



UV4growth

**COST-Action FA0906
1st Annual Network
Meeting,
Szeged, Hungary, 7-9 February 2011**

Abstracts



TÁMOP-4.2.2-08/1-2008-0001

Published by the Biological Research Center of the Hungarian Academy of Sciences
ISBN 978-963-508-606-1



ESF provides the COST Office through an EC contract



COST is supported by the EU RTD Framework programme

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This book may be cited as: Abstracts of the 1st Annual Meeting of COST Action FA0906 UV4growth, Szeged, Hungary, 7-9 February 2011

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About COST

COST- the acronym for European Cooperation in Science and Technology- is the oldest and widest European intergovernmental network for cooperation in research. Established by the Ministerial Conference in November 1971, COST is presently used by the scientific communities of 35 European countries to cooperate in common research projects supported by national funds.

The funds provided by COST - less than 1% of the total value of the projects - support the COST cooperation networks (COST Actions) through which, with EUR 30 million per year, more than 30 000 European scientists are involved in research having a total value which exceeds EUR 2 billion per year. This is the financial worth of the European added value which COST achieves.

A "bottom up approach" (the initiative of launching a COST Action comes from the European scientists themselves), "à la carte participation" (only countries interested in the Action participate), "equality of access" (participation is open also to the scientific communities of countries not belonging to the European Union) and "flexible structure" (easy implementation and light management of the research initiatives) are the main characteristics of COST.

As precursor of advanced multidisciplinary research COST has a very important role for the realisation of the European Research Area (ERA) anticipating and complementing the activities of the Framework Programmes, constituting a "bridge" towards the scientific communities of emerging countries, increasing the mobility of researchers across Europe and fostering the establishment of "Networks of Excellence" in many key scientific domains such as: Biomedicine and Molecular Biosciences; Food and Agriculture; Forests, their Products and Services; Materials, Physical and Nanosciences; Chemistry and Molecular Sciences and Technologies; Earth System Science and Environmental Management; Information and Communication Technologies; Transport and Urban Development; Individuals, Societies, Cultures and Health. It covers basic and more applied research and also addresses issues of pre-normative nature or of societal importance.

Welcome to Szeged!

A warm welcome to the first network meeting of COST-Action FA0906 “UV-B radiation: A specific regulator of plant growth and food quality in a changing climate”.

Among plant scientists, perceptions of the biological impact of UV-B radiation have changed dramatically during the past 25 years. In the 1980s, following reports of thinning of the stratospheric ozone layer, UV-B radiation was mostly viewed as a stressor that had the potential to substantially decrease crop yields. The subsequent development and use of increasingly realistic UV-exposure techniques showed that UV-B mediated stress was a rare event. Now, there is an increasing awareness that UV-B is an important regulator of plant gene expression, metabolite profiles, and responses to climate change parameters, giving new insights in ecosystem function and generating novel potential for a more sustainable agriculture.

The aim of COST-Action FA0906 is to enhance understanding of UV-B mediated regulatory processes. Considering the altered perception of UV-B radiation, an important goal for this COST-Action is to develop an integrated vision of the role of UV-B radiation in regulating plant growth at organisational levels from genes through to habitats, across natural and agricultural systems, and to communicate this with potential stakeholders, including researchers, food industries, agricultural enterprises, and policy-makers.

This meeting of COST-Action FA0906 is a first step to enhance the European UV research domain. This meeting is just as much about generating collaborative interactions across disciplines, connecting different universities and countries, as it is about creating a stimulating research environment for our Early Stage Researchers (ESRs), many of which will give oral presentations and attend the special technical UV-workshop attached to this meeting.

A meeting such as this does not “simply happen”. We gratefully acknowledge the outstanding work by Prof. Éva Hideg and co-workers in organizing this meeting, the warm welcome offered by the Biological Research Centre Szeged, and the financial support by COST.

We wish you a productive and enjoyable meeting

Dr. Marcel Jansen
Chair FA0906

Prof. Åke Strid
Vice chair FA0906

Abstracts of keynote talks

Sun simulators for plant research

Andreas Albert

Helmholtz Zentrum, München, Germany

In view of the dramatic effects of global changes there is an urgent need for an improved understanding of the impact of climate changes and anthropogenic stress factors on growth, yield and quality of crop plants. The assessment of the plants' response requires the possibility to perform plant experiment under reproducible realistic climate scenarios. In particular – as several researchers have pointed out – a realistic risk assessment of UVB induced damages in plants can only be obtained if the experiments are performed under natural light and radiation conditions. This applies particularly to the balance between the UVB, UVA, and the visible or photosynthetic active component of solar radiation. The natural global radiation varies during the day and year by intensity and spectral composition, which has to be taken into account for a realistic simulation of the solar radiation. A suite of environmental simulation facilities, especially designed for this purpose, has been developed at our department at the Helmholtz Zentrum München using state-of-the-art techniques for lighting and filtering methods to obtain realistic and reproducible UV scenarios.

