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P2–LIGHT STRESS IN PLANTS

Organised by Giles Johnson and Kate Maxwell for the Plant Environmental Physiology Group on behalf of the SEB and the British Ecological Society, with proceedings to be published by the Journal of Experimental Botany. This session has been supported by ADC Bioscientific Ltd, Hansatech Instruments Ltd, PP Systems and Skye Instruments Ltd

P2.1 Role of beta-carotene in protection of Photosystem II against light stress

A.Telfer (Imperial College)

The photosystem II (PSII) reaction centre contains a heterodimer of two related proteins called D1 and D2 which bind all the cofactors involved in primary and secondary electron transfer leading to water oxidation and plastoquinone reduction. They also bind two beta-carotene molecules, which, unlike carotenoids in other photosynthetic reaction centres and light harvesting systems, do not quench chlorophyll triplet states or act as efficient light harvesting pigments. They are, however, important structurally and do quench singlet oxygen offering some protection against light-induced oxidative damage. Additionally both beta-carotenes are redox active, and can re-reduce the highly oxidising chlorophyll known as P680, which under normal circumstances oxidises water. This 'side-reaction' seems to be important in the photoprotection of PSII against damaging oxidative reactions. It involves redox interactions with cytochrome b559 and chlorophyll(s) bound within the reaction centre. The beta-carotene radical cation, if not rapidly re-reduced by cytochrome b559 or Chlz, is very unstable and the possible destabilisation of the PSII reaction center, if it is irreversible bleached, will be discussed. The oxidation of the PSII reaction centre carotenes will be discussed in the light of recent X-ray structure models, which identify a number of carotenoid molecules in the PSII core complex.

P2.2 The products of the *psbE-F-L-J* operon control photosystem II assembly and unidirectional electron flow to the plastoquinone pool

J. Meurer, C. Dal Bosco (Ludwig-Maximilians-Universität), I. Ohad (The Hebrew University of Jerusalem) and R.G. Herrmann (Ludwig-Maximilians-Universität)

The highly conserved plastid *psbE-F-L-J* operon encodes low molecular weight, single transmembrane proteins of photosystem II (PSII). Inactivation of each of these genes in tobacco plants affects specifically the assembly and/or activity of PSII. Inactivation of PsbE and PsbF, the cytochrome *b559* α and β subunits, respectively, prevents assembly of PSII. The Δ*psbJ* and Δ*psbL* mutants are obligatory heterotrophes while harboring partially active and highly light sensitive PSII complexes. The Δ*psbJ* mutant exhibits low oxygen evolution. Forward electron flow from $Q_A^{\bullet -}$ to plastoquinone via $Q_B^{\bullet -}$ is hindered promoting back electron flow due to changes in the redox potentials of these electron carriers. In contrast, Δ*psbL* mutants readily reduce plastoquinone. However, the Q_B site affinity for quinones and DCMU is altered. Following reduction of the plastoquinone pool by a single 1s light-pulse, Q_A is re-reduced giving rise to an exceptional second maximal fluorescence emission in complete darkness. Lowering the temperature decreases reduction of Q_A by plastoquinol in the Δ*psbL* mutant whereas it increases the lifetime of $Q_A^{\bullet -}$ in the Δ*psbJ* mutant. Thus, both processes are energy dependent, compatible with electron flow uphill a potential gradient. We conclude that PsbL prevents reduction of PSII by back electron flow from plastoquinol protecting PSII from photoinactivation, whereas PsbJ regulates the redox potentials of Q_A and Q_B electron carriers and thus, reduction of plastoquinone. Therefore, both proteins contribute substantially to ensure unidirectional forward electron flow from PSII to the plastoquinone pool and thus, the efficiency of the

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light-induced charge separation in the photosynthetic process.

P2.3 Oxidative stress in photosystem II: influence of the redox potential of the quinone acceptor on the yield of singlet oxygen formation

A. Krieger-Liszkay and C. Fufezan (University of Freiburg)

Photo-generated reactive oxygen species in herbicide-treated photosystem II were investigated by spin-trapping, using 2,2,6,6-tetramethyl-piperidine as trap. While production of $^{\bullet}OH$ and $O_2^{-\bullet}$ were herbicide-independent, $^1O_2$ with a phenolic was twice that with an urea herbicide. This correlates with the reported influence of these herbicides on the redox properties of the semiquinone $Q_A^{-\bullet}$. Herbicide binding influences the redox potential (E_m) of the Q_A/Q_A^- redox couple. Phenolic herbicides lower the E_m by 45 mV, while DCMU raises it by 50 mV. Earlier work shows that phenolics increase photodamage, while DCMU protects against it. This is explained by the hypothesis that $^1O_2$ is produced by charge recombination reactions that are stimulated by herbicide-binding and modulated by the nature of the herbicide. When phenolic herbicides are bound, charge recombination at the level of $P_{680}^{+\bullet}Pheo^{-\bullet}$ is thermodynamically favoured forming ^3Chl and hence $^1O_2$. With urea herbicides this pathway is less favourable and a greater proportion of the $P_{680}Q_A^-$ radical pair decays by an alternative, less damaging, route. Furthermore we measured the temperature dependence of $P^+Q_A^-$ recombination kinetics by flash-induced absorption spectroscopy in the presence of DCMU and phenolic herbicides. Some of the phytotoxic properties of phenolics may be explained by the fact that they render PSII ultrasensitive to light due to high yield of $^1O_2$ formation.

P2.4 Light-induced turnover and sequential reassembly of photosystem II subunits

E-M. Aro, A. Rokka, M. Suorsa and N. Battchikova (University of Turku)

PSII centres are susceptible to photodamage at all light intensities. Damaged PSII centers, however, do not usually accumulate in the thylakoid membrane due to a rapid and efficient repair mechanism. The main target of photodamage is the reaction centre D1 protein, with less frequent damage also to the D2 and PsbH proteins. Repair of PSII via turnover of the damaged protein subunits is a complex process involving (i) highly regulated reversible phosphorylation of PSII subunits, (ii) monomerization and (iii) migration of PSII complexes from grana to stroma lamellae, (iv) partial disassembly of the PSII core monomer, (v) highly specific proteolysis of the damaged protein subunits and finally (vi) a multistep replacement of the damaged protein copies with de novo synthesized ones followed by (vii) reassembly and dimerization of the PSII complexes. During PSII repair on stroma thylakoids, the D1 protein becomes co-translationally assembled with a complex comprised of D2, Cyt b_{559} and most probably also PsbI. Subsequently CP47 becomes reassociated, followed by an assembly of PsbH together with at least two other chloroplast-encoded LMW subunits (PsbM and PsbT) and a nuclear-encoded PsbR. Mutant studies have further suggested that the PsbL and possibly also PsbJ subunits assemble to PSII at this same stage. Then CP43 associates with the PSII complex concomitantly with PsbK and the 33 kDa OEC protein. Such PSII core monomers undergo dimerization and are again found in the grana membrane region.

