomatal conductance, and a slightly higher intercellular CO_2 concentration were observed in stressed plants. For the chlorophyll fluorescence parameter Fv/Fm, Fv_0/Fm_0, ETR and qP, there were no differences between stressed and control plants, but qN showed a reduction in stressed plants.

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Acknowledgements: Supported by Bio-Green 21 from RDA and GRRC project.

Photoinactivation of photosystem II in the dehydrated leaves of green pepper

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Introduction: In this study, we investigated that the role of changes in PSII functionality for the increased susceptibility to high light when photoinhibition is given under water stress and demonstrated that exacerbated photoinhibition in the dehydrated plants is accompanied with no incremental damage to PSII.

Material and Methods:
Plant material: *Capsicum annuum* L. (cv. New Town no. 3) plants were grown in a potting mixture and watered every second day. Photoinhibitory and dehydartion treatment was done either by exposing the detached leaves directly to the light of 900 μmol m^{-2} s^{-1} suplied by halogen lamp or by immersing the roots in the Hoagland solution containing 5% (W/V) PEG-6000. Water potential of leaf was measured using dewpoint microvoltmeter (HR-33T, Wescor, USA).
Measurement of Chl fluorescence & O_2 evolution: O_2 evolution and Chl fluorescence were measured simultaneously using leaf discs of 3.5 cm-diameter at 25 °C in a Hansatech (Kings Lynn, UK) LD2 leaf disc chamber with a Walz PAM Chl fluorometer (Effeltrich, Germany) and a Clark type electrode connected to Hansatech O_2 electrode control box.

Results and Discussion:
1. Without incremental damage to PSII as evidenced by no further reduction in both Fv/Fm and functional PSII contents.

2. Deviation from linearity between Pmax and functional PSII contents in the dehydrated leaves under high light in contrast to photoinhibited leaves further demonstrates that incremental damage to PSII is not involved in the increased susceptibility.

3. Magnified inhibition may be due to the impaired electron flow to NADP+ itself or malfunctioning of Calvin cycle.

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Acknowledgements: supported by Korean Research Foundation Grant (KRF-2004-050-C00020).

Photosynthetic responses of *Betula ermanii* to environmental stress

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Introduction: The effects of understory dwarf banboo (*Sasa kurilensis*) on the growth of overstory trees were studied in a dense secondary forest of *Betula ermanii* in northern Japan. Plants compete for resources such as light, soil water and nutrients. In dense forest, taller plants suppress the growth of smaller ones because of light interception. Although understory *Sasa* can not shade overstory trees, it is likely to affect the growth of overstory trees through below-ground competition for soil resources (water and/or nutrients) [1]. Two plots were established in a dense *Betula ermanii* forest with *Sasa* in the understory, and *Sasa* was removed in one plot (removal plot) and *Sasa* was intact in the other plot (*Sasa* plot). This study was investigated in the field to elucidate how photosynthetic capacity of levels of *Betula* plants responds to environmental stress caused by *Sasa*.

Material and Methods: This study was carried out in the Uryu Experimental Forest of Hokkaido University in northern Japan. *Betula ermanii* samples were collected in the field between July 2002 and October 2002. CO_2 assimilation rate and chlorophyll fluorescence of fully expanded mature leaves were measured simultaneously using a portable photosynthesis system (Li-6400, Li-Cor, Nebraska, USA) equipped with leaf chamber fluorometer. Leaves were immediately frozen in liquid nitrogen in the field and stored at -80 °C in the laboratory until analysis. Pigments concentrations were determined by HPLC (LC-10AVP, Shimadzu, Kyoto, Japan). Enzyme activities were measured spectrophotometrically at 25 °C in a temperature-controlled cuvette.

Result and Discussion: Leaves of *Betula ermanii* in the *Sasa* plot have higher CO_2 assimilation rates and larger area. Value of maximum quantum yield of PSII (F_v/F_m) are used as the index of photoinhibition. Decreases in F_v/F_m are not observed neither in removal plot nor in *Sasa* plot. Excess excitation energy are dissipated as heat by de-epoxidation in xanthophyll cycle [2] .The ratios of de-epoxidation in xanthophyll cycle were high regardless of the presence/absence of *Sasa*. Even in the enzyme activities of the enzymatic antioxidant system, any difference was not seen.
It is thought that *Betula ermanii* defends against photoinhibition regardless of the presence of *Sasa*. In particular, the damage of the photosynthesis with excess excitation energy has been avoided by increasing the amount of the xanthophyll cycle pigments which dissipate energy as heat. It is thought that photostress doesn't affect the difference of the CO_2 assimilation rate by the presence of *Sasa*.

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Photosynthetic response of young soybean plants during drought stress

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Introduction: Recently, chlorophyll fluorescence is widely used in analyzing the photosynthetic apparatus and understanding the mechanism of photosynthesis and the mechanism by which a range of environmental factors alter photosynthetic activity under both biotic or abiotic stresses. Fluorescence parameters have been applied in rapid identifying injury to leaves in the absence of visible symptoms and a detailed analysis of change in photosynthetic capacity. Therefore, chlorophyll fluorescence is also frequently used as a potential indicator of environmental stress and a screening method of tolerant plants. We investigated the fluorescence induction (FI) in soybean leaves and isolated chloroplasts.

Material and Methods: After a 30-min dark period in ambient conditions in the laboratory, chlorophyll *a* fluorescence in soybean leaf was measured using a pulse-amplitude modulated fluorometer (Fluorcam, CZ). Measurements of minimal (F_0) and maximal (Fm) fluorescence yields allowed determination of the optimal quantum yield (Fv/Fm), the ratio ($Fm - F_0$)/Fm being used to calculate the maximal potential efficiency of PS II of dark adapted leaves. Leaves were then irradiated by actinic radiation (110 μmol m^{-2} s^{-1}), and saturation pulses of 3,500 μmol m^{-2} s^{-1} were also triggered repeatedly (every 20 s) during approximately 6 min. For assessment of light use efficiency, leaves harvested on illuminated plants were gradually exposed to higher irradiance (2 min at each intensity: 50 to 1,850 μmol m^{-2} s^{-1}). The operational PS II quantum yield (Y) and relative electron transport rate (RETR) were calculated using, respectively, the following formulas according to [3]: $Y = (F'm - Ft)/F'm$ and $RETR = Y. PAR$.

Results and Discussion: The growth, photosynthetic gas exchange and chlorophyll fluorescence were studied in experimental plants grown under salt stress. The stressed plants showed decreased plant weight, leaf area, plant height although they had similar leaf number. Chlorophyll content was significantly reduced in stressed plants after 2 weeks of drought as compared to control plants. Decreased net photosynthetic rate, transpiration and stomatal conductance, and a slightly higher intercellular CO_2 concentration were observed in stressed plants. For the chlorophyll fluorescence parameter Fv/Fm, Fv_0/Fm_0, ETR and qP, there were no differences between stressed and control plants, but qN showed a reduction in stressed plants.

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Acknowledgements: Supported by RIS and GRRC project.

Toxic compounds and herbicides

Effect of Cd and Ni on chlorophyll content, leaf size and anatomy of *Origanum vulgare* L and *Thymus vulgaris* L

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Introduction: Heavy metals are widespread pollutants and their effect on aromatic and medicinal plants is of key importance for their growth and often a limitation factor for disposal in the global market. The two species of the Lamiaceae family, *Origanum vulgare* L. and *Thymus vulgaris* L. were exposed to the arising concentrations of Cd (0-160 ppm) and Ni (0-1600 ppm) in the soil to analyse chlorophyll content, size and anatomical structure of leaves under the stress conditions.

Material and Methods: The chlorophyll content was examined one month before plants harvest. The concentrations of chlorophyll *a*, *b* were determined by measuring absorbance at 663 nm, 646 nm and 540 nm in the diode array spectrophotometer Hewlett Packard model 8452. The quantity of chlorophylls was calculated by [1]. Statistical analysis of the data was performed by ANOVA (SPSS, Version 10.0 package). Studies of anatomical structure of leaves were carried out on paraffin slides, stained with Hematoxylin and Eosin.

Results and Discussion: Chlorophyll content in *Origanum vulgare* L. and *Thymus vulgaris* L. increased at the 20, 40, 60 and 80 ppm of supplied Cd. Opposite pattern was found at Ni treatments, where reduced chlorophyll content was recorded. In both species the size of leaves decreased more remarkably at Ni than Cd treatment. A negative correlation between leaf size and chlorophyll content was found at Cd treatment, which means that chloroplast remained the same but the leaf cells failed to expand; whereas a positive correlation was in thyme plants at Ni-supplied soil, indicating reduction in chlorophyll synthesis caused probably by toxic effect of Ni. Both heavy metals affected the leaves' anatomical structure of experimental species. Lamina thickening in Cd supply was due to palisade parenchyma, while in Ni-treated plants it was due to the increase of spongy parenchyma. This phenomenon also has been reported with other heavy metals [2, 3]. At the highest concentrations of Cd and Ni, thyme was affected more than oregano plants.

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Assimilation pigments, chlorophyll *a* fluorescence, cysteine, non-protein thiol compounds and membrane lipid peroxidation levels in the lichen photobiont *Trebouxia erici* in response to copper and cadmium

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Introduction: Lichens are environment sensitive organisms, which can acquire pollutants, heavy metals including, from the atmosphere as well as substrate. Physiological response of lichen photobionts to heavy metals including alterations of growth rates, pigment content, mineral uptake, membrane integrity, dehydrogenase activity, activity of photosystem II, or levels of intracellular proline [1-3].