Action spectra, lamps and solar radiation

Pedro J. Aphalo¹, Titta Kotilainen^{1,2}, Anders Lindfors^{3,4}, Riitta Tegelberg^{1,5}, Luis O. Morales¹, Sari Siipola¹ and Matthew T. Robson¹

¹Dep. Biosciences, University of Helsinki, Finland

²Valoya Oy, Lapinkylä, Finland

³University of Edinburgh, School of Geosciences, Edinburgh, UK

⁴Climate Change Research, Finnish Meteorological Institute, Helsinki, Finland

⁵Faculty of Science and Forestry, University of Eastern Finland, Joensuu, Finland

Action spectra describe the effectiveness of radiation of different wavelengths in eliciting a given response. They make it possible to distinguish between responses dependent on different photoreceptors. They can also be used as Biological Spectral Weighting Functions (BSWF) when calculating biologically effective UV doses. This step is unavoidable when comparing the effects of UV radiation from sources with different emission spectra, as is the case of most lamps and the sun. We have assessed how well action spectra commonly used as BSWFs match phenolic accumulation in response to UV-A and UV-B radiation, obtaining evidence that there is strong variation even among closely related metabolites. This result indicates that at least two photoreceptors interact to activate these responses, several metabolites being under very delicate and partly independent control. Consequently, fine grained physiological studies require light sources faithfully simulating sunlight. On the other hand, our simulation studies revealed that coarse grained responses like growth are in many cases little affected by the use of either one of the generalized plant action, plant growth or erythral spectra, for calculating the doses from lamps used to simulate ozone depletion.

Overview of the significance of UV radiation superimposed on the main drivers of climate change

Janet F. Bornman

University of Waikato, Hamilton, New Zealand

Key climate factors affecting ecosystems are increased CO₂, temperature, changes in precipitation patterns and anthropogenic nitrogen loads. Superimposed on these drivers are the changes in levels of solar UV-B radiation (280-315 nm) resulting from stratospheric ozone depletion and other atmospheric changes. Globally, the total column ozone is predicted to return to 1980 levels between 2025 and 2040, with some deviation expected in southern and northern mid-latitudes, and the poles, where recovery will likely take longer. However, climate change is also altering exposure of ecosystems to UV radiation, independently of stratospheric ozone, as exemplified by increased aridity, reduced cloud cover in some regions, decreased air pollution (e.g. in the N. hemisphere), and deforestation. Consequently, UV irradiance will not necessarily follow stratospheric ozone recovery. Some of the consequences of the complex interactions between climate drivers and UV radiation include accelerated carbon loss to the atmosphere; changed rates and quality of litter decomposition; altered species distributions; contrasting effects on plant chemistry, plant herbivory, and patterns of growth and energy allocation. These environmental conditions are likely to change patterns of acclimation and resilience.

Checkpoint control in plants

Lieven De Veylder

VIB / Universiteit Gent, Belgium

Plants are sedentary, and so have unavoidably close contact with agents that target their genome integrity. To sense and react to these threats, plants have evolved DNA stress checkpoint mechanisms that arrest the cell cycle and activate DNA repair machinery to preserve the genome content. Although the pathways that maintain DNA integrity are largely conserved among eukaryotic organisms, plants put different accents on cell cycle control under DNA stress and might have their own way to cope with it.

Two decades of enhanced UV-B exposure in the Swedish sub-arctic: responses of a heath community

Dylan Gwynn-Jones¹, David Comont¹, Jenny Bussel¹, Mary Dimambro¹, Catherine Butcher¹, David Causton¹, Lars Olof Björn², Ulf Johanson², Mats Sonesson³ and Terry V Callaghan⁴

¹Institute of Biological Environmental and Rural Sciences, Aberystwyth University, Ceredigion, SY23 3 DA, WALES, UK

²Department of Cell and Organism Biology, Lund University, SE-223-62 Sweden

³Plant Ecology and Systematics, Ecology Building, Lund University, SE-223 62 Lund

⁴Abisko Scientific Research Station, Abisko, SE-981-07 Sweden.

This study aimed to examine the long term (nearly two decades) effects of enhanced UV-B simulating a 15% on a sub-arctic heath community. The experiment was based in the Abisko valley in Northern Sweden (68°N). Treatments involved UV-B fluorescent lamps housed on aluminium frames using square wave timers with the lamps filtered either by glass (Control) or pre-burnt cellulose acetate (+UVB). The experiment commenced in May 1991, the heath has been subsequently exposed to enhanced UV-B levels every growing season from mid May to mid September until 2010. This presentation considers long term impacts of UV-B at the plant and community levels exploring growth responses of dwarf shrub species and point cover measurements of community change. In later years, physiological measurements are considered including soil respiration, shrub photosynthesis, stomatal conductance and leaf secondary metabolite chemistry. Through detailed assessment of community components we interrogate how long term UV-B exposure affects sub-arctic heath communities.

UV-B induced morphogenesis; structure and function

Marcel A.K. Jansen^{1,2}, Kathleen Hectors², Els Prinsen³ and Yves Guisez²

¹Department of Zoology, Ecology & Plant Sciences, University College Cork, Ireland;

²Laboratory of Molecular Plant Physiology & Biotechnology, University of Antwerp, Belgium

³Laboratory of Plant Growth & Development, University of Antwerp, Belgium

Exposure to UV-B radiation is an unavoidable consequence of the photosynthetic light requirement of plants. Molecular-physiological analysis of the UV-B acclimation response in *Arabidopsis thaliana* shows parallel changes in phenylpropanoid levels and plant architecture. UV-induced morphological changes include decreased rosette diameter, decreased inflorescence height and increased numbers of flowering stems, accentuating that chronic UV-B treatment induces a redistribution of growth rather than a cessation. Gene expression profiling in UV-acclimated *Arabidopsis*, using microarrays, indicates possible morphogenic roles for auxins, brassinosteroids and gibberellins. In *Arabidopsis*, IAA is accumulating in the proximal half of UV-irradiated leaves suggesting a redistribution of IAA along the proximal-distal axis. In parallel, UV-B induces accumulation of flavonoids. Flavonoids are not only important in UV-screening, but are also internal modulators of IAA transport. This suggests that UV-B induced changes in flavonoid accumulation and auxin homeostasis should not be considered in isolation, and that UV-B induced changes in morphology could be modulated as a consequence of the crosstalk between these two key UV-acclimation responses.