P2.5 Role of FtsH proteases in photoprotection and the removal of damaged protein from the thylakoid membrane of the cyanobacterium *Synechocystis* 6803

P.J. Nixon (Imperial College)

Photosystem II (PSII) is the multi-subunit pigment protein complex which catalyses water oxidation in higher plants, algae and cyanobacteria. When plants and cyanobacteria are exposed to excess light, their photosynthetic performance declines in a process termed photoinhibition. A key target for light-induced damage is the D1 subunit of PSII. PSII homeostasis is maintained through the operation of a D1 repair cycle to replace damaged D1 by a newly synthesised copy. Only when the rate of damage exceeds the rate of repair is net loss of PSII activity observed. As yet the protein components involved in the replacement of damaged D1 *in vivo* are ill-defined. Possible proteases involved in D1 degradation are the FtsH and DegP (or HtrA) families of protease. In *Synechocystis* sp. PCC 6803 there are four FtsH homologues, designated slr0228, slr1390, slr1604 and sll1463. All four members contain potential ATP-binding and Zn^{2+}-binding sites characteristic of this type of protease. Photoautotrophic growth of an insertion mutant lacking FtsH (slr0228) was found to be extremely sensitive to visible light stress. D1 degradation *in vivo* was determined to be much slower in this mutant than the WT, and no D1 fragments were detected. In contrast, D1 degradation was relatively unimpaired in a mutant lacking the three members of the DegP/HtrA family of proteases. Immunochemical detection of FtsH in His-tagged PSII preparations isolated from *Synechocystis* suggested that FtsH was capable of binding to PSII. Together these results are consistent with a model in which damaged

D1 is removed through a large FtsH complex, and degraded in a highly processive reaction. I will also present other data to support the hypothesis that FtsH (slr0228) has a general role in the removal of unassembled proteins in the thylakoid membrane

P2.6 Reaction center quenching and photoprotection of photosystem II

N. Huner and A. Ivanov (University of Western Ontario)

Abstract not supplied

P2.7 Control of the chlorophyll excitation density in the photosynthetic membrane

A.V. Ruban and P. Horton (University of Sheffield)

The structure and function of the light harvesting system of photosystem II (LHCII) shows dynamic properties that are adapted to the large fluctuations in the external environment and developmental state of the plant. Structural organization of LHCII *in vivo* could determine the efficiency of light harvesting by means of controlling the cross-section and chlorophyll excitation density, developed as a result of a long-term adaptation. Therefore the study of the integrated antenna provides a fundamental background for understanding flexible nature of light-harvesting. Adaptive tuning of light harvesting can also occur on the fast-time scale as a result of ΔpH formation and xanthophyll cycle activity in antenna. The process, called NPQ (qE) displays the kinetic features of an allosterically regulated co-operative process, involving a switch from energy dissipative to a non-dissipative state. This process is second order and is associated with absorption changes showing alterations in chlorophyll and carotenoid environment. *In vivo* spectroscopy has shown features of zeaxanthin 'activation' by altering its physico-chemical properties. Similar alterations have been found in *in vitro* experiments showing zeaxanthin binding to isolated PsbS protein. Such binding could make zeaxanthin an effective excitation quencher in antenna and PsbS a 'vector' delivering zeaxanthin into the NPQ site. Alternatively PsbS can be involved in sensing the proton gradient and modulating or triggering fast LHC antenna dynamics leading to NPQ. Studies *in vitro* reveal the extent of the dynamic capacity of each of the LHCII components. Regulation of the organization of the light harvesting system in different ways may be important not only in determination of NPQ capacity and dynamics, but also in the more sustained qI type of energy dissipation, which can play major photoprotective role in some higher plants and diatom algae.

P2.8 Molecular genetics of nonphotochemical quenching

K.K. Niyogi, X-P. Li, H-S. Jung and T. Golan (University of California, Berkeley)

A feedback de-excitation mechanism (qE) regulates photosynthetic light harvesting by thermal dissipation of excess absorbed light energy, which can be measured as a component of nonphotochemical quenching of chlorophyll fluorescence. qE depends on a low lumen pH, de-epoxidized xanthophylls, and a specific xanthophyll-binding photosystem II protein, PsbS. The characterization of *Arabidopsis thaliana* mutants and transgenic plants has helped to show the importance of these factors. The expression level of the PsbS protein was demonstrated to be a determinant of qE capacity, but PsbS-dependent enhancement of qE did not depend on the peripheral antenna proteins of PS II, suggesting that PsbS interacts functionally with the core antenna of PS II. To examine the hypothesis that species-dependent variation in qE capacity may be related to PsbS, we surveyed variation in qE capacity among different accessions of *A. thaliana*. However, in two selected strains with either high or low qE capacity, the variation was not due to differences in PsbS expression but was attributable to multiple genes. Mapping of quantitative trait loci (QTL) revealed two major QTL affecting qE capacity, neither of which mapped to the chromosomal position of any genes identified previously by forward genetics. The results of site-directed mutagenesis experiments indicated that PsbS binds protons (and zeaxanthin) in excess light, leading to de-excitation of singlet excited chlorophyll and harmless thermal dissipation of absorbed light energy.

P2.9 Physiological characterization of State transitions in *Chlamydomonas reinhardtii*

G. Finazzi (IBPC)

State transitions have been originally described as a short-term chromatic adaptation that allows plants and algae to respond to changes in the spectral quality of light, by varying the relative absorption cross-section of Photosystem II (PSII) and photosystem I (PSI). State transitions involve the reversible redistribution of the light-harvesting antenna (LHCII) between PSI and PSII. This occurs via the phosphorylation of LHCII by a kinase, which is activated when the PQ pool is reduced. The cytochrome b_6f complex is involved in the activation of the kinase, through the binding of plastoquinol to its Qo site. We have studied the physiological consequences of State transitions in *C. reinhardtii.* We have shown that a strict cause-effect relationship exists between the redistribution of the LHCII complexes and

the commutation between linear electron flow (involving both PSII and PSI) and of cyclic flow, which implies only PSI. The former can be observed in State 1, and the latter in State 2. We show that physiological conditions are intermediate between the two states, and consequently both linear and cyclic flow coexist in the same chloroplast. This suggests that cyclic flow may contribute to the synthesis of the 'extra' ATP required to allow CO_2 assimilation in this organism. Consequences of this phenomenon for both optimisation of photosynthetic CO_2 assimilation, and protection from light stress in *Chlamydomonas* will be discussed.

P2.10 State transitions – an example of acclimation to low-light stress

C.W. Mullineaux (University College London)

State 1–state 2 transitions ('state transitions') are a rapid physiological adaptation mechanism that adjusts the way absorbed light energy is distributed between Photosystem I and Photosystem II. They occur in both green plants and cyanobacteria, although the light-harvesting complexes involved are very different. I will discuss which aspects of the mechanism are conserved in green plants and cyanobacteria, and which may be different. We have shown that phycobilisome mobility is necessary for state transitions in cyanobacteria. We have identified a conserved cyanobacterial gene (*rpaC*) that plays a very specific role in state transitions. There is still debate about the physiological role of state transitions. Comparison of the growth properties of the *rpaC* deletion mutant with the wild-type gives us a way of directly addressing the question. We find that state transitions are physiologically important only at very low light intensities: they play no role in protection from photoinhibition. Thus state transitions are a way to maximise the efficiency of light-harvesting at low light intensities.

P2.11 The flexibility of the light reactions and the response of chloroplasts to changing environmental conditions

D.M. Kramer, A. Kanazawa, T. Avenson, T. Takizawa, O. Kiirants, J. Cruz and G.E. Edwards (Washington State University)

Plant photosynthesis performs the remarkable feat of converting light energy into the precise balance of usable chemical forms, a process that involves taming highly reactive intermediates, while preventing photodamage. This requires an efficient and robust apparatus that is flexible in its responses to dramatically changing environmental conditions. It also requires that output of the energy-storing light reactions be matched with the demands of metabolism. This presentation addresses the mechanisms by which this flexibility is achieved, and focuses on the dual role of the proton motive force (*pmf*) both in driving the synthesis of ATP via the thylakoid ATP synthase, and in initiating antenna down-regulation, via the q_E mechanism. It is argued that chloroplasts need two types of flexible mechanisms. Type I flexibility is responsible for modulating the output ratio of ATP:NADPH, to meet the biochemical demands of the plant. Cyclic electron flow around photosystem I (CEF1) and the water-water cycle are thought to be important Type I flexibility mechanisms, and operate by augmenting proton flux over that provided by linear electron flow (LEF). Type I mechanisms will also help initiate q_E, but with the (sometimes unwanted) side-effect of altering ATP:NADPH output ratio. On the other hand, Type II flexible mechanisms only changes the sensitivity of q_E, without altering the ATP:NADPH balance. We discuss evidence, from direct spectroscopic assays and mutant strains of *Arabidopsis*, for three specific Type II mechanisms, changes in the conductivity of the ATP synthase to protons (g_{H^+}), changes in the partitioning of the *pmf* as electric field ($\Delta\psi$) and ΔpH, and the composition of the antenna complexes. We conclude that both Types I and II flexibility mechanisms are essential for efficient photosynthesis.