Material and Methods: Cysteine, reduced glutathione and phytochelatin content were determined by HPLC [4] in the lichen photobiont cells exposed for 24 hours to selected cadmium and copper concentrations (0, 0.1, 1, 5 and 10 μM) in 5 mM HEPES buffer. Assimilation pigment composition, chlorophyll *a* fluorescence (F_V/F_M) and malondialdehyde concentration were selected as markers for assessment of heavy metal stress.

Results and Discussion: Both tested metals caused concentration dependent increase of phytochelatins synthesis, mainly with short length chain (PC_2-PC_4). However, tested metals differed in degree of biological response. Redox reactive metal copper significantly altered assimilation pigment composition, chlorophyll *a* fluorescence and degree of membrane lipid peroxidation. Cadmium, on the other hand, did not alter significantly pigment composition and degree of membrane lipid peroxidation, although decreased readings for chlorophyll *a* fluorescence parameters.

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Electrochemical biosensor based on Photosystem II for detection of photosynthetic herbicides

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Introduction: Despite newly emerging environment-friendly products available on the market, classical photosynthetic herbicides still represent risk for the nature and health. Recently, we have developed a prototype of the electrochemical biosensor based on immobilized Photosystem II useful for pre-screening of herbicide presence in soil [1]. Here, we present an up-to-date state of our biosensor set-up consisting of a microflow system vessel, a semiautomatic control unit specially designed for amperometric measurement of PS II activity, and optionally a portable computer.

Material and Methods: The PSII complex was isolated from the thermophilic cyanobacterium *Synechococcus elongatus* and immobilised on the surface of the platinum working electrode centred in the middle of radially oriented three-electrode screen-printed electrode system Pt:Ag/AgCl (BVT Technologies Ltd. Brno). A simple immobilisation procedure using cross-linking of PSII preparation in glutaraldehyde-BSA-glycerol matrix was used. The electrode system is mounted into the electrode slot of the Microflow System (MFS, BVT Technologies Ltd, Brno) specially designed for monitoring PSII activity. The experimental set-up consists of the driving pump rotor submerged into a 10-mL vessel and a red LED placed in front of the electrode slot. The liquid is pumped through a minichannel, passing by the working electrode and another, passageway channel provides sufficient mixing of the solution inside the vessel. The control unit enables to provide a semiautomatic measurement process as well as the data acquisition and processing (BVT Technologies Ltd, Brno).

Results and Discussion: Typical measuring protocol consists of short light pulses (5 sec) when the signal (measured as electrical current in tens to hundreds of nA) on the electrode reflects reoxidation of an artificial electron acceptor reduced by electrons from the PSII complex. When the light is switched off, the signal decreases close to the initial value. The light pulses are followed by 180-sec dark periods. A peak to baseline difference value is acquired. When a herbicide is added to the medium, a decrease of signal peaks during the illumination period is observed due to the blocked electron transport between the PSII complex and artificial electron acceptor. The signal height during illumination is herbicide concentration dependent. In this way, calibration curve can be constructed to estimate concentration of herbicides in natural samples. Recently this system was used to assay a urea-type herbicide isoproturon in field soil extracts [2].

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Acknowledgements: This work is supported by the Czech Science Foundation, project 522/03/0659 and by the Ministry of Education, Youth and Sports, project MSM6007665808.

Photosynthetic activity of some medicinal plants under cadmium stress

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Introduction: Medicinal plants are widely used in pharmaceutical, food and cosmetic industries. Many widely used pharmaceuticals are derived from plants and other natural sources, or are based on traditional knowledge of an herbal remedy. Slovakia region, including 250 species of medicinal plants growing free in nature in sufficient amount, could be a significant source of drugs for almost whole Europe. For our study two most frequently used medicinal species *Matricaria recutita* (L.) Rausch. and *Salvia officinalis* L. which differ not only in metal accumulation into plant organs [1-3] but also in the effect of Cd on their biochemical and physiological characteristics were chosen.

Material and Methods: Seeds of *Matricaria recutita* (L.) Rausch., tetraploid cv. Goral and Lutea and *Salvia officinalis* L., cv. Primorska and Krajova were sown on the surface of standard soil and grown in the growing chamber at mean air temperature 25 ^{o}C, photosynthetic active radiation 80 μmol m^{-2} s^{-1}, photoperiode 16 h day/8 h night, and mean air relative humidity 80 %. Three weeks old seedlings were picked up (1 plant per pot with standard soil) and transferred into the greenhouse. Afterwards the six weeks old plants were exposed in hydroponia with Hoagland solution at pH = 5.5 with different concentrations of $Cd(NO_3)_2.4\ H_2O$ (0 – 120 μmol L^{-1}) in a growth chamber at the same conditions as described above. After 7 days the lengths and dry mass of the shoots and roots, as well as net photosynthetic rate and mitochondrial respiration rate (for chamomile cultivars) were gasometrically measured. For determination of Cd content in aboveground and underground parts of studied plants AAS was used.

Results and Discussion: Production characteristics of studied medicinal plants and cultivars (length and dry mass of roots and shoots) were not significantly changed after Cd application in investigated concentration range. This indicated high tolerance of studied species against Cd. The roots of medicinal plants accumulated higher Cd concentration than the shoots and metal accumulation in both plant organs increased with increasing external Cd concentration. *S. officinalis* species cv. Krajova accumulated higher Cd amount in the roots than cv. Primorska, however metal mobility into the shoots was higher for the second cultivar. The both chamomile cultivars accumulated into shoots two times more metal than *S. officinalis* indicating that chamomile species are Cd hyperaccumulators. Moreover, the higher Cd concentrations induced in chamomile plants the formation of inflorescence. Application of 12 μmol L^{-1} Cd decreased assimilation pigment content in new formed leaves, however at higher Cd application (> 60 μmol L^{-1}) new formed leaves (containing extremely high Cd concentrations) were dark green coloured, without necrosis symptoms. This phenomenon could be connected with induction of cytokinin synthesis. To compare photosynthetic activity of control chamomile plants, cv. Lutea possess higher value of the net photosynthetic rate (P_N); shoot respiration rate (R_D shoot) was comparable with that of cv. Goral plants but root respiration rate (R_D root) for cv. Lutea was much more lower. At 12 μmol Cd L^{-1} application in both chamomile cultivars P_N significantly decreased indicating negative effect of the metal on photosynthetic processes. On the other hand, R_D root as well as R_D shoot were not affected by cadmium treatment. Studied medicinal plants cultivated in the pots with soil under green-house conditions treated during two months with 60 μmol Cd L^{-1} accumulated lower amount of Cd than plants hydroponically cultivated 7 d at the same Cd concentration.

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Acknowledgement: . Grant No. 1/0100/03 of Slovak Grant Agency VEGA

Photosynthetic activity in *Brassica juncea* L. under cadmium stress

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Introduction: Plants can accumulate heavy metals, when grown in soils with high metal concentrations, from natural or anthropogenic sources. Cadmium is one of the most toxic heavy metals that can be taken up by plants and translocated to the shoots [1], namely to the leaves. The aim of this work was the investigation of photosynthetic activity in *Brassica juncea* L., under cadmium stress. The results of pigment contents, oxygen evolution and fluorescence measurements have been analysed and compared with control plants.

Material and Methods: Control and cadmium plants were grown in a solid medium during 3 to 4 weeks. Cadmium plants were submitted to 50 µM CdSO4. Pigment contents were determined in 100% methanol extracts. Photosynthetic activity was evaluated as O_2 evolution using a gas-phase oxygen electrode. A PAM portable modulated fluorometer was used to measure chlorophyll fluorescence parameters.

Results and Discussion: Several damages were observed on cadmium treated plants when compared with control ones. At the end of experimental time (3weeks) fresh and dry weight were lowered. Pigment contents were reduced about 40%. However, the ratio of chlorophyll *a* to chlorophyll *b* increased. Photosynthetic activity showed a pronounced reduction on cadmium plants. Fluorescence measurements revealed a decreased in PSII efficiency (Fv/Fm) when compared to control plants [2]. The values obtained for Y, qP, qN fluorescence parameters seems to indicate a reduction in photosynthetic electron transport in plants under cadmium stress [3].