Arabidopsis UVR8: a Putative UV-B Photoreceptor

Gareth I. Jenkins, Katherine Baxter, Bobby A. Brown, Catherine Cloix, Monika Heilmann and Andrew O'Hara

Plant Science Group, Institute of Molecular Cell and Systems Biology, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Low fluence rates of UV-B stimulate the expression of genes involved in various processes, including UV-protection, through a 'photomorphogenic' UV-B signaling pathway. The aim of our research is to understand the processes of photomorphogenic UV-B perception and signal transduction that regulate transcription.

We reported that *Arabidopsis* UV RESISTANCE LOCUS 8 (UVR8) defines a UV-B-specific, photomorphogenic signaling pathway that orchestrates expression of a range of genes with vital UV-protective functions. The transcription factors HY5 and HYH are key effectors of the UVR8 pathway. UVR8 is a 7-bladed propeller protein that binds to chromatin via histones at the *HY5* locus, and at other target gene loci, providing a mechanistic basis for its involvement in regulating transcription. UV-B exposure promotes the rapid nuclear accumulation of UVR8 and stimulates an interaction between UVR8 and the COP1 protein that is required for transcriptional regulation.

Our recent experiments indicate that UVR8 functions as a UV-B photoreceptor. Data supporting this conclusion, and showing how particular regions and amino acids of UVR8 are important in its function will be presented.

The Effects of UV Radiation on Sauvignon blanc Berry Biochemical Composition

Scott Gregan, Jason Wargent¹, Linlin Liu, Andriy Podolyan, Jim Shinkle², Rainer Hofmann, Chris Winefield, Mike Trought³ and **Brian Jordan**

Lincoln University, Christchurch, New Zealand; ¹Massey University, Palmerston North, New Zealand; ²Trinity University, Texas, USA; ³Marlborough Wine Research Centre, Blenheim New Zealand

The objective of this research programme is to understand the effects of UV radiation on the biosynthesis of important aroma compounds (such as methoxypyrazines and thiols), amino acids and flavonoids in Sauvignon blanc grapes. This study is particularly significant when the viticultural management practice of leaf removal to reduce disease pressure is considered. UV-B increases the overall levels of flavonoids throughout development and also causes qualitative changes in skin flavonoids that take place between veraison and harvest. UV-B *per se* does not have a significant effect on the amino acid composition of the grapes during development. In contrast, the levels of certain amino acids in the berries relate to the presence of leaves remaining over the fruiting zone. UV-B does not influence the methoxypyrazine levels at harvest, although UV-B may reduce levels early in development. Gene expression activity for methyl transferase is being determined to characterise methoxypyrazine biosynthesis further. We are also characterising the LOX-HPL biochemical pathway that is involved in thiol aroma formation in addition to wound and pathogen responses. Wines have been made from these treatments and show differences in appearance and taste from the initial pressings to the final product.

Ecophysiological aspects of UV-B mediated changes in non-volatile secondary plant compounds and corresponding gene expression in Brassicaceae

Inga Mewis¹, Franziska Beran², Christian Ulrichs², Rita Zrenner¹, Angelika Krumbein¹, Erich Glawischnig³ and Monika Schreiner¹

¹Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Quality Department, Großbeeren, Germany; ²Humboldt-Universität zu Berlin, Faculty for Agriculture and Horticulture, Division Urban Plant Ecophysiology, Berlin, Germany;

³Technical University Munich, Institute for Genetics, Freising, Germany

UV-B effects on plant biochemistry have been usually linked with changes mostly increases in phenolic compounds. However, little is known about UV-B mediated changes of other non-volatile secondary metabolites such as glucosinolates (GS) and the phytoalexin camalexin present in Brassicaceae. Short-term application of UV-B (0.15 Wh m⁻²) to *Arabidopsis thaliana* Col-0 plants and broccoli sprouts (*Brassica oleracea* var. *italica*) increased aliphatic GS significantly already after one day, whereas indolyl GS remained unchanged. The UV-B protectants kaempferol and quercetin increased in treated broccoli sprouts as expected. A novel finding is the induction of camalexin by UV-B in *A. thaliana* plants. To study the impact of UV-B on plant defense against insects, bioassays were conducted with the specialist *Pieris brassicae* L. and the generalist *Spodoptera exigua* (Hübner). The larval performance of the specialist was poor on UV-B treated plants, whereas the generalist growth better on plants pre-exposed to UV-B. Comparative microarray analysis with *A. thaliana* and broccoli sprouts revealed, that UV-B leads to a decrease of most genes associated with photosynthesis, while most plant defensive genes were activated. Combination treatments resulted in a generally stronger gene response.