P2.12 Regulating photosynthetic electron transport to avoid oxidatve stress

G.N. Johnson and A. Golding (University of Manchester)

Exposure of plants to environmental stress (extreme temperatures, drought, high light) is liable to expose them to the risk of oxidative damage. We have examined how regulation of the photosynthetic electron transport chain (ETC) might protect plants from such damage. When plants are exposed to drought or high light, the conductance of the ETC is lowered, lowering the rate at which electrons arrive at the photosystem I (PSI) reaction centre. We have observed that this effect can be mimicked in vivo by incubation of thylakoid membranes with the thiol reducing agent dithiothreitol (DTT), which mimics the effects of thioredoxin. The mid point potential of the component reduced is close to that of ferredoxin and NADPH, making this suitable as a regulatory pathway controlling the redox poise of PSI acceptors. Under conditions of low CO_2 or drought, where linear electron transport is down regulated, we observe an increase in the rate of cyclic electron transport. This increase is brought about by the activation of a pool of PSI centres which cannot be measured as turning over under optimal conditions. These centres represent approx. 30% of the total PSI in the leaf, a value corresponding to estimates of the proportion of PSI centres located in the stromal lamellae. The proportion of centres active shows a linear correlation with the extent of non photochemical quenching, supporting the notion that cyclic ET is activated in order to provide a pH gradient to drive this quenching.

P2.13 A role for leaf water status in tissue-specific perception of photo-oxidatuve stress and associated signalling in *Arabidopsis* leaves

N.R. Baker (University of Essex)

In *Arabidopsis* leaves high light stress induces rapid expression of a gene encoding a cytosolic ascorbate peroxidase (*APX2*), whose expression is restricted to bundle sheath cells of the vascular tissue. Imaging of chlorophyll fluorescence and the production of reactive oxygen species (ROS) indicated that *APX2* expression followed a localised increase in hydrogen peroxide resulting from photosynthetic electron transport in the bundle sheath cells. Leaf transpiration rate also increased prior to *APX2* expression suggesting that water status may also be involved in the signalling pathway. Abscisic acid (ABA) stimulated *APX2* expression. Exposure of ABA insensitive mutants (*abi1-1*, *abi2-1*) to excess light resulted in reduced levels of *APX2* expression and confirmed a role for ABA in the signalling pathway. ABA appears to act in concert with hydrogen peroxide to initiate *APX2* expression. This regulation of *APX2* may reflect a functional organisation of the leaf to resolve two conflicting physiological requirements of protecting the sites of primary photosynthesis from ROS and at the same time, stimulating ROS accumulation to signal responses to changes in the light environment.

P2.14 Roles for ascorbate in the regulation of photosynthesis

C.H. Foyer (Rothamsted Research)

Ascorbate, glutathione and tocopherol are three major low molecular weight antioxidants of plant cells. While tocopherol is hydrophobic and is found only in the lipid membranes, ascorbate and glutathione are hydrophilic accumulating to high concentrations in the chloroplast stroma and other compartments of the plant cell. It is generally accepted that there is a high degree of redox coupling between these antioxidants that has implications for regulation, function and signalling associated with photosynthesis and respiration. In terms of photosynthesis, tocopherol has an essential protective function counter-acting the harmful effects of singlet oxygen production at photosystem II (PSII). While it has long been recognised that ascorbate reduces and thus regenerates oxidised tocopherol, the effectiveness of tocopherol in protecting PSII and its interactions with ascorbate have only recently been demonstrated. Moreover, while reduced glutathione will always reduce oxidised ascorbate (dehydroascorbate), the degree of coupling between the ascorbate and glutathione redox couples varies in different cellular compartments. Antioxidants are crucial to the defence and function of both chloroplasts and mitochondria which use oxygen in essential energy exchange reactions. However, these metabolites not only limit the extent of accumulation of reactive forms of oxygen and hence prevent such phenomena as photo-oxidative damage and oxidative stress but they can act independently as signal-transducing molecules conveying information on oxidative load and redox-buffering capacity. For example, the regulation of a number of key genes associated with photosynthesis is modified in response to ascorbate abundance.

P2.15 Regulation of antioxidant defence gene expression in light-stressed Arabidopsis: mutants reveal a central role for glutathione

P.M. Mullineaux, L. Ball, G. Creissen, H. Reynolds (John Innes Centre), M. Fryer (University of Essex), N. Leyland (John Innes Centre), N.R. Baker (University of Essex) and S. Karpinski (Stockholm University)

A phenotypically normal *Arabidopsis* mutant was isolated that expresses the normally excess light-inducible *ASCORBATE PEROXIDASE 2* (*APX2*) under non-stress conditions. The mutated locus, *regulator of ascorbate peroxidase 2-1* (*rax1-1*) is a lesion in *GSH1*, which encodes the enzyme catalysing first step in glutathione biosynthesis, γ-glutamylcysteine synthetase (γ-ECS). This mutant has ca. 50% diminished level of foliar glutathione. Along with earlier more indirect evidence, firmly establishes glutathione as an important regulator of *APX2* expression under excess light stress conditions. From a study of *rax1-1* and other mutants, we conclude that glutathione metabolism, and no other antioxidant or ROS, control the configuration or poising of key abiotic and biotic stress defence genes under steady state conditions. When environmental conditions change (such as an increase in light intensity) and the plant is undergoing a transition from one steady state to another, it is the influence of total foliar redox state that is important. This affects the potency of a ROS-derived signal. The way in which glutathione metabolism might achieve this modulation of defence gene expression and its role in chloroplast-to-nucleus signalling will be discussed.

P2.16 What is acclimation for? On the significance of changes in chloroplast composition

R.G. Walters (University of Oxford), S. Bailey (University of Warwick), P. Horton (University of Sheffield)

It has long been recognised that plants vary the composition and organisation of the photosynthetic apparatus in response to the prevailing environmental conditions, with particular attention being paid to the responses to incident light. Under high light conditions there are increases in the levels of photosystems, electron transport and ATP synthase complexes, and enzymes of the Calvin-Benson cycle; conversely, under low light there is an increase in the relative levels of light-harvesting complexes (LHC) and in the stacking of thylakoid membranes to form grana. It has long been presumed that these changes are of adaptive significance, and in a few cases evidence been provided that this is indeed the case – an increase in photosynthetic capacity reduces susceptibility to photodamage, while changes in photosystem stoichiometry serve to optimise light utilisation. In contrast, it is much less clear what might be the benefit to the plant of other changes in chloroplast composition, such as in the levels of LHC. Thus, antisense plants lacking the major LHC complexes show little change in photosynthetic characteristics, apparently refuting the idea that they serve to increase light absorption cross-section. However, it is clear that when attempting to assess the extent to which a plant is acclimated to its environment, several conceptual and methodological pitfalls await the unwary investigator. Our recent studies relating chloroplast composition to photosynthetic function will be discussed, highlighting some of these issues, and our current understanding of the functional significance of acclimation will be reviewed.