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Physiological, biochemical, anatomical and ultrastructural aspects of photosynthesis in carnivorous pitcher plants *Nepenthes alata* and *Nepenthes mirabilis*

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Introduction: According to Givnish's cost/benefit model of carnivory [1]**,** carnivorous plants are restricted to environments with abundand supply of water and light, but poor in nutrients. In other environments, the cost of producing traps would exceed the benefits gained from prey. From photosynthetic point of view, cost represents extra energy requirement for respiration of carnivorous organs. If water and light are in a short supply or nutrients are abundand, carnivorous plants give up carnivory temporarily. It was found that carnivorous plants have low P_N [2]. *Nepenthes* is Asian pitcher plants which leaves are composed of assimilation part and trapping pitcher. This allow us to make a direct comparison between assimilation part and carnivorous trapping pitcher and to understand which features are involved in a carnivorous syndrome. This was not possible in former studies in *Drosera* and *Pinguicula* [2] in which the entire shoot is photosynthetic and captured organ.
Material and Methods: *Nepenthes alata* and *Nepentes mirabilis* were grown under greenhouse conditions in moss without fertilizer. We measured the rate of photosynthesis (P_N) and respiration (R_D) gasometrically in assimilation leaves and pitchers. Assimilation pigment concentration was determined spectrophotometrically, N, C and H concentration using CHN-method. Samples for light and electron microscopy were fixed in glutaraldehyde and OsO_4 and were embadded in Durcupan and double stained with toulidine blue and basic fuchsin (light microscopy) and uranyl acetate and lead citrate (TEM). Stomata density was determined using microrelief method.
Results and Discussion: P_N of assimilation leaves (P_{Nleaf}) ranged 24.4 – 42.3 nmol CO_2 g^{-1} d.w. s^{-1} and picher ($P_{Npitcher}$) ranged -2.4 – (-) 0.1 nmol CO_2 g^{-1} d.w. s^{-1} (CO_2 is evolved) at 300 μmol m^{-2} s^{-1} PAR, 25 °C and 660 mg CO_2 m^{-3}. The R_{Dleaf} of *N. alata* was significantly higher than $R_{Dpitcher}$. On the contrary, *N. mirabilis* had higher $R_{Dpitcher}$. In comparison with non-carnivorous plants we can conclude that P_N is lower and the pitchers represent the cost for plant. We estimated that the cost of carnivory is minimal 8% C of total carbon budget and it is increasing with decrease of light. We found a set of characteristics that are involved in low assimilation capacity of traps in both studied species. First, five times lower assimilation pigment concentration (chl*a*, chl*b*, carotenoids) as well as lower nitrogen concentration in the pitcher, although H and C concentrations did not differ significantly. Assimilation leaf has app. 10 times (*N. alata*) and 200 times (*N. mirabilis*) higher stomata density than the pitcher. This results in lower stomatal conductance for CO_2 in the pitcher. Similarly, we can predict lower mesophyll conductance in the pitcher, because mesophyll is compact with small portion of intercellular spaces without palisade layer. On the contrary, mesophyll of assimilation leaf is composed of palisade layer with numerous chloroplasts and spongious parenchyma with a well developed intercellular spaces. Therefore, lower P_{Nleaf} of *Nepenthes* in comparison with non-carnivorous plants is probably due to lower N concentration as consequence of nitrogen poor habitat. Chloroplasts in both tissue have well developed grana with numerous plastoglobuli and differences in chloroplasts between pitcher and assimilation leaf are rather quantitative than qualitative character. We predict, that some of the characteristics, that make photosynthesis inefficient, could be responsible for carnivorous function in nutrient poor habitat. Compact mesophyll could provide better symplastic transport of water with enzymes into the pitcher as well as better transport for nutrients obtained from prey. Very low stomata density is probably consequence of low assimilation capacity of pitcher, because respiration delivers sufficient CO_2 for P_N and thus pitcher is less dependent on atmosferic CO_2 concentration. Moreover, transpiration is not favoured in traps, because water is secreted into the pitcher as a digestive fluid.
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Salicylic acid - induced protection on photosynthesis to paraquat oxidative stress

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Introduction: Salicylic acid (SA) has been recognised as an endogenous regulatory signal in plants mediating plant defence against pathogens, ozone or UV light and plays a role in the plant response to adverse environmental conditions, such as salt and osmotic stresses [1]. The common link among different stresses is that they all produce oxidative burst. Paraquat (Pq) is a non-selective contact herbicide that causes extensive lipid peroxidation, chlorophyll breakdown, loss of photosynthetic activity, leakage of electrolytes, and loss in cell membranes integrity [2]. This study was undertaken to determine the physiological and biochemical changes in barley plants treated by SA during Pq-induced stress, to investigate whether this plant regulator is involved in induction of defence response, to elucidate the underlying mechanisms by which SA alleviates the Pq-induced inhibition on photosynthesis, and to test the hypothesis that this treatment might reduce the Pq injury on photosynthesis through their effect on detoxification of AOS.

Material and Methods: Barley seedlings (12d old) were supplied with 500 μM SA or 10 μM Pq via the transpiration stream and kept in the dark for 24 h. Then they were exposed to 100 μmol m^{-2} s^{-1} PAR and samples have been taken 1, 2, 3, and 6 h after the light exposure. Leaf gas exchange parameters, the activity of RuBPC and of the photorespiratory enzymes PG, GO, and CAT were determined as described by [3, 4].

Results and Discussion: Treatment of seedlings with SA alone resulted in decreased levels of Chl, photosynthetic (A) and transpiration (Tr) rates. Pq treatment led to a decrease in Chl and protein contents and to a very strong inhibition of A. Pq-treatment did not affect the activity of RuBPC but highly increased the activity of the photorespiratory enzymes. Pre-treatment of seedlings with SA fully blocked the inhibitory effect of Pq on A and provided protection against subsequent Pq-induced oxidative damage. This observation was confirmed by gas exchange parameters, Chl and protein content and by changes in lipid peroxidation, H_20_2 level, and electrolyte leakage. The relationship between SA and Pq toxicity and the degree of oxidative damage was examined by measuring the activities of several antioxidative enzymes such as SOD, APX, GR and POX. Treatment with 10 μM Pq reduced the activities of APX and GR. Pre-treatment with 500 μM SA for 24 h in dark highly improved the capacity of the antioxidative defence system and increased Pq tolerance. Considering the enhanced activity of the antioxidative enzymes and the decrease in the levels of H_20_2, lipid peroxidation and electrolyte leakage in pretreated with SA barley plants we could assume that the observed protection on photosynthesis was probably due to increased antioxidant capacity and improved cell permeability.

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Acknowledgments: Supported by NSF BG, grant No. B 1109.

The effect of micromolar concentrations of methyl viologen on photosynthesis and antioxidant system of barley leaves

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Introduction: Methyl viologen (MV, paraquat, 1,1'-dimethyl-4,4'-bipyrydinium dichloride) is a non-selective rapid action herbicide that catalyzes the electron transfer from iron sulfur cluster FeS_A/FeS_B of photosystem I [1] to molecular oxygen resulting in the accumulation of reactive oxygen species (ROS) in stroma of chloroplasts. An important pathway of ROS detoxification in chloroplasts is a reduction of H_2O_2 by reduced ascorbate (Asc) catalyzed by ascorbate peroxidase (APX). Under illumination Asc is regenerated (re-reduced) mainly non-enzymatically by reduced ferredoxin [2]. As MV competes for electrons with ferredoxin it inhibits the Asc regeneration. We studied the effect of μM concentrations of MV on photosynthesis and antioxidant system of barley leaves.

Material and Methods: Leaf segments of 8-day-old barley (*Hordeum vulgare* L, cv. Akcent) were infiltrated with 3 and 10 μM MV in Petri dishes by floating of segments on the solution surface. Leaf segments floating on distilled water were used as a reference. After 1 h incubation at given solutions in the dark the samples were exposed to white light (100 μmol photons m^{-2} s^{-1} (PAR)) for 4 h or they were kept in darkness (controls). Penetration of MV into the samples was investigated by monitoring the changes in ΔA_{820} after the onset of light (P700 oxidation). Changes in leaf gas exchange and chlorophyll fluorescence were also recorded. The capacity of the antioxidant defence system was estimated by using the measurement of the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT).

Results and Discussion: Prolonged MV treatment caused an acceleration of P700 oxidation (changes in ΔA_{820}) and a gradual inhibition of photosynthetic CO_2 assimilation (a decline of A_g). Only a slight inhibition of the photosystem II function (a slight decrease in the F_V/F_M ratio, unchanged non-photochemical fluorescence quenching) was observed. While the MV treatment led to a significant inhibition of APX activity the activities of SOD and CAT remained unchanged. Since APX is known to be labile in the absence of Asc (inhibition and degradation by H_2O_2) [3], we suggest that the observed reduction of APX activity (to 65% or 53% for 3 or 10 μM MV, respectively) was due to the Asc depletion. The enhanced request for Asc corresponded with the observed increase of GR (Asc regenerating enzyme) activity.
Further, it was found that MV action under illumination led to a stomatal closure. Recent studies shows that the redox state of ascorbate plays an important role in controlling of the abscisic acid- and H_2O_2-mediated stomatal closure [4, 5]. Our results indicate that the MV-induced oxidative stress on photosynthetic apparatus is initiated by the inhibition of Asc regeneration.

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Acknowledgements: Supported by the grant MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

Treatment with Salicylic acid decreases the effect of cadmium on photosynthesis in maize plants

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Introduction: Salicylic acid (SA) is a natural signal molecule for the activation of plant defence responses [5]. It has been shown that SA can improve the thermotolerance and heat acclimation [3], increase the chilling tolerance [4], and it plays a role in plant response to salt and osmotic stress [2], UV light and ozone [7, 8] or to paraquat-induced stress [1].
Investigations were carried out to study the possible role of salicylic acid (SA) in protection of photosynthesis to Cd-induced toxicity in maize plants raised on growth medium with various concentrations of cadmium chloride (0, 10, 15 and 25 μM). Here we explore the suggestion that SA has a protective effect on photosynthesis in maize plants by activation of antioxidant capacity of plants.

Material and Methods: Seeds of maize (*Zea mays* L., hybrid Norma) were sterilized and divided into two groups. One half of the seeds was presoaked in 500 μM SA solution only for 6 h and then the both groups were allowed to germinate for 4 days and were then grown for 10 days in Hoagland solution at 22/18 ^{0}C with a 16/8-h light/dark periods and 120 μmol m^{-2} s^{-1} PAR. All seedlings (without H_20 and SA controls) were transferred on Cd-containing solutions and grown for 10 days. The rate of $C0_2$ fixation and the activity of the carboxylating enzymes were measured as described by [6].