Functional interaction of the circadian clock and UV RESISTANCE LOCUS 8-controlled UV-B signaling pathways in Arabidopsis

Balázs Fehér¹, László Kozma-Bognár¹, Éva Kevei¹, Anita Hajdu¹, Melanie Binkert², Seth Jon Davis³, Eberhard Schäfer⁴, Roman Ulm², Ferenc Nagy^{1,4,5}

¹Institute of Plant Biology, Biological Research Centre, Szeged, Hungary; ²Department of Plant Biology, University of Geneva, Geneva, Switzerland; ³Max Planck Institute for Plant Breeding Research, Cologne, Germany; ⁴Institute of Biology II/Botany, University of Freiburg, Freiburg, Germany; ⁵School of Biological Sciences, University of Edinburgh, Edinburgh, UK

Circadian clocks regulate many molecular and physiological processes in *Arabidopsis* (*Arabidopsis thaliana*) with a 24 h period, allowing the timing of these processes to the most appropriate time of the day. The accuracy of timing relies on the synchrony of the clock and the environmental day/night cycle. Visible light is the most potent signal for such synchronization but light-induced responses are also rhythmically attenuated (gated) by the clock. Here we report a similar mutual interaction of the circadian clock and non-damaging photomorphogenic UV-B light. We show that low intensity UV-B radiation acts as entraining signal for the clock. UV RESISTANCE LOCUS 8 (UVR8) and CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) are required, but ELONGATED HYPOCOTYL 5 (HY5) and HY5 HOMOLOG (HYH), are dispensable for this process. UV-B responsiveness of clock gene expression suggests that photomorphogenic UV-B entrains the plant clock through transcriptional activation. We also demonstrate that UV-B induction of gene expression under these conditions is gated by the clock in a HY5/HYH-independent manner. The arrhythmic *early flowering 3-4* mutant showed non-gated, high level gene induction by UV-B, yet displayed no increased tolerance to UV-B stress.

UV-B acclimated crops: methods to assess bioavailability of carotenoids and other phytochemicals

Nora O'Brien

School of Food and Nutritional Sciences, University College Cork, Ireland

An *in-vitro* model for the measurement of bioavailability of nutrients from foodstuffs has been established. The model consists of an *in-vitro* digestion procedure which mimics gastric and duodenal digestion by the addition of enzymes and pH modification of the foodstuff. Carotenoid bioaccessibility is defined as the amount of ingested carotenoid that is available for absorption in the gut. Traditional food processing and preservation methods, especially thermal treatments, induce the formation of cis isomeric forms. Bioaccessibility of cis/trans isomers of carotenoids can be established using this model. The digestate is then applied to a transwell plate containing differentiated Caco-2 cells. Differentiated Caco-2 cells resemble cells of the small intestine. They are grown on a support which separates the culture dish into a top and a bottom chamber. The digested foodstuff is added to the top chamber. Nutrients present in the bottom chamber must pass through the Caco-2 monolayer and this is indicative of the amount which would be absorbed *in-vivo*. Effects observed in the model compared very favourably with observations from published human studies thus validating the *in vitro* bioavailability model.

Organismal responses to UV radiation: damage or regulation or what?

Nigel Paul

Lancaster University, UK

Plant responses to UV-B radiation have been the subject of relatively intensive research for around three decades. Most of that research has been conducted in the context of increases in UV-B radiation that would result from uncontrolled ozone depletion. While there is no doubt that understanding of responses to UV-B has been advanced enormously by this body of research, the focus on the effects of ozone depletion has also restricted the perception of the role of UV radiation under field conditions, as well as substantially influencing the design and interpretation of experiments. One perception widely held in the past, was the view of UV-B responses as the product of damage, but that is now changing rapidly with the growing recognition of the regulatory role of UV in many plant. In this talk, I will consider whether other commonly-expressed views about UV are overdue for re-assessment. Examples will include the focus on the effect (or perhaps lack of effect) of moderate increases in UV-B above the current ambient, the rather rigid differentiation between UV-B and UV-A, and the relationships between field and controlled environment experimentation with UV radiation.

UV-B perception and signalling in Arabidopsis

Luca Rizzini, Jean-Jacques Favory and **Roman Ulm**

Institute of Biology II, University of Freiburg, Germany

Department of Plant Biology, University of Geneva, Switzerland

To optimize their growth and survival, plants perceive and respond to UV-B radiation. However, neither the molecular identity of the UV-B photoreceptor nor the photoperception mechanism is known. An early molecular response to UV-B radiation involves the direct interaction between UVR8 and COP1, two key factors regulating UV-B-induced photomorphogenesis. This pathway secures plant acclimation to UV-B and thus endurance under sunlight. We will present data supporting a molecular mechanism underlying UV-B perception and the rapid UVR8-COP1 interaction.

The use of a single-monochromator diode array spectroradiometer for UV-radiation measurements

Lasse Ylianttila

STUK, Helsinki, Finland

The new so-called minispectrometers offer an alluring choice for numerous measurement applications. They are relatively cheap, small, robust (no moving parts) and they seem to be easy to use. The suitability of single-monochromator diode array spectroradiometer for UV-radiation measurements, especially UV-B, is evaluated. Special attention is paid for stray-light characterisation and a correction method for stray-light is presented. Also the effects of dark signal, linearity, cosine response and wavelength accuracy to the measurements are evaluated. Results for two minispectrometers are presented, one is Ocean Optics S2000 which have been in use in STUK for several years and the other one is a brand new Ocean Optics Maya. The spectra measured with these minispectrometers were compared to the spectra measured with a Bentham DM 150 double-monochromator spectroradiometer. By applying all the corrections a measurement uncertainty of approximately 10% can be achieved. Without corrections the measurement errors can easily exceed 50%. For the proper use of the instrument, the user should have knowledge of the instruments operating principles and potential error sources.