P2.17 Acclimation of photosynthesis to high irradiance in rice: gene expression and interactions with leaf development

E.H. Murchie, S. Hubbart and P. Horton (University of Sheffield)

Rice in tropical agricultural systems can be exposed to a wide range of light levels due to season and climate. The high levels of light seen in the dry season in the Philippines can cause photoinhibition even in well-watered plants. We have used rice as a model plant to study the long-term response of photosynthesis to high irradiance focussing on the composition of the photosynthetic apparatus and leaf morphology. Typical sun / shade differences in chloroplast composition are seen following growth in high compared to low irradiance: higher rates of photosynthesis, higher amounts of Rubisco, cytochrome b/f complex per unit leaf area, but lower levels of light harvesting complex proteins (LHCII) and total chlorophyll. However responses appear more complex when leaf developmental stage is considered. Using a system of transferring plants from low to high light in the laboratory we have studied responses that occur (1) pre-full leaf expansion and (2) post leaf expansion:
(1) We test the hypothesis that the establishment of a high light leaf anatomy in rice is dependent upon the division and expansion of leaf cells within the leaf sheath. (2) Acclimation of photosynthesis is limited by leaf *age*: transfer to high light post-expansion is characterised by alterations in LHCII but not Rubisco protein. We used microarray technology to analyse how the expression of different functional groups of genes, particularly those involved in photosynthesis, stress-responses and cell signalling, change when plants are transferred to high light at specific stages of leaf development.

P2.18 Light stress and the circadian clock

A.N. Dodd, C.T. Hotta (University of Cambridge), J. Love (University of Exeter) and A.A.R. Webb (University of Cambridge)

Plants are subjected daily cycles of transition from dark to light. The light/dark transition can be damaging to the plant and result in stress if mechanisms are not in place to deal with excess light energy. The plant circadian clock allows the plant to predict periods of the day when the plant is likely to experience light stress. The circadian network includes input pathways that entrain the clock to the external light/dark cycle, a molecular oscillator comprising negative feedback loop(s) of gene expression, and output pathways that communicate the estimate of time produced by the oscillator to clock-controlled components. We are using a combination of Ca^{2+}-based imaging, pharmacological studies, mutant analysis and whole-plant physiology to investigate the signal transduction pathway regulates cellular physiology. These studies have demonstrated that circadian regulation of physiology increases photosynthetic efficiency, growth and survival of plants. We will describe how circadian regulation of Ca^{2+} signalling contributes to protection of the plant from daily cycles of light stress, and increases photosynthesis.

P2.19 Cold-induced photoinhibition and the regeneration niche in temperate evergreen species

M.C. Ball, C. Holly and J.J.G. Egerton (Australian National University)

Temperate evergreens are subject to seasonal variation in temperature stress. The objective was to determine how seasonal variation in temperature stress affects light use in relation to carbon gain and growth. Snow gum seedlings (Eucalyptus pauciflora) were planted on north, south, east and west sides of vertical screens made from 50% shadecloth. Maintenance of a constant sky view factor caused all seedlings to experience similar minimum temperatures at night, although timing and interception of direct sunlight during the day differed between treatments. Exposure to freezing temperatures induced chronic suppression of Fv/Fm consistent with the appearance of the CHB feature in the chlorophyll a fluorescence emission spectra. During winter, south-facing seedlings maintained the highest Fv/Fm and suffered the least loss in canopy area. Upon transfer of all seedlings to common, fully exposed conditions in spring, seedlings that had over-wintered on south sides of screens showed the most rapid recovery of photosynthetic activity and sustained 50% higher growth rates during subsequent seasons than seedlings from other treatments. In contrast, growth of seedlings that remained in the treatments was greatest on south and west sides of screens. Warmer afternoon temperatures apparently enabled west facing seedlings to take advantage of high irradiance in carbon gain while shade in morning protected against adverse effects of high irradiance when low temperatures limited photosynthesis. These results show that variation in microclimate following forest fragmentation could account for enhanced establishment and growth of seedlings on south and west sides of parent trees.

P2.20 Winter Damage in upland *Calluna vulgaris*: The possible contribution of light stress

S.J.M. Caporn, J.A. Carroll, M. Pilkington, J. Edmondson, Y.Lei, L. Cawley (Manchester Metropolitan University)

The phenomenon of late 'winter browning' in upland *Calluna vulgaris* is well documented (Watson 1966), typically occurring during periods of cold dry an bright weather, and leading to progressive damage to the exposed shoot tips of the plants. This condition is generally considered to be due to a combination of frost damage, dessication injury and wind exposure. The exact mechanisms involved however, remain unclear, and there is a clear possibility that high light stress may also have a role to play in the development of this condition. Long-term field manipulation studies on upland *Calluna vulgaris* stands in North Wales have shown that winter damage is exacerbated in plants receiving increased inputs of nitrogen and phosphorus, indicative of a shift in phenology or physiological processes that could provide some insight into mechanisms involved in the winter damage process. The work presented here seeks to analyse the different components of winter damage in *Calluna* in greater detail, using chlorophyll fluorescence techniques to examine the possible impact on photosynthetic mechanisms, and to follow the interaction with phosphorus and/or nitrogen treatment.

Carroll, J.A., Caporn, S.J.M., Cawley, L, Read D.J., Lee, J.A. 1999. The effect of increased deposition of atmospheric nitrogen on *Calluna vulgaris* in upland Britain. New Phytologist 141: 423 – 431.
Watson, A., Miller, G.R., Green, F.H.W., 1966. Winter browning of heather (*Calluna vulgaris*) and other moorland plants. Transactions of the Botanical Society of Edinburgh 40: 195 – 203.

P2.21 Leaf movements and photoinhibition in relation to water stress in the field-grown beans

C. Pastenes (Universidad de Chile)

Photoinhibition in plants depends on the extent of light energy being absorbed in excess of what can be used in photochemistry and is expected to increase as environmental constraints limit CO_2 assimilation. Water stress induces the closure of stomata limiting the carbon availability at the carboxilation sites in chloroplasts and, therefore, results in an excessive excitation of the photosynthetic apparatus, particularly photosystem II (PSII). Mechanisms have evolved in plants in order to protect against photoinhibition such as non-photochemical energy dissipation; chlorophyll concentration changes in order to reduce the extent of the absorbed light; chloroplast movements, reducing the organelle and photosynthetic complexes exposure to light; increases in the capacity for scavenging the active oxygen species; and leaf movement or paraheliotropism, avoiding direct exposure to sun, thereby avoiding light and heat. In beans (*Phaseolus vulgaris* L.), paraheliotropism seems to be an important feature of the plant to avoid photoinhibition. The extent of the leaf movement is increased as the water potential drops, reducing the light interception and, therefore, maintaining a high proportion of reduced PSII centres. Photoinhibition in water stressed beans, measured as the capacity to recover Fv/Fm, is even lower than in well watered plants and leaf temperature is maintained below the ambient, despite the closure of stomata. Data is presented suggesting that even

though protective under water stress, paraheliotropism, by reducing light interception, affects the capacity to maintain high CO_2 assimilation rates along the day in well watered plants.
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P2.22 Role of photorespiration in photosynthesis response to elevated temperature under high light conditions