Results and Discussion: Exposure of plants to Cd caused a gradual decrease in shoot weight accumulation, the effect being higher expressed at plants treated with 25 μM Cd. Pretreatment of seeds with SA increased the weight of shoots up to 35% at 25μM Cd variants. The same tendency was observed for the chlorophyll level. The rate of $C0_2$ fixation was lower in Cd-treated plants and the inhibition was partially overcome in SA-pretreated plants. A drop in the activities of carboxylating enzymes (RuBPcarboxylase and PEP carboxylase) was observed for Cd-treated plants. Pretreatment with SA alleviates the inhibitory effect of Cd on the enzymes activity. Data suggest that biochemical factors are involved in the response of photosynthesis to Cd toxicity. The proline production, the rates of lipid peroxidation and electrolyte leakage highly increased in Cd-treated plants and the values of these parameters were much lower in SA-pretreated plants. Our results suggest that the phytotoxicity of Cd is mainly induced by oxidative stress and SA is involved in the defense responses of plants to Cd exposure.

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Acknowledgements: This work was carried out in the frame of cooperation agreement between Bulgarian Academy of Sciences and Hungarian Academy of Sciences and supported by NSF grand (1102, Bulgaria).

Polycyclic aromatic hydrocarbons as the factor affecting the Hill's reaction activity

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous organic pollutants in the environment which can act as potential carcinogen, mutagens and teratogens [1]. Plants as a dominant biotic component of terrestrial ecosystems take up and accumulate substantial portion of those compounds from the environment. PAHs can, depending on the time and intensity of influence, invoke acute, chronic and latent injury of plants, above all in an interaction with futher environmental factors. Long-term influence of toxicants can affect the structure and function of ecosystems - diversity of plant species, reduction of biomass production, reduction of O_2 production and/or the degradation of the natural environment. The primary targets where air pollutants injure plants are biomembranes and enzymes. Subsequently, the biochemistry, the photosynthesis, respiration and transpiration processes, and the structural and chemical components are affected.

The numerous photosynthetic processes, with the exception of pigment concentrations (considered under the metabolite section), may be useful as biomarkers of general effects, but these processes are affected by such a variety of environmental and physiological factors that they may be of only marginal utility as indicators of effects that can be associated with pollutants. Photosynthetic processes that could be considered include changes in electron transport, photophosphorylation, fluorescence characteristics, carbon assimilation, partitioning and allocation. Photosystem II (the damage on the level of D1 protein, primary electron donor - Tyr Z, secondary plastoquinone acceptor - Q_B and oxygen evolving centre - Hill's reaction activity) is inactived by a variety of stresses - high irradiance, extreme temperatures, drought, salinity, nutrient deficiency, air pollutants and chemicals as heavy metals and herbicides [2]. Many of these same effects have been observed with nonherbicide organics, e.g. polycyclic aromatic hydrocarbons.

The aim of our experiments was to evaluate the effect of the polycyclic aromatic hydrocarbon fluoranthene on the Hill's reaction activity, the chlorophyll fluorescence parameters and the content of photosynthetic pigments in pea plants.

Material and Methods: Pea plants (*Pisum sativum* L., cv. Garde) were used as plant material and cultivated for 18 and 25 days in nutrient solution. Fluoranthene (FLT) (*Supelco*, USA) was applied in the concentration of 0.01 and 1 mg l^{-1} into the nutrient solution. The content of photosynthetic pigments (chlorophyll *a* and *b*, carotenoids) was determined spectrophotometrically (UV-VIS Spectrophotometer, Shimadzu, Japan). For the extraction of pigments was used 100% acetone. The calculation was made according to Holm [3]. Chlorophyll fluorescence parameters (F_0, F_V/F_M, Φ_{II}; for the definition see [4]) were determined from a slow (Kautsky) induction kinetics of chlorophyll fluorescence, recorded by PAM-2000 portable fluorometer (*Walz*, Germany). Hill's reaction activity was measured spectrophotometrically at 630 nm as the amount of DCPIP reduced by the chloroplast suspension at an irradiance of 400 μmol m^{-2} s^{-1} PAR. Results were then processed with software STATISTICA 6 (StatSoft, Inc.®).

Results and Discussion: The applied concentrations of FLT (0.01 and 1 mg l^{-1}) significantly affected primary photochemical processes of photosynthesis of pea plants:

- decreasing Hill's reaction activity
- increasing F_0 and decreasing F_V/F_M and Φ_{II}
- decreasing the content of chloroplhyll *a*, *b* and carotenoids.

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Nitric oxide targets photosynthetic electron transport in pea leaves

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Introduction Plants not only respond to ambient levels of nitric oxide (NO), but also generate NO by nitric oxide synthase-like isozymes and other abiotic ways. In the past years, this gaseous free radical has been implied as an important signaling molecule in many physiological processes. NO induces elongation in root segments; it also stimulates seed germination and de-etiolation, as well as inhibiting hypocotyl elongation. Recently it has been shown to mediate plant defense responses against pathogens. Nevertheless, the role of NO in photosynthesis is not completely revealed. In previous studies, the regulating activity of NO in photophosphorylation in chloroplasts was clearly demonstrated. NO inhibits electron transport and light-induced ΔpH formation across thylakoid membrane. The NO donor, S-nitroso-*N*-acetylpenicillamime (SNAP) reversibly reduces ATP synthesis [1]. Interestingly, the optimal quantum efficiency (F_v/F_m) was not altered by increasing levels of NO in isolated thylakoid membranes, while others showed reduced quantum efficiency in intact potato leaves upon such treatment [2]. It was found that photochemical quenching (qP) and the effective quantum efficiency (Φ_{PSII}) decreased while non-photochemical quenching (NPQ) remained unchanged following treatment with the NO donor, sodium nitroprusside (SNP). In our study, we focused on the potential effects on photosynthetic electron transport using quenching analysis of chlorophyll *a* fluorescence.

Materials and methods *Pisum sativum* L. cv Rajnai Törpe plants were grown in the greenhouse for 2 weeks in a modified Hoagland solution. The youngest fully expanded leaves were excised and the petioles were submerged in Petri dishes containing distilled water, NO donor molecules and scavenger chemicals with various concentrations. Chlorophyll fluorescence of PS II of pea leaves was measured with a PAM fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Q_A^- reoxidation kinetics was followed by double-modulation fluorometer (Photon Systems Instruments, Brno, Czech Republic). Xanthophyll cycle pigments were determined by HPLC.

Results and Discussion Previous electron paramagnetic resonance (EPR) studies on transition metals in electron transfer components showed that NO can replace bound bicarbonate at different binding sites. NO has a strong affinity to iron in heme and non-heme proteins *e.g.* the non-heme iron complex in PSII [3]. In order to investigate the effect of NO, the Q_A^- reoxidation kinetics was examined at different NO donor concentrations. The flash induced fluorescence relaxation curves were deconvoluted using a fitting function with two exponential components and one hyperbolic component. In the presence of SNP, the relative amplitudes and the half-lifetimes of the fast and middle phases changed, indicating the effect of NO on Q_A^- reoxidation via forward electron transport in PSII centres both which contained bound plastoquinone molecule and which had an empty Q_B site at the time of the flash. As NO seems to bind to different binding sites of electron transport components, it is not easy to distinguish and identify the individual modulations in the complex chlorophyll *a* fluorescence kinetics. Fast induction kinetics showed an increase in F_o and F_I levels upon the addition of a NO donor, and the altered area over the curve between F_o and F_m indicated a reduced pool size of PS II electron acceptors. In addition, rising NO donor concentrations caused an increase in NPQ values, which, in a certain high range, decreased again. Analysis of NPQ recovery indicated that energy-dependent fluorescence quenching (qE) followed the changes in NPQ values, while the photoinhibition component (qI) constantly increased. The rate of violaxanthin de-epoxidation was followed by HPLC. Every measurement was tested with specific NO scavengers, cPTIO and haemoglobin, which inhibit the effect of NO in each experiment.

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Affection of photosynthesis in lichens by photomodified polycyclic aromatic hydrocarbon fluoranthene

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Introduction: Terrestrial ecosystems are affected by a number of noxious compounds including polycyclic aromatic hydrocarbons (PAHs). Many PAHs are highly toxic, mutagenic and/or carcinogenic. They are formed during incomplete combustion and pyrolysis of organic matter (fossil fuels, wood and plastic) [1]. The chemical properties and biological activity of PAHs can be altered both abiotically and biotically. One of the most important abiotic factors is short-wave radiation (UV-A and UV-B). During photomodification, mostly photooxidation, PAHs are structurally altered to a complex mixture of (more than 20) compounds, mainly oxidation products, which may be more toxic than the parent compounds due to the combination of increased solubility, reactivity and bioavailability [2]. PAHs, their metabolites and products of their photomodification can affect structures and functions at the cellular and subcellular levels. The plasma membrane and inner membranes are the first targets, followed by changes in enzyme activity [3] and leading to an affection of membrane-bound processes, e.g. photosynthesis or respiration. Inhibition of photosynthesis is often a key mechanism of the toxicity of pollutants (heavy metals, NO_X, SO_2 or organic compounds) in plants and a modification of photosynthetic activity is often used as a "bioindicator" of contamination effects [4]. Many recent studies have used vegetation which bioaccumulates air pollutants for monitoring studies. Lichens have been often used as bioindicators or as bioaccumulators of environmental pollution by heavy metals and some organic pollutants. Fluoranthene (FLT), one of the most frequent PAHs in the environment of the Czech Republic, can affect all stages of higher plant growth and development through injury or change of main physiological and biochemical processes (e.g. primary processes of photosynthesis). Free diffusibility of lichen thalli enables quick penetration of toxic compounds from atmosphere to the photosynthesizing cells and, therefore, the responses of lichens to air pollution could be more sensitive than in the case of higher plants. Induced chlorophyll fluorescence measurement is becoming a valuable, non-destructive procedure with which it is possible to measure changes associated with photosystem II (PSII) of plant or lichen photosynthetic apparatus.
The aim of our experiments was to evaluate the effect of the intact and photomodified form of fluoranthene on chlorophyll fluorescence parameters in two foliose lichen species.