Abstracts of oral presentations in parallel sessions

UV-B radiation on rice – Impact on the photosynthetic performance

José Cochicho Ramalho¹, António Eduardo Leitão¹, Maria Manuela Abreu da Silva²,
Fernando José Cebola Lidon³

¹Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Instituto de Investigação Científica Tropical, Oeiras, Portugal; ²ESE Almeida Garrett, Grupo Universidade Lusófona, COFAC, Lisboa, Portugal; ³GDEH, Faculdade de Ciências e Tecnologia, Univ. Nova de Lisboa, Caparica, Portugal.

The impact of UV-B radiation on photosynthetic parameters was studied in *Oryza sativa* L. cv. Safari plants irradiated (biological effective UV-B of 20.825 kJ m⁻²) between 8 and 14 days after germination. Following the inhibition of isoprenoids contents, gas exchange measurements showed dramatic decreases of more than 80% in net photosynthesis, stomatal conductance and photosynthetic capacity, 1 day after the end of UV-B irradiation. Non-photochemical quenching further decreased suggesting the failure of thermal dissipation mechanisms, which was accompanied by severe reductions on photochemical efficiency of photosystem (PS) II, photochemical quenching and estimation of quantum yield of linear thylakoid electron transport, given by fluorescence analysis. In accordance with these patterns, at a thylakoidal level, significant deleterious effects were observed in the rate of electron transport involving PS I and II (to ca. 34 and 50%, respectively), with the contents of galactolipids strongly decreasing (mostly due to significant monogalactosyldiacylglycerol peroxidation coupled to ethylene synthesis), whereas the opposite occurred with phospholipids. Gas exchange measurements and PSs functioning one week later showed the prevalence of negative effects in irradiated leaves, although they were absent in the new developed ones. As the UV-B effects on the photosynthetic apparatus were mostly limited to the irradiated leaves, the modulation of the photosynthetic performance in leaves grown after UV-B exposure is presented and discussed.

Changes in spectral reflectance characteristics of two barley varieties in response to combined effect of UV and PAR

Alexander Ač, Daniel Kováč, Karel Klem, Petr Holub, Otmar Urban

CzechGlobe – Centre for Global Change Impact Studies, Academy of Sciences of the Czech Republic, Czech Republic

The main objective of this study was to evaluate the relationships between physiological responses of barley plants to ultraviolet radiation (UV) and leaf spectral characteristics (reflectance). The synergic effects of photosynthetically active radiation (PAR) and UV on the flavonols content (*in vivo*), chlorophyll fluorescence and gas-exchange parameters after 7 days acclimation were studied in two barley varieties with different sensitivity to UV (Barke and Bonus). Leaves of different age classes were measured by spectroradiometer FieldSpec3 (ASD, USA).

Enhanced UV radiation (ca 200% of ambient intensity) combined with low PAR intensity (ca 25% of ambient PAR) led to significant changes in spectral reflectance of both varieties; however, these changes were negligible in ambient PAR intensity. These changes were more pronounced in sensitive variety Barke and they were closely related to the reduction of light saturated CO₂ assimilation rate (R² for reflectance intensity at 690 nm was R²=0.94) and steady state chlorophyll fluorescence (R² for reflectance intensity at 390 nm was R²=0.64). However, the relationships between photosynthetic parameters and reflectance indices were strongly influenced by leaf age. Tight correlation between reflectance intensity at around 390 nm and flavonols content was found (R² = 0.75).

Acknowledgement: This work is part of the research supported by grant 522/09/0468.

The pitfalls of UVB treatments in grapevine studies

Anhalt Ulrike CM, Forneck Astrid

University of Natural Life Science, Department of Applied Plant Sciences and Plant Biotechnology, Institute for Horticulture, Fruit-Growing, and Viticulture, Vienna, Austria

A great challenge is to plan an appropriate and strong design for grapevine studies especially with the aim to study molecular changes of grapevine under UVB stress. Studies showed already that additional stress to UVB radiation, like heat or drought, can influence the result of UVB studies negatively. Since grapevine plants react immediately to stress, and especially to additional stress, it is necessary to focus on stable experimental conditions. Grapevine has the disadvantage that it is less manageable than e.g. smaller *Arabidopsis* plants and less is known about UVB stress genes than in *Arabidopsis*. But it is as well a perennial plant and it has the advantage to study annual effects and transmission effects of UVB stress. Since it is one of our most important agricultural crops and has economic importance it is very important to carefully analyze the molecular background of UVB stress. Transcriptomic and proteomic approaches can help to find genes related directly to stress or which are involved in stress response pathways (Jellouli *et al.* 2010, Plant Mol Biol Rep).

This contribution will give a look into pitfalls of UVB studies and tries to give solutions in grapevine UVB stress experiments.