O. Stasik (National Academy of Sciences of Ukraine) and H.G. Jones (University of Dundee)

It is widely accepted that photorespiration acts as a protective mechanism that dissipates of energy excess under stress conditions that limit CO_2 access to carboxylation sites. An aim of this work was a study of the role of photorespiration in the response of photosynthesis to elevated temperature under bright light and high air humidity that prevents reduction in stomatal conductance (Gs) and substomatal CO_2 concentration (Ci). Plants of two wheat varieties known as drought-tolerant and two known as drought-sensitive were grown in a well-conditioned greenhouse at day/night temperature – 24/19 °C and natural + artificial light (high pressure sodium) varying from 200 (morning and evening) to 1000 (sunny midday) μmol m^{-2} s^{-1} of PAR at the leaf level. A recently fully expanded 4^{th} or 5^{th} leaf was exposed to PPFD of 1500 μmol m^{-2} s^{-1} at 25 °C and 350 ppm CO_2 in air. Increasing the leaf temperature to 38 °C at 21% O_2 in air for 10 min resulted in a sharp decrease in CO_2 assimilation rate (A) for sensitive varieties but only a slight decrease for tolerant varieties. After 1 h of high temperature treatment, the assimilation activity was inhibited by 60–66% in sensitive varieties and 27–37% in tolerant ones. These differences were not related to changes in Gs and Ci that both were higher in sensitive varieties. In contrast, chloroplast metabolism parameters, initial slope of A/C_i curve (attributed to Rubisco activity), maximum A at saturated CO_2 concentration (reflecting RuBP regeneration activity in Calvin cycle), initial slope of light response curve (quantum efficiency of CO_2 assimilation), quantum efficiency of photosystem 2 ((Fm′-Fs)/Fm′), were reduced in sensitive varieties in much greater extent than tolerant ones. Treatment of leaves at 38 °C under non-photorespiratory conditions (2% O_2 in air) increased the inhibitory effect on CO_2 assimilation for tolerant varieties and diminished it for sensitive ones. This large decline in assimilation activity for tolerant varieties corresponded with a reduction in all the above-mentioned metabolic parameters but not in stomatal conductance or Ci. The results suggest that the role of photorespiration in the photosynthetic response to abiotic stress may be more complex than as an energy dissipator under photoinhibitory conditions and strongly depends on interactions with other protective mechanisms. High photorespiratory activity is essential constituent to realize the tolerance ability for wheat varieties adapted to growth in arid environment.

P2.23 Light stress in host-parasitic angiosperm associations

W.E. Seel (University of Aberdeen), J-M. Geniez (Université Montpellier) and D.D. Cameron (University of Aberdeen)

Parasitic plants steal resources from host plants, reducing the capacity of the latter to grow and reproduce. In addition to direct withdrawal of resources from the host the parasite may also induce changes in host plant photochemistry. This has previously been demonstrated in the *Striga*/maize association, where the parasite (*Striga*) generally has a very large effect on the host. We have studied the interaction between *Rhinanthus minor* and several of its many different host species. Here the impact of the parasite is less dramatic than that of *Striga*. We show, however, that parasite-induced reductions in both cholorophyll content and photochemical efficiency can occur in infected hosts. Photochemistry of apparently tolerant hosts (defined by no significant biomass surpression) show reduced photosynthetic efficiency at low levels of actinic light, while the main effect on susceptible hosts occurs at high levels of actinic light. We also examined the impact of the host on parasite photochemistry, and found that ‘good’ host species promote photochemical efficiency in the parasite, and that ‘poor’ hosts significantly reduce the photochemical efficiency of the parasite.

P2.24 Interactions between water deficits and sun-shade acclimation in Sitka spruce seedlings

K. Black, P. Davis, P. Doherty and B. Osborne (University Collage Dublin)

Gas exchange and chlorophyll fluorescence techniques were used to evaluate the acclimatory capacity of Sitka spruce seedlings to water deficits under sun and shaded conditions. The onset of water deficits resulted in a decreased stomatal conductance and net photosynthesis in plants exposed to full sunlight and 50% shade. However, carbon gain limitation occurred earlier in sun plants than shade plants due to a more rapid decline in stomatal conductance associated with lower leaf water potentials and higher leaf to air vapour pressure deficits (D). In sun grown seedlings exposed to water deficits there was also a decrease in Fv/Fm and ΦPSII after prolonged stomatal closure. Although stomatal conductance recovered in these seedlings, following re-wetting, net photosynthesis was still inhibited and there was a high degree of non-photosynthetic energy dissipation (NPQ) and a reduction in both Fv/Fm and ΦPSII. In contrast, shade seedlings exposed to water deficits showed a transient decrease in stomatal conductance and full recovery of photosynthesis and ΦPSII following re-wetting. Analyses of fluorescence quenching relaxation kinetics suggested that NPQ in sun plants exposed to water deficits was primarily associated with slowly relaxing quenching and photoinhibitory processes. When shade seedlings were exposed to water deficits, NPQ was associated with rapidly relaxing quenching and photoprotective processes. These results suggest that lower light levels and a reduced D, under shaded conditions, 'protected' photochemical pathways against inactivation while exposure to full sunlight resulted in photodamage following stomatal closure.

P2.25 The interaction of water deficits and exposure on plants growing on exposed limestone pavement

P. Moran, R. Turner and B. Osborne (University College Dublin)

In situ measurements made in the Burren, an area of open Karst limestone, located in Co. Clare, Ireland, indicated that the substrate volumetric water content (VWC) was comparable to semi-arid habitats, despite the prevailing oceanic conditions. This is due to minimal storage of water associated with the limestone aquifer. Following heavy rainfall events, which occur regularly every 6–9 days during the summer, there were transient increases in VWC, followed by a rapid decline (with rates of up to 0.5% per hour). . Chlorophyll fluorescence measurements carried out on *Teucrium scorodonia*, one of the most commonly occurring plants on the open pavement, before and after one of these events (23mm of rain in 3 days) indicated that increases in VWC were associated with a 20% increase in ΦPSII/ decrease in NPQ. Laboratory experiments yielded similar results. In addition, maximum photosynthetic rates showed a similar response to VWC, but of greater magnitude (a 10 fold increase in A_{max} following a simulated rainfall event). Under typical field conditions (VWC of approximately 15% and PAR values ranging between 200 and 2000 μmol $m^{-2}s^{-1}$ on the day measurements were made), photosynthesis had a relatively low light saturation point (between 200 and 300 μmol $m^{-2}s^{-1}$). These results suggest that limited water availability, rather than exposure, may have an important role to play in governing the species composition of the Burren limestone pavement.

P2.26 Xanthophyll cycles in photoacclimation and photoprotection: deep shade leaves of a tropical tree legume (Inga spp.) deploy the slowly reversible accessory lutein epoxide cycle in photoacclimation and the rapidly reversible violaxanthin cycle in photoprotection

S. Matsubara (Institute Phytosphere), M. Naumann (Technical University Darmstadt), R. Martin (Carnegie Institution), C. Nichol (University of Edinburgh), U. Rascher (Carnegie Institution), T. Morosinotto, Roberto Bassi (Universita di Verona), and B. Osmond (Biosphere 2 Center & Australian National University)

Plant leaves exposed to light intensities from darkness to full sunlight tune photosynthetic activity over four orders of magnitude in photon flux. The photosynthetic apparatus maintains high efficiencies of light use in shade, while engaging reversible mechanisms for tolerating excess light (photoprotection), for repair of damage (photoinactivation) that inevitably accompanies this most energetic of biological processes, and for photoacclimation in response to sustained changes in light environment. The well-known b-xanthophyll cycle (violaxanthin + antheraxanthin + zeaxanthin) has a key role in photoprotection and photoacclimation. Surprisingly, deeply shaded leaves of a tropical tree legume (Inga spp.) display a catena of responses, achieving photoprotection within minutes without detectable xanthophyll interconversion in pigment protein complexes of light harvesting antennae, and photoacclimation within hours by irreversibly converting extraordinarily high levels of a-xanthophyll lutein epoxide to lutein, while simultaneously engaging the b-xanthophyll cycle. Our data focus attention on the still enigmatic role of lutein in these processes.