Material and Methods: Foliose lichens *Lasallia pustulata* (L.) Mérat. and *Umbilicaria hirsuta* (Sw. ex Westr.) Hoffm. were used as plant material. The intact and photomodified form of fluoranthene (*Supelco*, USA) was applied in the concentration of 0.1, 1 and 5 mg l^{-1}. Chlorophyll fluorescence parameters (F_0, F_V/F_M, Φ_{II}; for the definition see [5]) were determined from a slow (Kautsky) induction kinetics of chlorophyll fluorescence, recorded by PAM-2000 portable fluorometer (*Walz*, Germany) before FLT exposure and after 1, 2 and 3 days of exposure. Results were then processed with software STATISTICA 6 (StatSoft, Inc.®).

Results and Discussion: The obtained results demonstrated, that the applied concentrations of FLT (1 and 5 mg.l^{-1}) affected primary photochemical processes of photosynthesis of algae in both lichen species (increasing F_0, decreasing F_V/F_M and Φ_{II}). Photomodified form of FLT appeared to be more toxic than the intact form. Among selected lichen species, *Umbilicaria hirsuta* exhibit more sensitive response than *Lasallia pustulata*.

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Organism interactions and photosynthesis

Presymptomatic visualization of *Phaseolus vulgaris – Pseudomonas syringae* interaction by chlorophyll fluorescence imaging.

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Introduction: Kinetic imaging fluorometry is a non-invasive tool for detecting biotic stress of plants [1, 3, 4]. Imaging kinetic fluorometer FluorCam [2] (www.psi.cz) was used to map fluorescence transients over a bean leaf infected by two pathovars of *Psedomonas syringae*.

Material and Methods: Three light intensities were used to elicit chlorophyll fluorescence (90, 320, 640 μmol m^{-2} s^{-1}). Simultaneously, portable photosynthesis system Cirras-1 (www.ppsystems.com) was employed to monitor integral CO_2 uptake. The parallel monitoring of leaf heterogeneity by fluorescence and of integral leaf performance by gas exchange was aimed at dissecting the role of spatial and temporal heterogeneity of photosynthesis.

Results and Discussion: Here, we show that the fluorescence imaging method revealed the bacterial infection much earlier than visual symptoms occurred. The time spreading infection caused distinct patchiness over the leaf already 3 and 5 days after infection visible only in fluorescence imaging. Visual symptoms appeared 14 days after the inoculation.
The protocols with low light levels were more potent to detect the infection than the high light protocols. Opposite was true for the CO_2 assimilation measurements. Overall, fluorescence was more sensitive that the gas exchange measurements. The strongest fluorescence signatures of the infection were found typically during the fluorescence decline from the transient maximum level ca 4s after the increase of the incident irradiance.

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Photosynthesis in populations, ecosystems and extreme environments

Applicability of reflectometric approach to the study of structural and functional characteristics of lichens

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Introduction: In contrast to many reflectometric studies done in plant leaves or stands, only a very limited amount of experimental work has been done to characterize diffuse spectral reflectance of lichens and utilize the results in lichen physiology [1,2,4]. The thalli of lichens are structures substantially different from plant leaves in many respects. Green layer of single-cells photobionts is covered by highly reflexive *cortex*, formed by fungal hyphae fused together by polysaccharidic substances. Our preliminary experiments were focused on determination of potential negative effect of cortical structures on physiological interpretation of spectral reflectance measurements.

Material and Methods: Two foliose aerophytic lichen species (*Umbilicaria hisuta* and *Lasallia pustulata),* were used for measurements of changes of spectral reflectance using a portable reflectometer UNISPEC (*PP Systems, USA*) equipped with a detector *MMS1/NIR enhanced (300-1100 nm)*, with an internal light source (halogen lamp), and bifurcated foreoptics. The measurements were repeated at different intrathalline water status of the samples. In a subset of the samples the upper cortex was mechanically removed. Several reflectance indices were derived from spectral reflectance curves using *UniWin* software: *water index, structural independent pigment index, chlorophyll content index, and photochemical reflectance index*, for details see [3].

Results and Discussion: In spite of extremely high increase in total reflectance of thalli of the two tested lichen species during desiccation, caused mainly by structural changes in the upper cortex, it was possible to derive useful information by a narrow-band analysis. The depression in reflectance around 970 nm and its derivative characteristics were the most reliable predictors of water status. They were in close correlation with both the water content and the water potential of the thalli within a broad range of hydration from full saturation to critical water shortage at which photosynthetic processes were inactivated. Determination of chlorophyll content and of the photochemical reflectance index (which correlates with the epoxidation state of the xanthophyll cycle pigments) from spectral reflectance data was also not much influenced by presence of the upper cortex and its optical changes during dessication. It is possible to conclude that even in lichens with thick and highly reflexive upper cortex it is possible to derive from rapid and non-invasive spectral reflectance measurements reasonably accurate information necessary for a more complex investigation of their physiology.

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Photosynthesis under natural CO_2 enrichment

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Introduction: Since early nineties, the sites with natural atmospheric CO_2 enrichment (natural CO_2 springs, mofettes) have been efficiently used in CO_2 research and helped to improve predictions on long-term plant response to increased atmospheric CO_2 concentration [1]. While this holds true mainly for plant water relations, the data on photosynthetic performance of mofette plants seem to be as inconsistent as results from experiments with artificially doubled CO_2 [1, 2, 3]. Gas-exchange measurements on the plants grown under naturally doubled atmospheric CO_2 concentration revealed no clear photosynthetic down-regulation [1, 2]. On the other hand a clear inhibition of photosynthesis was observed when plants were studied along the higher soil [CO_2] gradient [3].

Material and Methods: Plants growing at natural CO_2 springs are, in any case, exposed to extreme gaseous environment. Beside high fluctuations of air CO_2 and unstable gaseous conditions, there are many other environmental factors that can easily modify photosynthetic response under elevated CO_2. These would include modified mineral- and water availability, temperature effects, changed gaseous regime in the soil (high [CO_2] and low [O_2] effects) and others. Due to the direct and indirect action of elevated CO_2 on below- and above-ground plant organs the causal analysis of the effects observed on the level of photosynthesis is not simple.

Results and Discussion: In this paper the experience on photosynthetic response obtained from the Italian and Slovenian natural CO_2 springs will be reviewed and compared. Possible adaptive photosynthetic strategies of autotrophic plants growing under extreme CO_2 conditions will be discussed.

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Estimation of physiologically-active time in mosses based on in-situ temperature profile data from maritime Antarctica

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Introduction: In maritime Antarctica, lichens and mosses represent major components of vegetation cover in numerous coastal vegetation oases. Several attempts using fluorometric [1] and microclimatological measurements [reviewed by 2] have been made to assess physiologically active time available for these poikilohydric organisms to perform photosynthesis and biomass production. Here, we present the estimation of physiologically-active time in Antarctic mosses during Austral summer of 2004/2005.

Material and Methods: A year-round measurements of microclimatological characteristics were performed at the Galindez Island 65 °15 'S, 64 °16 'W, maritime Antarctica) in the vegetation oasis located close to the summit of the Woozle Hill (51 m a.s.l.). Two neighbouring plots were selected for the long-term study: (1) top of a small rock, and (2) shallow depression surrounded by rock formations. At the first plot, Cu-Constantan thermocouples were installed into a moss cusion formed by *Chorisodontium* sp. and *Polytrichum* sp. at the depths of 2, 5, 10, 15, and 20 cm. Temperature was measured each 1 h and stored into a multichannel MiniCube VX datalogger (Environmental Measuring Systems, CZ). At the plot two, a set of thermocouples was inserted into the same depths of moss carpet that was expected to be under snow cover for a longer time and supplied by more water from melted snow during Austral summer.

Results and Discussion: The investigated moss cusion was covered by snow throughout the Austral summer of 2004. Melting of snow started by Nov 10, and ended by Nov 20. Since the re-activation time of full photosynthetic activity is about 24 h of rehydration [3], the physiologically active period of the moss located at the rock started by Nov. 21. For the 2004/2005 Austral summer, the period of physiological activity might be estimated 110 d. The period was, however, not continuous, interrupted with few days when, due to full sunshine and a high air temperature, the upper surfaces of moss cusions became dry and thus limited in physiological activity. At the second plot, the role of surrounding rock microrelief was of a great importance. Due to long-lasting snow cover and stagnant water persisting during part of Austral summer, the physiologically active time was only about 40 d.

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Acknowledgements:
The research reported in this contribution was supported by the GAČR project 522/03/0754 and the Long-term Research Plan VZ MSM 07/98:143100007.

Stress bioindication for spruce forest ecosystems

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Introduction: The work on the research proposed in the APVT Project „Analysis of causes of massive decline of spruce forests in boundary regions in northern Slovakia and proposal of appropriate management measures" includes a multi-disciplinary research into spruce forest dieback in the region Horný Spiš – running under a preliminary title "non-specific dieback of spruce forests". The concerned research has been focussed on physiological status of beech trees distinguished according to three developmental stages (seedlings, young growth and adult trees). It is out of question that the direct cause of the decline of forest woody plants is the insufficiency and disturbances in their physiology. Consequently, there immediately arises a question about the origin of the physiological disturbances [1].