The response of insects to UV radiation

David Ben-Yakir, Yehezkel Antignus, Yossi Offir and Dror Hadar

Agricultural Research Organization, The Volcani Center
Polysack Plastic Industries, Nir-Yitzhak, Israel

Insects use optical cues for host finding and flight orientation. UV- radiation at 350-370 nm is perceived by most insects and it is used by them for orientation and navigation. UV-light stimulates flight activity in aphids, whiteflies and thrips, and its absence reduces their dispersal rates. There are many reports that indicate that covering crops with plastics or screens containing UV-blocking additives provides a greater protection against insect pests than standard cladding materials. High levels of UV-reflection deter insect landing. Therefore, UV-reflecting mulches and reflective nets are known to be effective means to reduce insect pests' infestations. UV- radiation at 290-320 nm (UVB) is perceived by few insects and it usually deters most insects. Direct exposure to UVB can strongly decrease the survivorship and reproduction of insects. Exposure of host plants to UVB often induces plant defences against insect pests.

UV-irradiation provokes generation of superoxide on cell-wall polygalacturonic acid

Jelena Bogdanović Pristiv and Ivan Spasojević

Institute for Multidisciplinary Research, Beograd, Serbia

Superoxide dismutases (SOD) present in the apoplast and the cell wall imply extracellular generation of superoxide whose source and effects remain to be fully understood. We show using electron paramagnetic resonance (EPR) spectroscopy employing DEPMPO spin-trap capable of differentiating between hydroxyl ($\cdot\text{OH}$) and superoxide ($\cdot\text{O}_2^-$) radicals that galacturonic acid in pectin from the cell wall can produce $\cdot\text{O}_2^-$ by transforming $\cdot\text{OH}$ radical, which is generated via UV-mediated hydrolysis, in the presence of oxygen. UV-B system was composed of ultrapure water solution of H_2O_2 (2 mM) exposed to UV ($10 \text{ J m}^{-2} \text{ s}^{-1}$ at 254 nm for 90 s) with or without PGA (15 mg/mL). In the absence of PGA only the production of $\cdot\text{OH}$ was observed, while the presence of PGA led to the production of significant amounts of superoxide. Superoxide produced in the apoplast is dismutated by SOD to H_2O_2 which may enter into surrounding healthy tissue to initiate gene expression and modulate enzyme activity crucial for subsequent phases of defense and adaptation to UV-provoked stress. Our results, imply that cell wall has an initial role in the signaling cascades important for the defense against UV damage. This may explain the presence of $\cdot\text{O}_2^-$ and SOD in the apoplast and cell wall.

Towards a complete understanding of Arabidopsis transcription factor – promoter element interactions

Lauri Vaahtera¹ and Mikael Brosché^{1,2}

¹Department of Biosciences, University of Helsinki, Helsinki, Finland

²Institute of Technology, University of Tartu, Tartu, Estonia

Due to their sessile lifestyle plants are constantly confronted with unfavourable growth conditions, for example extreme temperatures or pathogen attack. Plant adaptation and acclimation require activation of appropriate defences against external stresses and include a large reprogramming of gene expression through regulation of transcription. The executors of the transcriptional response are gene specific transcription factors binding to target promoter elements. Despite their importance, the target promoter element for most transcription factors is unknown. Here we have used multiplexed massively parallel SELEX (systematic evolution of ligands by exponential enrichment), an *in vitro* method that determines the DNA binding affinity and sequence specificity of a transcription factor, to a selected group of *Arabidopsis thaliana* transcription factors. HY5 and HYH, two transcription factors important for regulation of UV-B induced changes in gene expression, bind to identical promoter element motives.

The effect of UV-B stress and elevated CO₂ on rhizosphere processes

Jenny Bussell, Dylan Gwynn-Jones, John Scullion and Gareth Griffith

IBERS, Aberystwyth University, Aberystwyth, SY23 3DA, UK.

UV stress impacts the soil rhizosphere via plants through effects on their belowground resource allocation, and modified root exudation. Consequently, such plant-microbe interactions affect the soil microbial communities, often causing a shift in the associated mycorrhizal community. Other environmental factors, such as elevated atmospheric CO₂, can further influence this mycorrhizal response and soil microbial diversity by increasing the carbon inputs to the root and soil system, with a shift to carbohydrate rich exudation.

This ongoing study used a fully factorial glasshouse experiment to explore how UV stress and elevated CO₂ (650ppm) interact to impact on the root development and mycorrhizal associations of the sub-arctic grass *Calamagrostis purpurea*. This species naturally occupies soil with limited or not easily phytoavailable sources of phosphorus. It was hypothesised that treatment of the plants with UV would decrease plant access to inaccessible forms of phosphorus (FePO₄), but that this effect may be mitigated by elevated CO₂ treatment. The presentation will describe available experimental outcomes and will discuss more widely UV and elevated CO₂ impacts in the rhizosphere system.

Exploring latitudinal variation in UV radiation and climate: impacts on a model grass system

Comont, D.¹, Abaigar, J. M.², Albert, A.³, Aphalo, P.⁴, Figueroa, F. L.⁵, Gaberscik, A.⁶, Gausch, L. L.⁷, Hauser, M. T.⁸, Jansen, M.⁹, Kardefelt, M.¹⁰, Luque, P. C.⁵, Neubert, S.⁸, Olivera, E. N.², Olsen, J.¹¹, Robson, M.⁴, Schreiner, M.¹², Sommaruga, R.¹³, Strid, Å.¹⁴, Torre, S.¹¹, Turunen, M.¹⁵, Veljovic-Jovanovic, S.¹⁶, Verdaguer, D.⁷, Vidovic, M.¹⁶, Wagner, J.¹³, Winkler, J. B.³, Zipoli, G.¹⁷, Gwynn-Jones, D.¹