P2.27 Xanthophyll cycle dynamics in a tropical dry forest canopy

C. Nichol (University of Edinburgh & Jena), O. Veenedaal, B. Mantlana, O. Shibistova and J. Lloyd (Jena)

The seasonal and diurnal dynamics of the xanthophyll cycle, photosynthesis and the Photochemical Reflectance Index (PRI) were investigated in an African semi-arid woodland. A clear reduction in ecosystem CO_2 flux occurred between March (the wet season) and June (the dry season), but this was not associated with any significant difference in the de-epoxidation state (DEPS) of the xanthophyll cycle between the seasons. Although diurnal changes in DEPS and xanthophyll pigments occurred, significant correlations existed only with PRI and canopy light use efficiency (Fc) and DEPS but not with the ecosystem CO_2 flux (NE). The Normalised Difference Vegetation Index (NDVI) was also correlated with Fc and DEPS but not NE in both the wet seasons and dry seasons. Although PRI also showed a strong significant correlation with NDVI, neither index was correlated with total carotenoids. Contrary to expectation neither the total pool size of carotenoids or DEPS appear to change significantly between the dry wet and dry season June. This was despite marked reductions in canopy NE.

P2.28 Congeneric comparisons of the architecture of New Zealand shrubs with contrasting growth habits in relation to light harvesting

R. Christian, D. Kelly and M.H. Turnbull (University of Canterbury)

A role for the divaricating shoot habit in optimising light harvesting for photosynthesis was investigated in *Aristotelia fruticosa*, *Coprosma propinqua*, and *Corokia cotoneaster*. Comparisons were made with congeners lacking the habit. Shrubs were grown in full sun or behind vertical screens of shadecloth transmitting c. 25, 52, or 73% sunlight. Phylogeny influenced the expression of the divaricate habit but few architectural traits were significantly affected by shading. Leaf numbers per unit stem length increased and decreased with canopy depth in divaricates and nondivaricates, respectively. Overall, divaricates had lesser leaf size, stem diameters, leaf area per unit crown area (leaf area index, LAI), heights relative to crown diameters, lengths of the primary stem relative to the remaining stems, stem biomass per unit stem length, and leaf area per unit shoot biomass, but greater numbers of apices per unit crown volume than their nondivaricate congeners. Traits were consistent with weak apical control in divaricates and the hypothesis that the habit enhances penumbral effects to create a more even within-canopy light environment. The low leaf area per unit shoot biomass in divaricates limits the value of simple measures and models of light penetration based on traits such as LAI, and challenges the hypothesis that the habit provided a selective advantage through enhanced net canopy photosynthesis.

P2.29 Photoinhibition and resistance to winter damage in stay-green amenity grasses

A.P. Gay, E.L. Smith, A. Kingston-Smith, D. Thorogood, M. Roca, H. Ougham, H. Thomas, C. James, T. Davies and L. Moss (IGER, Aberystwyth)

Good visual appearance is important for grasses for sports and amenity purposes. Recently the incorporation of stay-green genes (which prevent yellowing during leaf senescence) into *Lolium perenne* has improved the appearance of amenity varieties. However, in winter 2002-3, severe scorch occurred on some stay-green *Lolium perenne* lines in the field. The damage occurred during cold, bright weather which followed a dull, relatively warm period. A possible cause of the damage was photoinhibition and subsequent photo-oxidation, which may be more prevalent in those stay-green lines which retain more chlorophyll. Thus, pure-breeding and segregating stay-green populations with a range of tolerances to field scorch were studied. They were exposed to mild, dull conditions which were then changed to brighter, colder conditions in a growth room. During the experiment the maximum quantum yield of PSII (Fv/Fm) and leaf damage were recorded. Leaf samples were taken for measurement of chlorophyll and its catabolites and oxidative damage to proteins. Susceptibility to damage in the field and mean Fv/Fm of populations in the growth room were correlated. There was direct evidence of the presence of oxidised chlorophyll compounds but oxidative damage to proteins was less obvious. It was concluded that photo-oxidation was the likely cause of the damage observed in the field. The data will be discussed in terms of our ability to improve resistance to winter scorch in stay-green amenity grasses.

P2.30 Interactions between sunflecks and global change: the influence of elevated CO_2, light environment and temperature on the responses of dipterocarp seedlings to sunflecks in tropical rain forests

M.C. Press, J.D. Scholes and A.D.B. Leakey (University of Sheffield)

Abstract not supplied

P2.31 Photoinhibition and water stress in Mediterranean woody seedlings: estimating effects in sun and shade phenotypes

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Mediterranean plants are exposed to high light and water stress, a particularly stressful combination for seedlings that cannot access the water table during the summer. Despite the increasing amount of ecophysiological information on stress responses of Mediterranean plants, only scant data is available for the interacting effects of high light and drought and even less for the differential responses of sun and shade phenotypes. Chlorophyll fluorescence and gas exchange were measured in two-year old seedlings of four Mediterranean trees (*Quercus ilex, Q. coccifera, Pistacia lentiscus* and *P. terebinthus*) during a drought simulation at the end of the summer where watering was restrained and seedlings were drying out slowly under 50–60% sunlight. Half of the seedlings were grown in full sunlight and the other half in the shade (6% sunlight). Half of each combination species-phenotype was exposed to high light during the six central hours of the day, every other day, using halogen lamps in addition to ambient light (leaf level PPFD 2100–2350 μmol $m^{-2}s^{-1}$). The two oak species tolerated both high light and water stress significantly better than the other two species, surviving longer, in drier soils, and exhibiting a less pronounced photoinhibition. Shade plants exhibited a larger photochemical efficiency decrease than sun plants in response not only to high light but also to water stress. However, shade plants survived for significantly longer periods of time, probably due to their lower transpiration rate, indicating that survival under high light and water stress is not necessarily linked to leaf-level physiological performance.

P2.32 Photosynthetic performance at high light: has its ecological significance been exaggerated?

B. Osborne, P. Moran, S. Ryan, K. Black and G. Lanigan[1] (University College Dublin)

Consistently over recent years differences in the ecology of species and populations have been largely based on photosynthesis or related characteristics. This approach has been driven more recently by improvements in technology and by a need to provide mechanistic predictors for ecosystem carbon flux determinations and global change investigations. Most of these studies have, however, been correlative and while they are providing increasing information on photosynthetic mechanisms *per se*, they do not necessarily identify characteristics that determine success in a given environment. Based on literature evidence photosynthesis at high light in either crop or wild plants is poorly selected for. Our measurements on *Mycelis muralis* and *Teucrium scorodonia* indicate that populations of these species can exist over markedly contrasting environments without any major differences in photosynthesis or photoregulatory processes. Plants can grow successfully at high light despite being unable to utilise a major portion of the available radiation and, in some cases, successful populations actually show lower photosynthetic rates under ecologically significant conditions in the laboratory than unsuccessful populations. This indicates a remarkable plasticity in the ability of the photosynthetic apparatus to adjust to widely different environments. There may, however, be genetic differences between the populations that are associated with variations in exposure, but the physiological basis of these difference are not known. It is our contention, therefore, that more ecologically relevant studies are required on populations and transplants, utilising the benefits of modern technology, before we can ascribe differences in success to photosynthesis or related processes.