Material and Methods: In this contribution we present the results of auxiliary analyses of physiological characteristics (analyses of chlorophylls, analyses of chlorophyll fluorescence, analyses of selected nutrient minerals) used with proficiency as bioindicators of stress to woody plants, yet before the first visual manifestations of the damage are evident. We have primarily focussed on the comparison between the physiological status of selected sample trees growing in a locality with extraordinarily damaged and dying spruce forests (locality „Hliníky" in the Spiš region) and trees growing in a locality without visible damage symptoms (research monitoring plot in the Predná Poľana Mts.). At the same time, we also established a jar experiment in six varied replications. We primarily aimed at monitoring soil properties and their influence on the health status over the two-year study period (2004 – 2005). The measurements of chlorophyll fluorescence were carried out using a portable fluorometer PEA (Hansatech, Norfolk, UK) and a FMS2 system (Hansatech, Norfolk, UK). The samples of needles intended for analysis of chlorophylls and carotenoides were first homogenized in a homogenizer and then analysed in a 80 % water solution of acetone. The absorbance values were measured by means of spectro-photometry (apparatus Cintra 6.5, GBS, Australia). The concentration values of the individual photosynthetic pigments were calculated based on the adjusted relation according to [2].

Results and Discussion: Based on the evaluation and comparisons of the chlorophyll content values between the examined sampled trees and between the experimental plots, we can conclude that the chlorophylls concentrations in the assimilatory organs were found significantly lower on the airborne-pollutants loaded plot in the region Spiš, in comparison with the control locality in the Poľana Mts. At the same time, we observed an increasing trend in the ratio of chl*a*/chl*b* on the loaded plot (Spiš) in comparison with the control plot in Poľana. The values measured for the most important parameter – rapid kinetics of chlorophyll fluorescence F_v/F_m, evidently show that two adult sample trees (from 10 analysed) growing on the loaded „Hliníky" (Spiš) are under the limit of physiological disturbances (experimentally determined threshold value of 0.725) and one sample tree is very close to this critical limit. In the case of young spruce trees (age of 20 years), the critical limit for physiological disturbances has not yet been exceeded, not even in one single case. This fact indicates about better current physiological status of the spruce young growth in comparison to the adult trees at the same locality.

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Acknowledgements: Supported by GA APVT – 510193 02, VEGA – 2/4159/04.

Seasonal variability of photosynthetically active radiation incident on vegetation cover at Vernadsky Station, Galindez Island, Antarctica

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Introduction: For photosynthesis, growth and productivity of mosses and lichens forming Antarctic vegetation oasis, availability of water is of major importance due to their poikilohydric character. They are physiologically active and capable to utilize incident solar radiation in photosynthetic processes only if they are in a wet state. For the estimation of summer-season (snow-free) productivity, simultaneous measurements of their hydration status and radiation regime are required. Detailed long-term measurements of photosynthetically active radiation (PAR), however, are scarce in the region of the Antarctic Peninsula. Moreover, episodes with high PAR levels are limited there due to oceanic character of climate typical of numerous days with cloudiness. In the presented study, we report number of prevailingly sunny days with a special attention to the occurrence of days with a very high PAR (> 1500 μmol m^{-2} s^{-1}) incident at a vegetation oasis at the Galindez Island, maritime Antarctica.

Material and Methods: The radiation data were taken within the period of 2004-2005 at the Ukrainian Station Vernadsky, Galindez Island, Argentine Islands (65°14'43"S, 64°15'24"W). The below-specified sensors were installed in two dominating vegetation components: (1) moss bench dominated by *Chorisodontium* sp. formed in a shallow depression surrounded by stony belt and (2) clumps of fruticose lichens (mainly *Usnea* sp.) growing at the rocky edge of a coastal cliff. The measurements were accomplished using a PAR sensor manufactured by the EMS Brno (CZ) and by a pyranometer CM-6B produced by the Kipp & Zonen (the Netherlands). Both of these sensors were supplemented by a CV2 ventilation system with a heater (Kipp & Zonen, the Netherlands) for prevention of precipitation of dew and frost on the sensor glass dome. The parameters were sampled each 15 s and the data were stored as 10 min averages. The results are presented at the level of daily and seasonal fluctuations. The possible effect of light regime on physiologically active time of moss and lichens cover was evaluated with using of an every week photo-documentation, surface and soil temperature data at the both stands.

Results and Discussion: The annual regime of the daily sum of PAR and global solar radiation had due to the basic extraterrestrial factors (distance the Earth from the Sun and the zenith angle of the Sun) a typically single wave with the highest values around the summer solstice (*e.g.* 11 December 72.481 mol m^{-2} d^{-1} and/or 34.862 MJ m^{-2} d^{-1}; 21 December 73.214 mol m^{-2} d^{-1} and/or 35.287 MJ m^{-2} d^{-1}). The daily sums of the incoming radiations fluctuated greatly according to changes in the weather conditions, and essentially replicated the high variability of the cloudiness. During the summer season (October – March) was determined 57 days with the PAR values higher than 1500 μmol.m^{-2}.s^{-1} and only 13 days with the PAR values exceeded the level of 2000 μmol m^{-2} s^{-1}. The PAR of 2000 μmol m^{-2} s^{-1} and higher often occurred for short-time interval of 10 or 20 min within a day. Only in one case (17 December), the level of 2000 μmol m^{-2} s^{-1} had a duration over 40 min. Due to PAR reflection from episodic snow deposition on the vegetation surface during summer season, the true number with high PAR incident on the lichens and mosses was reduced. The analysis of the snow depth data confirmed that the moss cover of *Chorisodontium* sp. developed in shallow depression could be exposed by the highest values of PAR only three times during summer (10, 22 January and 6 February). Contrastingly, the lichen cover with *Usnea* sp. situated on the rocky edge where exposed 12 days.

Acknowledgements: Supported by the Research Goal of Czech Ministry of Education, Youth and Sports, MSM 143100007.

Variation of spectral composition of photosynthetically active radiation penetrating into the Norway spruce canopy

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Introduction: The distribution of radiation at any depth in canopy depends not only on the overlying leaf area index but also on the solar elevation and the portion of diffuse to direct solar irradiance above the canopy [1]. Radiation interacts with plant organs through absorption and scattering. These processes vary widely in various parts of the solar radiation spectrum and depend on the leaf structure, leaf age, angle of incident radiation, etc. [2]. In presented contribution we focused on characterization of the changes of spectral composition of photosyntheticaly active radiation (PhAR) within the crown layer of dense Norway spruce stand. The effects od diffuse index and solar elevation angle on the spectral characteristics of radiation penetrating into the canopy are discussed.

Material and Methods: All measurements were carried out within Norway spruce stand [*Picea abies* (L.) Karst.] on the Experimental site Bílý Kříž (Moravian-Silesian Beskydy Mts., Czech Republic) during 2004 using portable spectroradiometer LI-1800 (Li-Cor, USA) for spectral measurements and LAI-2000 (Li-Cor, USA) for leaf area index estimation along with sensors LI-190SA, LI-200SA (Li-Cor, USA) and Kipp-Zonen (Holland) connected with data-logger (Delta-T, England) for irradiance measurements. Measurements of the spectra of incident solar radiation were performed above the canopy (H) and in two layers of the canopy characterized with LAI = 7,3 +/- 0,8 (middle layer – S) and LAI = 12,3 +/- 0,7 (lower layer – D)

Results and Discussion: Above the canopy the relative representation of the blue spectral region of PhAR was significantly higher under the cloudy sky conditions (characterized by the diffuse index DI > 0.7) in comparison with clear sky (DI < 0.3). This difference was more pronounced during late afternoon (at low elevation angle) that correspond with the fact that the spectral composition of direct solar radiation strongly depends on solar elevation angle, whereas that of diffuse radiation (radiation during cloudy days) is almost independent. Surprisingly, within the canopy the opposite difference between spectra characterising cloudy and clear days was observed. As expected the penetration of the PhAR into the canopy results in relative increase of the green spectral region. However, the influence on the penetration of the blue and red light into the canopy strongly depends on both diffuse index and solar elevation angle. On the contrary to cloudy days, the penetration of blue and red light was adversely dependent on the solar elevation angle for clear sky. As a consequence, under cloudless conditions (during late afternoon) the relative representation of the blue PhAR region (400-430 nm) within canopy may be almost twofold in comparison with PhAR spectrum above the canopy. This is quite surprising taking into account the fact that the canopy layer transmittance for direct radiation was similar during noon and late afternoon (0.44% and 0.42%, respectively).

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Acknowledgements: This research was supported by GAČR (No. 526/03/1104), IGA PřF OU (No. 06/2005) and EU research program CARBOEUROPE (VaV/640/18/03). We thank P. Limberková for help with measurements.