¹IBERS, Aberystwyth University, Aberystwyth, UK; ²University of La Rioja, Logrono, Spain; ³Helmholtz Zentrum München, Neuherberg, Germany; ⁴University of Helsinki, Helsinki, Finland; ⁵University of Málaga, Málaga, Spain; ⁶University of Ljubljana, Ljubljana, Slovenia; ⁷University of Girona, Girona, Spain; ⁸University of Natural Resources and Life Sciences, Vienna, Austria; ⁹University College Cork, Cork, Ireland; ¹⁰Abisko Scientific research station, Abisko, Sweden; ¹¹Norwegian University of Life Sciences, Ås, Norway; ¹²Leibniz-Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany; ¹³University of Innsbruck, Innsbruck, Austria; ¹⁴Örebro Life Science Center, Örebro, Sweden; ¹⁵University of Lapland, Rovaniemi, Finland; ¹⁶Institute for multidisciplinary research, Belgrade, Serbia; ¹⁷C.N.R. Ibimet, Firenze, Italy

Perennial ryegrass (*Lolium perenne*) seedlings were grown at 14 European locations across a latitudinal gradient spanning 37 to 68°N. Seedlings planted in nutrient enriched vermiculite were grown outdoors over five weeks between the 29th June and the 3rd August 2010. At each location there were three treatments – open, filtered with cellulose acetate (UV transparent) and filtered with Mylar (UV opaque). Plants were regularly watered and outdoor climatic conditions were monitored at nearby meteorological stations. The aim of the experiment was to assess the significance of ambient UV radiation to *L.perenne*, both at each location and across the gradient in terms of aboveground biomass, tiller number, and the level of UV protective plant pigments. Material was further screened using metabolite fingerprinting (FT-IR spectroscopy) to assess local, regional and latitudinal variation in total plant chemistry. Data presented will explore and interpret the complex variations in growth and chemistry looking at local responses and the latitudinal gradient explored.

The interaction of UV-B radiation treatment with drought and selenium addition in two buckwheat species

Barbara Breznik, Mateja Germ, Nataša Dolinar, Ivan Kreft and **Alenka Gaberščik**

Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia

The study evaluates the interaction of elevated UV-B radiation with drought and selenium treatments in *Fagopyrum esculentum* and *Fagopyrum tataricum*. Plants were exposed to UV-B (UV-B) radiation, drought (D) and selenium (Se) at different levels and in different combinations: (1) control (C); D; UV-B(+); UV-B(+), D; (2) C; Se1; UV-B(+); UV-B(+), Se1; (4) C; Se2; UV-B(+); UV-B(+), Se2; (5) C; Se2; D; UV-B(+), D. The following parameters were monitored: chlorophyll *a+b* content (Chl *a+b*), potential and effective photochemical efficiency (PE), transpiration rate, terminal electron transport (ETS) activity, plant architecture and biomass production. An interaction between UV-B(+) and D was observed. UV-B(+) mitigated negative effects of D on PE and biomass of tartary buckwheat. UV-B(+) affected biomass of common buckwheat less during D in comparison to UV-B(+) treatment only. Se treatment ameliorated negative effects of UV-B (+) which was not the case for D. Se addition improved Chl *a+b*, PE and biomass production of radiated common buckwheat, irrespective to Se concentration. High Se dose improved PE and biomass production of radiated tartary buckwheat. Common buckwheat showed higher susceptibility to UV-B(+) and D in comparison to tartary buckwheat.

Plant strategies of UV-B absorbing compounds production

Mateja Germ, Tadeja Trošt Sedej and Alenka Gaberščik

Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia

The contribution summarises partly published results on plant strategies of UV-B absorbing compounds (AC) production. In all cases we used the same UV-B treatment and analysis procedures. Enhanced UV-B radiation increased the production of UV-AC in *Ceratophyllum demersum*, *Tropaeolum majus* and *Fagopyrum esculentum*. No relation was found in *Ranunculus trichophyllus*, *Myosotis scorpioides*, *Potamogeton alpinus*, *Myriophyllum spicatum*, and *Picea abies*. Some plant species contained saturated amounts of UV-AS, and enhanced doses did not exert the increased production. In *Hypericum perforatum* and *Fagus silvatica* the production increased with the elevation level. The lowest total amount was found in aquatic plants. In amphibious species emerged specimens produce higher amounts than submerged ones. Woody plants produced higher amounts in comparison to herbaceous plants. The difference was observed when we compared evergreen and deciduous trees. The former had higher UV-AS contents. Data analyses revealed three main strategies of UV-AC production: (1) the production of UV-AC with increasing UV-B radiation dose, (2) the increase during growing season and (3) saturated amount of UV-AC in high and unpredictable radiation environment.

Theoretical study of the UVR8 protein structure, its tryptophan amino acid array and its role as a UV-B photoreceptor

Min Wu¹, **Elin Grahn**², Leif A. Eriksson¹ and Åke Strid²

¹School of Chemistry, National University of Ireland - Galway, Galway, Ireland

²Örebro Life Science Center, School of Science and Technology, Örebro University, Sweden

The protein UV resistance locus 8 (UVR8) is known to be involved in the specific UV-B response resulting in regulation of a large number of genes in plants. However, the precise mechanism for the function of the protein is not yet known. We have built a homology model of the protein UVR8 from *Arabidopsis thaliana*, and subjected the model to energy minimization. As predicted from sequence alignments, the model has a seven-bladed β -propeller fold. The final model shows that a large amount of the highly conserved tryptophans in UVR8 are clustered on one face of the globular protein structure. After obtaining the model, a quantum chemical calculation of the excitation spectra was performed based on the structure and relative orientation of the tryptophan residues. The calculated spectrum shows two peaks, corresponding to absorption maxima around 280 and 300 nm, a result in accordance with the previous experimental studies on regulation of gene expression. In summary, the distribution of the conserved tryptophan residues in the UVR8 model, together with the involvement of these residues in the UV absorption as calculated using quantum chemical methods, suggest that UVR8 itself could act as the UV-B receptor molecule in plants absorbing at both 280 and 300 nm.