P2.33 Photoactivation of photosystem II: 23 kDa protein as metallo-chaperone

N. Bondareva and A. Krieger-Liszkay (University of Freiburg)

Photosystem II (PSII) is synthesised in vivo in an inactiveform lacking the Mn Cluster. The Mn cluster is assembled in a light-dependent process, so called photoactivation. Active PSII underlies a permanent dis- and reassemble process and the synthesis of a new Mn cluster is essential. Before the degradation of the D1 protein takes place, the extrinisc proteins of PSII (33 kDa, 23kDa and 17kDa proteins) and the Mn ions are released into the lumen. It is an open question where Mn is located during PS II degradation and how Mn is delivered to the new assembled photosystem. According to our hypothesis, one of the extrinsic proteins, the so-called 23 kDa protein, located on the lumen side of PSII, is able to bind Mn and delivers it to PSII. When the extrinsic proteins are removed together with the Mn from PSII, no free $Mn2+$ signal is detected in X-band EPR measurement. EPR and intrinsic fluorescence measurements of isolated extrinsic proteins provided the information that Mn is attached to the 23kDa protein. High Field EPR spectra of the isolated 23kDa protein from spinach and also of the recombinant protein showed that Mn is indeed bound to this protein. Photoactivation of Mn-depleted PSII with a Mn-reconstituted mixture of the 23 and 17 kDa proteins was successful. A Mn/PSII ratio of 4:1 was sufficient, while, in the absence of these proteins a ratio of 100 Mn per PSII was required.

P2.34 Leaf expansion and development in response to light intensity. Comparison of different genotypes of *Arabidopsis thaliana*

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The amount of absorbed radiation has a large influence on development, expansion and architecture of plants. We propose a model to analyse the phenotypic response to light intensity for different genotypes of *Arabidopsis thaliana*, studying wild ecotypes and mutants. 3D virtual plants were built for eight genotypes and various light treatments between 2.5 and 17.5 mol m^{-2} d^{-1}. Columbia and its mutants *serrate* and *rotundifolia* are presented as a sample of the studied genotypes. The 3D representations allowed accurate estimations of the amount of absorbed radiation by the plants, which is not physically measurable. A reduction of light intensity affected leaf area, delayed leaf initiation lengthened the duration of leaf expansion, and decreased maximum relative expansion rate. For all the ecotypes, robust positive relationships related to the amount of absorbed radiation were obtained for (i) leaf initiation rate and (ii) relative expansion rate. The duration of expansion was negatively related to the amount of incident radiation. Responses to the amount of incident or absorbed radiation were significantly different among the studied ecotypes. When comparing ecotypes and their corresponding mutants, specific changes of the plant physiology have been found. For instance, the *serrate* mutation disrupted the sensitivity of leaf initiation rate to absorbed radiation, and the *rotundifolia* mutation increased it. In contrast the response of relative expansion rate was similar for all the genotypes. The model presented here allows us to further the knowledge of the genotype-environment interaction and the phenotypic variability in response to light intensity.

P2.35 Photodamage of electron transport chain under heat stress

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High temperature is considered to be one of the most important environmental factors influencing on the photosynthetic reactions in plants. The molecular mechanism of thermoinduced damage of electron transport chain is unknown. On the basis of similarity of stress reaction of photosynthetic apparatus under high temperature and high light it is probable that the mechanism of thermoinhibition is suitable to the photodamage. The seedlings of different ages are characterized by various rates of the photosynthetic reactions, therefore it is possible to suppose, that they could possess different stability to the stress impact. In this connection the effects of heat shock (40°C, 3 h) on the photosynthetic activity of 4-, 7- and 11-day-old barley seedlings were studied. The rate of CO_2 gas exchange in young leaves was not changed under heat shock while in 11-day-old seedlings the high temperature affected both quantum yield and maximum rate of CO_2 fixation. By means of PAM fluorometry was show that the inhibition of the photosynthetic electron transport was caused by the impairment of photosystem II activity and the increase of plastoquinone pool reduction. The cause of the increase of plastoquinone pool reduction was the diminution of oxidizing ability of cytochrome b_6/f complex and the decrease of plastoqiunone pool size. The preheating of 4-day-old leaves at 40°C led to a marked decrease of the nonphotochemical quenching (NPQ) of chlorophyll fluorescence. This was related mainly to the lowering of ΔpH-dependent quenching mechanisms. Thus the high temperature inhibited the photosynthetic reactions in young leaves by the decrease of both electron transport and the proton gradient on the thylakoid membranes. In 11-day-old seedlings NPQ and energy-dependent quenching of chlorophyll fluorescence was increased by high temperature. The suppression of photosynthesis by heat shock in old leaves was caused first of all by the reduction of CO_2 uptake due to closing of high amount of stomata and the decrease of Rubisco activity. The following events were the impairment of ATP and NADPH consumption by the Calvin cycle and the increase of the proton gradient on the thylakoid membranes. As a result the level of plastoquinone pool reduction was also raised up. The mechanism of damage of electron transport chain under increased ΔpH can be similar to photodamage that induced the degradation of pigment-protein complex of PS II and D1 and D2 protein. Really, the breaking of D1 and D2 protein was shown in 11-day-old leaves under high temperature. Thus the heating of young leaves inhibited the photosynthesis by reduction of the electron transport rate while in old leaves the enzymes of Calvin cycle was mainly limited. The thermoinhibition of electron transport in old leaves was caused by structural damage of PS II and excessive plastoquinone pool reduction similar to photodamage.

P2.36 Does systemic signalling protect against excess excitation energy in *phaseolus vulgaris* l. and *zea mays* l?

N. D'Ambrosio, L. Vitale, A. Virzo De Santo (Università di Napoli)

In the past years numerous light-sensing mechanisms have been identified in plants but only recently some signalling pathways have been linked to excess excitation energy. The aim of this work was to establish whether the systemic acquired acclimation observed in *Arabidopsis thaliana* is a general phenomenon in plants allowing low light-exposed leaves to develop photoprotective responses induced from distal leaves. Plants of *Phaseolus vulgaris* and *Zea mays* were exposed to 1800 μmol photons m^{-2} s^{-1} for 1, 5–3 hours ('primary leaves') keeping during this period some parts of plants to 200 μmol photons m^{-2} s^{-1} ('secondary leaves'). Later secondary leaves were exposed to the same high-light treatment of primary leaves. Measurements of gas exchange and chlorophyll fluorescence were performed in primary and secondary leaves before (starting condition) and after exposing to high light intensity to evaluate the responses of photosynthetic apparatus to light stress. The obtained results show that A_N, Φ_{PSII}, q_P and F_v/F_m decreases significantly ($P < 0.05$) in *P. vulgaris* leaves of both group after light treatment compared to starting condition but no significant differences were found between the two groups of leaves after light stress. In *Z. mays*, A_N, Φ_{PSII} and q_P decreases significantly ($P < 0.05$) only in primary leaves and the F_v/F_m decrease after light stress in secondary leaves was significantly lower than that of primary leaves. In conclusion, though here only an ecophysiological approach is applied, the data reported seem to suggest that the systemic acquired acclimation is not a general phenomenon in plants.

P2.37 Light stress accommodation and induction of PAL-dependent anthocyanin synthesis in some high altitude plants of Bhutan Himalayas

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Anthocyanins are believed to be important protectants against UV irradiation. In flavonoid metabolism, activities of the key phenylpropanoid branch-point enzymes PAL (Phenylalanine Ammonia Lyase) and CHS (Chalcone synthase), and expression of their respective genes, are found to be induced by external factors like exposure to UV-B irradiation stress in fruit peels of both wild and cultivated peaches of Bhutan.

Sunlight mediated anthocyanin synthesis and PAL induction in wild peach peel of Bhutan was compared with the acclimated cultivars of peach of this region. The anthocyanin induction in peel of acclimated cultivars is mediated exclusively by the ultraviolet-B (UV-B) component of sunlight, which was reduced by about 27% by a terminal far-red light pulse and was restored by a red light pulse, indicating the role of phytochrome in modulation of anthocyanin level. The sunlight mediated first PAL peak was induced by phytochrome and was seen in both wild and cultivated peaches, the second PAL peak, inducible by UV-B only, was expressed by the cultivated peaches alone.

Onset of anthocyanin synthesis in ripening peaches, exposed to high sunlight, coincided with a coordinated increase in expression of PAL and CHS genes suggesting the involvement of regulatory genes in light stress accommodation in peaches.