Emerging techniques, novel approaches

Thermographic and fluorescence imaging studies on virus-infected plants

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Introduction: We have previously shown that tobamovirus infection induces an inhibition of photosystem II (PSII) electron transport in *Nicotiana benthamiana* plants, disturbing the oxygen-evolving complex (OEC) [1] [2], which is one of the main targets of viral infection in the chloroplast. In the present work, *Nicotiana benthamiana* plants were infected with the Spanish and Italian strain of the Pepper Mild Mottle Virus (PMMoV-S and –I). Both strains induce curly of the young leaves at 7 post-infection days (dpi) and stunting of the plant at 14 dpi. Leaves that were fully expanded at the infection time remain asymptomatic. Plants infected with PMMoV-I are able to recover from their symptoms at 21 dpi. Fluorescence and thermographic imaging (FI, TI) are suitable for non-invasive follow-up of plant stress and can monitor changes in the plant physiological state before symptoms appear [3]. We have obtained images of different fluorescence parameters such as non-photochemical quenching (NPQ) and quantum yield from infected plants, measuring chlorophyll red fluorescence emission by means of the FluorCam (PSI, Brno, Czech Republic) [4]. In addition, thermal and fluorescence images were monitored with the equipment described in [5]. Our goal is to obtain *disease signatures* for this plant-pathogen interaction.

Material and Methods: *Nicotiana benthamiana* plants were infected with PMMoV-S and PMMoV –I; infection was followed at different dpi using the FluorCam [4] and the robotized imaging equipment [5]. Virus-induced fluorescence and thermal changes were monitored in asymptomatic leaves from PMMoV-infected plants, as well as in their corresponding control.

Results and Discussion: The fluorescence images obtained during the *quenching analysis* by means of the FluorCam revealed a spatial and reproducible NPQ pattern in infected asymptomatic leaves at different infection points. In addition, NPQ was the parameter providing the highest contrast between infected and non-infected plants; NPQ patterns differed also in PMMoV-S and PMMoV –I infected plants. Thermographic imaging showed increase in leaf temperature in asymptomatic leaves, which could correspond to the NPQ pattern in the same leaves. Thermal effect expanded over the whole leaf in the last infection steps. Control plants showed a more uniform temperature pattern.

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Application of multi-color fluorescence and reflectance imaging for localization of UV shielding and propagation of virus infection

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Introduction: Imaging of leaves provides information about distribution, localization and pattern of signals which can be used for studying differences and changes in leaves with a much higher statistical confidence than the usual point measurements [1, 2]. By choosing the spectral bands of excitation and detection one is able to characterize the leaf sample in terms of photosynthetic activity, pigment composition, leaf tissue properties and structure [3].

Material and Methods: In the multi-color fluorescence imaging system the sample is illuminated with flashes from a Xenon lamp with either a UV or a blue filter for fluorescence and a grey filter for reflectance measurements. The images are acquired by an intensified camera synchronized with the Xenon flashes. Different filters are used depending on the type of measurement for detection in the following bands: 440 nm, 520 nm, 550 nm, 690 nm, 740 nm and 800 nm [3].
Sun and shade leaves of a free standing beech tree (*Fagus sylvatica* L.) were used for screening of UV shielding. To follow the course of infection with the tobacco mosaic virus (TMV) with resistant tobacco plants (*Nicotiana tabacum* L.) grown in the greenhouse were analyzed.

Results and Discussion: The protection against UV radiation acquired by the sun and shade leaves have been detected by comparing the chlorophyll fluorescence F690 and F740 excited with blue and UV. UV shielding of the epidermis is expressed as log (^{B}F690/^{UV}F690). A higher value has been found for sun leaves and in particular the upper (adaxial) side. Within the lower side of the sun leaf and the upper side of the shade leaf the highest value for the ratio log (^{B}F690/^{UV}F690) and thus the highest UV-shielding was found at the leaf rim.
The propagation of virus infection within a leaf of a resistant tobacco plant was followed as appearance of spots at the site of TMV infection [4]. It could first be detected as blue-green fluorescence excited with UV and as chlorophyll fluorescence excited with blue light. Later on the chlorophyll fluorescence excited with UV and the reflectance at 800 nm showed the symptoms of TMV-infection. Finally, the effect of TMV became visible as detected by the reflectance at 550 nm.

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Acknowledgement: This study was financed by European community in the Research Training Network Project "STRESSIMAGING".

The xanthophyll cycle in higher plants and green algae: its role in the photosynthetic apparatus

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Introduction: Light dependent conversion of violaxanthin to zeaxanthin, the so-called xanthophyll cycle was shown to serve as a major, short-term light acclimation mechanism in higher plants. The role of xanthophylls in thermal dissipation of surplus excitation energy was deduced from the linear relationship between zeaxanthin formation and the magnitude of nonphotochemical quenching [1]. Unlike in higher plants, the role of xanthophyll cycle in green algae (*Chlorophyta*) is ambiguous since its contribution to energy dissipation can significantly vary among species e.g. [2, 3]. Elucidation of the xanthophyll role in nonphotochemical quenching is a magnificent example of the way how correlative biological evidence drives new biophysical research.

Methods: Irradiance, chlorophyll fluorescence and liquid chromatography measurements were used to monitor photochemical activity and xanthophyll content.

Results and Discussion: We have studied the role of the xanthophyll cycle in the adaptation of several species of green algae (*Chlorella, Scenedesmus, Haematococcus, Chlorococcum, Spongiochloris*) to high irradiance. The xanthophyll cycle was found functional in all tested organisms; however its contribution to nonphotochemical quenching is not as significant as in higher plants. In green algae, this conclusion is supported by three facts: (i) the content of zeaxanthin normalized per chlorophyll was significantly lower than that reported from higher plants, (ii) antheraxanthin + zeaxanthin content displayed different diel kinetics than nonphotochemical quenching, and (iii) there was no such a linear relationship between nonphotochemical quenching and the antheraxanthin + zeaxanthin content as shown in higher plants.
We assume that algae rely on other dissipation mechanism(s), which operate along with the xanthophyll cycle-dependent quenching.

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Acknowledgement:
This work is **s**upported by the Ministry of Education, Youth and Sports of the Czech Republic, project MSM6007665808.

Spectroscopic analyses of weakly absorbing samples using an integrating cavity absorption meter

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Introduction: Absorption spectrophotometry, a standard tool for quantitative analysis, suffers from two drawbacks: lack of sensitivity and vulnerability to scattering. It has been pointed out more than once that the solution to these problems lies in using a reflecting cavity as a sample holder. Due to multiple reflections at the cavity wall, the effective pathlength becomes considerably larger than the diameter of the cavity, and scattering losses are eliminated because scattered light is prevented from escaping the detector. Though much effort has been spent in analyzing and improving the performance of such a device, often called an integrating cavity absorption meter (ICAM), a simple strategy for deducing the absorbance of the sample is still lacking [1-4].

Material and Methods: Measurements were carried out with a spherical ICAM (~80 mm in diameter) made of glass that has a neck of about 15 mm, pointing upwards, which serves a dual purpose: exit port for the emerging light, and a passageway for the sample. The outer surface of the ICAM was coated with a silver layer, which provides almost uniform reflectivity over the whole visible range. A window (~8 mm in diameter) on the great circle of the sphere was left uncovered for light input, while the light output was picked up via a light-guide inserted into the neck. In order to protect the silver coating the sphere was covered with a white vitrified paint and then mounted into a box which held it fixed and also provided access to the optical window. A 24 V, 150 W tungsten filament lamp served as a light source which was focussed onto the input window of the sphere. The light emerging via the neck was collected by an optical fibre and led to a triple-grating polychromator equipped with an intensified diode array.

Results and Discussion: The distortion effects of the ICAM on absorption spectra of non-scattering solutions were analyzed. A correction method for calculating the absorption spectra is also discussed. It is shown that this correction function depends on the optical density of the solution, instead of the concentration of the sample alone. The corrected, true spectra are similar within 5 % difference when the concentration of the solution was changed over a wide range. The calibration and correction method presented in this paper is general, but it can be concluded, that every ICAM–detector system pair needs their own calibration procedure. We also demonstrate that in case of scattering samples the use of the ICAM can not just enhance the sensitivity but also reduces scattering related spectral distortions. As the sample concentration in the sphere is increasing the intensity of the monitoring light is being attenuated both directly (absorption) and indirectly (effective pathlength), therefore this method is best suited to measure weakly absorbing samples.

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Acknowledgements: This work was supported by the GVOP-3.3.1.-2004-04-0055/3.0 programme.

Three-dimensional reconstitution of thylakoid membrane system in granal chloroplasts

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Introduction: In the chloroplasts of mesophyll cells of C_3 and C_4 plants the thylakoid membranes exhibit a large lateral heterogeneity. Two types of thylakoid are distinguished, granum (stacked) and stroma (unstacked) thylakoids, which form a three-dimensional network. Although the spatial relationship between the granum and stroma membranes, multiple helices of stroma lamellae wound around the cylindrical grana has been known for more than two decades [for review, see 1], most textbooks and research papers continue to contain erroneous 3D models and simplified schemes. This work presents a detailed computer model, based on various electron microscopic observations, and the first approach to determine the 3D structure of this intriguing multilamellar system by electron tomography.

Material and Methods: Spinach chloroplasts were isolated and embedded by conventional electron microscopic procedures. Thick sections (250 nm) were cut, stained and placed in an HVEM (high voltage electron microscope). Pictures were taken at different tilt angles and analysed as described earlier [2].

Results and Discussion: The electron tomogram shows that the interconnections of stroma thylakoids and granum compartments shift upward on the right side and downward on the left side of the granum. This finding is consistent with the helical arrangement of the stroma thylakoids around the granum. However, the length of the junctions appeared to vary in a wide range, and we also observed some stacked thylakoid membrane pairs, on both ends of the grana, which did not participate in the helical arrangement. These data reveal flexibility of the structure and plasticity of the membrane organization, and also shed light on the self-assembly of the granal structure during ontogeny.

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Acknowledgements: This work was supported by grant from the Hungarian Fund for Basic Research (OTKA T42696).