P2.38 Stomatal and non-stomatal limitations of photosynthesis in three C4 grasses under water stress

A.E. Silva, A.S. Soares, A. Bernardes da Silva, J. Marques da Silva, M.C. Arrabaça

(Universidade de Lisboa)

The water stress effect in the photosynthetic metabolism of three C4 grasses with different decarboxylating mechanisms, *Paspalum dilatatum* (NADP-ME), *Cynodon dactylon* (NAD-ME) and *Zoysia japonica* (PEPCK) was studied. Water *deficit* was imposed by different polyethylene glycol 4000 (PEG_{4000}) concentrations in the nutrient solution and the leaf relative water content (RWC) was used as a reference to compare their physiological and biochemical responses. The net photosynthetic rate (A) decreased with the RWC in the three C4 grasses and the O_2 evolution rate showed a linear decrease with RWC in *P. dilatatum* and *C. dactylon* but was relatively constant in *Z. japonica* under moderate stress, decreasing only for lower RWC values. These results and those of PEPC and Rubisco initial activities suggest that the decrease of A under water stress conditions in *P. dilatatum* could be explained by stomatal and non-stomatal limitations, whereas in *C. dactylon* metabolic limitations, namely the Rubisco initial activity, could have a predominant role in the inhibition of A. In *Z. japonica* the decrease of A seems to be mainly due to stomatal limitations in moderate water stress conditions, while non-stomatal limitations became more important for lower RWC values. Carbon isotope discrimination tended to increase in *P. dilatatum* and *Z. japonica* under severe water stress. On the contrary, in *C. dactylon* this parameter was kept constant, suggesting that this species has a higher capacity to maintain some physiological and biochemical activity even when the water content in the tissues is reduced.

P2.39 Different responses to water stress in three C4 grasses

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The response of three C4 grasses of different metabolic sub-types, *Paspalum dilatatum* (NADP-ME), *Cynodon dactylon* (NAD-ME) and *Zoysia japonica* (PEPCK) under water stress conditions was studied. Water stress was induced with different concentrations of polyethylene glycol 4000 (PEG_{4000}) and leaf water *status* was monitored through the relative water content (RWC). RWC decreased with the increase of PEG_{4000} concentration in all species, but this decrease was more pronounced in *C. dactylon* and less in *Z. japonica*. In fact, under similar PEG_{4000} concentrations *C. dactylon* was the species with the lower RWC and *Z. japonica* with the higher one. In *C. dactylon* RWC decreased steeper with a low reduction of nutrient solution water potential, contrasting with what was found for the other two species. The decrease of specific leaf area under water stress was more pronounced in *Z. japonica* because the stressed leaves curled in this species. In *P. dilatatum* the soluble sugars content showed some variation with water stress, including an increase of sucrose content. Results suggest that the three C4 grasses studied have different responses to water stress, being *C. dactylon* (NAD-ME) more sensitive to the decrease of water availability. *P. dilatatum* (NADP-ME) and *Z. japonica* (PEPCK) could maintain a higher RWC by increasing the content of osmoprotective compounds or by curling the stressed leaves, respectively.

P2.40 Regulation of electron transport in *beta vulgaris* exposed to high irradiance: a different influence of non photorespiratory conditions and elevated CO_2

C. Arena, A. Virzo De Santo and N. D’Ambrosio (Università di Napoli)

Any limitation of photosynthesis may determine an excess of excitation energy to PSII reaction centres inducing photoinactivation. The regulation of electron transport activity through other alternative processes to CO_2 assimilation is crucial to dissipate the photons excess and to prevent photodamages. The aim of this work was to assess the different influence of non photorespiratory conditions and of elevated CO_2 concentrations on electron transport flow in de-excitation of PSII at high irradiance. Simultaneous measurements of gas exchange and chlorophyll fluorescence were performed in *B. vulgaris* plants exposed for 4 h to 1000 μmol photons m^{-2} s^{-1} at three different atmospheric conditions: 1) 400 ppm CO_2 and 21% O_2 considered as control, 2) 400 ppm CO_2 and 2% O_2, 3) 1000 ppm CO_2 and 21% O_2. The photosynthetic rate increased significantly at 400 ppm CO_2/2% O_2 and at 1000 ppm CO_2 compared to control. In despite of similar photosynthetic rates, a higher ETR was found at 1000 ppm CO_2 than 400 ppm CO_2/2% O_2. At non photorespiratory conditions, by reducing processes consuming O_2, higher reduction state of Q_A and NPQ value were observed compared to control and 1000 ppm CO_2. At elevated CO_2 concentration the highest photochemical activity, sustained also by processes consuming O_2, contributed to keep PSII photochemical efficiency significantly higher than control and 400 ppm CO_2/2% O_2. This suggests that elevated CO_2 concentration could be more effective than non-photorespiratory conditions to mitigate light stress in *Beta vulgaris* plants.

P2.41 The roles of the psbs protein and carotenoids in the regulation of light harvesting in green plants

S. Crouchman, M. Wentworth, A.V. Ruban and P. Horton (University of Sheffield)

The light harvesting proteins of photosystem II (PSII) have evolved to allow maximum light capture and utilisation, and also play a key role in the regulation of light harvesting. A dynamic system of control is needed to maintain the redox potential and level of excitation of photosystem II under changing environmental conditions, in particular short-term fluctuations in light intensity. Regulation is achieved by dissipation of excess light energy in a process known as nonphotochemical quenching. The major component of this process is qE; a rapid, reversible form of quenching dependent upon ΔpH and the de-epoxidation state of the xanthophyll cycle carotenoids zeaxanthin and violaxanthin.A 22kDa PSII protein called PsbS is essential for formation of qE *in vivo*, although its site and mechanism of action are currently unknown. Zeaxanthin also plays a crucial role in qE. Indeed, *in vivo*, lack of either PsbS or zeaxanthin results in a decrease in efficiency and magnitude of dissipation. Formation of qE is associated with a change in absorbance at 535nm (ΔA_{535}), caused by a shift in the absorption of zeaxanthin molecules, possibly due to their binding to PsbS. Recent experiments have shown that PsbS can bind zeaxanthin, and that the formation of this activated complex may therefore be the origin of ΔA_{535}. We will present our progress in further reconstitution of the complex *in vitro*, along with simultaneous measurements *in vivo* of absorbance and chlorophyll fluorescence in a variety of *A.thaliana* mutants.

P2.42 The effect of water availability on the performance of *Corylus avellana* and other limestone pavement species in the Burren, Ireland

S. Ryan, P. Moran and B.A. Osborne (University College Dublin)

The Burren is an exposed limestone region situated on the West Coast of Ireland. Despite a high rainfall, there is minimal storage of water due to the porous nature of the limestone substrate. Preliminary results have indicated that plants are sensitive to water availability via rainfall. Hazel scrub is characteristic of the Burren vegetation and appears to be growing at the expense of other species in these areas. To assess the significance of the water availability on the performance of hazel we have used assessments of fluorescence and photosynthesis on plants growing *in situ* and *ex situ* coupled with measurements of soil water content, shoot water potential and other climatic variables. The laboratory data collected exhibited approximately a 40% decline in the ratio of variable to maximum fluorescence (Fv/Fm) for the plants subjected to water deficits. The maximum photosynthetic rate (Pmax) reduced by approximately 85% when water was withheld for 16 days. In the field Fv/Fm values did not vary dramatically over the measuring period. *In situ* hazel may acclimate to the seasonal variations in soil water content; plant performance could also be influenced by other climatic and biotic factors. Further site measurements and implementation of some small scale watering treatments *in situ* could provide more detailed information about the relationship between water limitations and plant performance in the Burren.