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Photosynthesis, respiration and cell death at high temperature stress

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Introduction: A leaf temperature above 45°C caused visible damage to leaves, when it was applied for a longer time, as well as when the temperature rose continuously with 1°C per min up to this value [1], while leaves can recover from short pulses of high temperature [2]. As changes in photosynthetic parameters as well as cellular damage were to be observed as depending on temperature as well as on the time at this temperature, we applied short heat pulses between 30 and 48°C and of no more than 5 min to intact leaves of *Phaseolus vulgaris* L.

Material and Methods: Temperature changes were performed in a leaf cuvette. The upper side of the leaf was glued to the glass window of the chamber water-jacket by starch gel to enhance the temperature exchange between the leaf and thermostatted water, so that temperature changes were completed within about 15s. Chlorophyll fluorescence, CO_2 uptake or respiration and transpiration where measured continuously, oxygen evolution was measured before and after the treatment. Possible damage on cellular level was made visible after the treatment by use of the dye Evans Blue, that enters only cells with changed plasmalemma permeability [3], referred to as blue-stained cells.

Results and Discussion: CO_2 assimilation had its maximum between 30 and 35°C and declined steeply at temperatures higher than 40°C. In dark, respiration in all cases increased immediately after temperature rise, showed a peak after about 100s and then declined slowly. When the leaf was returned to 22°C, respiration returned to its original value within 5 to 10 min. Simultaneously with respiration, F_0 fluorescence increased, reached a plateau after 100 to 150s and remained stable. Only after return to 22°C, F_0 declined steeply, but, even after 1 h, remained on a somewhat higher level than before the temperature treatment. At temperatures higher than 40°C, oxygen evolution and photosynthetic electron transport declined. These parameteres did not recover within the time of experiment (up to 2h).
Three different versions of damage were observed. (i) After shorter exposure or lower temperature than 5 min at 44°C or 4 min at 48°C, no blue-stained cells were found. (ii) When this threshold for damage occurance was only just exceeded, single blue-stained cells were observed. Their number increased with time, and this progress could continue for more than 2 hours after the heat treatment. (iii) After sustained high temperature, large areas of blue-stained cells were observed. In leaf disks infiltrated with 3,3'-diaminobenzidine 4 HCl (DAB), cells stained reddish-brown were detected after heat treatment for 4 and 5 min at 48°C. They showed a similar distribution pattern as cells stained with Evans Blue. DAB is used for detection of H_2O_2, which is known to be produced in tissues as a stress response [4] and can trigger programmed cell death [5].

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Acknowledgements: Supported by Deutsche Forschungsgemeinschaft (Research Grant Hu 830/3-1)

Snow and ice cyanobacteria and algae and their succession to deglaciated environments

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Introduction: Cyanobacteria and algae play a key role in the Arctic and the Antarctic ecology as primary producers. Recession and melt down of glaciers is one of the most important phenomenon of the global climate warming. The microbial primary succession processes to deglaciated areas were studied only sporadically. They include three steps; immigration, colonisation - establishment, and development (this part can include several stages). Cyanobacteria and algae play a key role in these processes. They fix carbon - nitrogen and accumulate mineral nutrients and energy in their cells. These accumulated substances and energy are later used by other organisms participating in primary succession processes. In climatically favourable conditions the primary succession processes can be very quick. In contrary, under unfavourable conditions, these processes can be slow, taking place for long period of time.

Results and Discussion: The review of present state of knowledge together with results of three research projects localised in various parts of the Arctic and the Antarctic will be introduced.

Detection of heat production as a measure of non-radiative de-excitation of plants as indicator of photosynthetic activity

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Introduction: Non-radiative heat production, chlorophyll fluorescence and photochemistry are the forms to which energy is transferred during de-excitation after absorption of light. Kitajima and Butler [1] stated that under optimum conditions 78% of the absorbed energy is converted into photosynthesis, 20% into heat and 2% into chlorophyll fluorescence. The detection of the variation of the red chlorophyll fluorescence (Kautsky effect) is a common technique to study changes in photosynthetic activity. The decrease (or quench) of the maximum chlorophyll fluorescence reached when the reaction centres are closed in the dark adapted state is usually divided into the 'photochemical quench' the part directly related to the photosynthetic activity and the remaining 'non-photochemical quench'. The non-photochemical quench is derived from the missing chlorophyll fluorescence and described as 'heat production', 'thermal dissipation' or 'non-radiative decay'.
Methods: Only few publications describe real measurements of heat production. One of the reasons is that heat produced during non-radiative dissipation is in the order of 10^{-5} °C which can hardly be detected by conventional temperature sensors. There are different techniques to study the heat production of plants during non-radiative dissipation (photoacoustic, photothermic, beam deflection) [2].
Results: Increased heat production after photoinhibition has been proven by photoacoustic measurements [3-5]. The comparison of the induction kinetics of chlorophyll fluorescence and heat production lead to varying results [5-9]. Heat production was shown to increase at the end of a dark-recovery after light-induced induction [10]. Cyclic electron transport [11-13] could be measured by means of photoacoustic sensors. Non-radiative heat production can be detected not only with leaves, but also with chloroplasts [14] and thylakoid fragments [13, 15, 16]. The various aspects of studying photosynthesis by the detection of non-radiative heat production has been summarized [2, 17, 18].
Conclusion: Obviously, heat production is neither negligibly low nor constant or parallel to chlorophyll fluorescence. Therefore one should not deduce non-radiative dissipation from chlorophyll fluorescence measurements but carry out measurements of non-radiative heat production to fully understand the distribution and usage of absorbed light energy.
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Chronic photoinhibition plays only a minor role in the world's most successful bryophyte genus

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Introduction: Peat mosses (*Sphagnum*) cover vast areas of earth's land surface. By producing a peculiar peatland environment, they often induce unfavourable water-saturated soil conditions for vascular plants and form treeless, sun-exposed habitats [1]. This evokes the hypothesis that they show a long-term adaptation to full sun irradiance. When pristine mires are drained and forested, bryophyte species composition changes only partially, thus indicating that some species are able to acclimate to shade conditions. To test the characteristics of sun and/or shade plants in peat mosses, we screened photosynthetic properties of mosses growing both in open and shaded mire habitats, cf. [2].

Materials and Methods: We collected samples of six *Sphagnum* species in a pristine and a drained part of a mire, i.e., in sun and shade habitat. The mosses were kept in a growth chamber for 10 days under mild light conditions corresponding to those in the drained mire (220 $\mu mol\ m^{-2}\ s^{-1}$). We measured PPFD response of CO_2 assimilation (IRGA; Li6400, LICOR, USA), photochemical chlorophyll fluorescence (ChlF) parameters (FluorCam, PSI, CZ) and contents of chlorophylls and total carotenoids (spectrophotometrically) in 6-cm^2 moss samples.

Results and Discussion: *Sphagnum* mosses showed a broad span of maximum rate of photosynthesis, which increased along the sun - shade environmental gradient. This variable was highly correlated with maximum quantum yield of photosynthesis (r = 0.92; both based on CO_2 exchange), indicating, together with the similar level of PPFD saturation (~ 2000 $\mu mol\ m^{-2}\ s^{-1}$), reduced amount of PSII reaction centres in the sun samples. These results are consistent with those on ChlF; the maximum quantum yield of photosynthesis correlates well with that of PSII (F_V/F_M, ranging between 0.46 and 0.75; r = 0.81), as well as with Φ_{PS2}. Pigment content parameters showed, if any, a weak correlation with gas exchange and ChlF parameters, implying no typical biochemical adaptations to the contrasting habitat's light status. Only the photosynthetically efficient samples were lower in total carotenoids indicating their inefficiency in preventing photoinhibition and photodamage in the full sun. However, this chronic state can also be ascribed to the ambient level of UV-B radiation. The natural light conditions in open mires are evidently harmful to the photosynthetic apparatus of dominant *Sphagnum* plants. This is so in spite of those conditions having been induced by the *Sphagnum* mosses' ability to create a competitively favourable environment just for themselves. *Sphagnum* mosses therefore do not stand in need of a fully efficient photosynthesis when growing in open mires.

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Visualization of titanium stress in spinach by kinetic imaging fluorometry

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Introduction: The biological effect of Titanium (Ti) on spinach was studied in hydroponic experiment on defined nutrient solutions, determining the influence of high Ti concentration in nutrient solution on Fe stressed and non-stressed plants.

Materials and methods: Besides classical methods of nutrient experiments evaluation (measurements of plant growth and chemical analysis) we measured the heterogeneity of fluorescence transients over a leaf in static and dynamic light environments. For this purpose, we used kinetic imaging fluorometry (FluorCam, Photon Systems Instruments, Ltd., www.psi.cz).
First, we measured quenching of chl fluorescence in low light (50 $\mu mol.m^{-2}.s^{-1}$) and high light (200 $\mu mol.m^{-2}.s^{-1}$). Secondly, the dynamic environment was induced by a harmonically modulated irradiance. It was shown earlier that such irradiance leaded to far-from-equilibrium photosynthesis that differed from the activity attained in the steady-state conditions [1].

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PHOTOSYNTHESIS AND STRESS

Biophysical and Biochemical Methods in Photosynthesis Research Central-European Conference.
Brno, Sept. 15–16, 2005. Book of Abstracts.

edited Miloš Barták

Published by Masaryk University in Brno, 2005

1st edition, 2005
Printed by the Publishers of Masaryk University, Brno - Kraví hora

55-961A-2005 02/58 6/Př

ISBN 80-210-3762-8