Transgenerational stability and mechanisms of UV-B induced epigenetic changes in Arabidopsis thaliana

Christina Lang-Mladek¹, Olga Popova², Kathrin Kiok², Marc Berlinger¹, Claudia Jonak², Werner Aufsatz², Christian Luschnig¹ and **Marie-Theres Hauser**¹

¹BOKU- University of Natural Resources and Life Sciences , Vienna, Austria

²Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria

The survival of plants inevitably requires the response to daily and seasonal UV-B changes. Apart from short-term responses plants because of their sessile life style developed long-term strategies allowing them to adapt their genome to persistent elevated UV-B exposures. There is evidence that UV-B can heritably alter the frequency of somatic recombination even in non-stressed progeny possibly by interfering with epigenetic regulation (1,2). We show that several transcriptionally silenced and epigenetically controlled loci become activated after UV-B radiations. We demonstrate that the release of gene silencing occurs without loss of global and local DNA methylation (3). We show that histone modifications are significantly altered and that mutants in chromatin remodelling factors exhibit different UV-B sensitivities. UV-B induced reactivation of the silenced loci remains detectable in two subsequent generations without further exposure to UV-B. However the heritable reduction of epigenetic control is transient and returns in the third generation and upon prolonged seed storage to control levels. Therefore we suggest a mechanism that erases the epigenetic memory and might be involved to protect plant (epi)genome stability upon increased UV-B radiation.

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This project is supported by GENome Research in AUstria (GEN-AU) and the Austrian Science Foundation (FWF).

Negative feedback regulation of UV-B induced photomorphogenesis in Arabidopsis

Marc Heijde^{1,2}, Henriette Gruber¹, Werner Heller³, Andreas Albert⁴, Harald K. Seidlitz⁴ and Roman Ulm^{1,2}

¹Faculty of Biology, Institute of Biology II, University of Freiburg, D-79104 Freiburg, Germany; ²Department of Botany and Plant Biology, University of Geneva, Sciences III, CH-1211 Geneva 4, Switzerland; ³Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, D-85764 Neuherberg, Germany; ⁴Department of Environmental Engineering, Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, D-85764 Neuherberg, Germany

Survival of plants in sunlight requires UV-protective responses. Plants respond to low levels of UV-B radiation with a coordinated photomorphogenic response that allows acclimation to this environmental stress factor. The key players in this UV-B response are COP1 (an E3 ubiquitin ligase), UVR8 (a β -propeller protein), HY5 (a bZIP transcription factor) and the more recently identified RUP1 and RUP2 proteins (WD-40 repeat proteins). We have shown previously that an elevated UV-B-specific response is associated with dwarf growth, indicating the importance of balancing UV-B-specific signaling. Recently, published work has identified a particularly important role emerging for the UVR8 protein - a primary candidate for the long-sought UV-B photoreceptor in plants. We will present novel data on how the RUP proteins function as crucial negative regulators of UVR8 action, providing new insight into the mechanism of UV-B-induced photomorphogenesis.

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Evolving facility for studies on wavelength dependence on plant response to UVB radiation

Anu M. Heikkilä¹ and Petri Kärhä²

¹Finnish Meteorological Institute, R&D / Climate Change, Helsinki, Finland

²Aalto University, School of Science and Technology, Metrology Research Institute, Espoo, Finland

A facility originally designed and constructed for ageing of polymeric materials with spectrally resolved UV radiation is presented. The facility could possibly be modified to study wavelength dependent responses of plants to UVB radiation and derive various action spectra similar to photodegradation of materials.

The basic structure, principles of operation, and results of careful metrological characterization of the facility will be described. A 1000-W Xenon arc lamp, providing a spectrum closely following the solar terrestrial spectrum, is used as a UV radiation source. A holographic flat-field grating is used to resolve the output of the lamp into narrow bands of wavelength. The spectrally resolved radiation is reflected onto the sample exposure plane of 21 cm in width and 1.5 cm in height. The wavelength of the radiation over the exposure area ranges from 250 to 500 nm. The FWHMs of the bands may be adjusted by selection of the slit width of the spectrograph.

The potential of the facility to serve studies on plant response to UVB radiation is evaluated and the requirements of modification set by such studies on the facility are outlined. Aspects of future development enabling versatile studies on the processes of adaptation to changing UVB radiation conditions are discussed.

UV-B mediated changes in enzyme activity inducing flavonoids of white asparagus spears (*Asparagus officinalis* L.)

Susanne Huyskens-Keil, Ines Eichholz, Christian Ulrichs, Nina Beesk and Sascha Rohn
Humboldt-Universität zu Berlin, Faculty of Agriculture and Horticulture, Department
Urban Plant Ecophysiology, Berlin, Germany

The flavonoids quercetin and its derivatives are outstanding dietary antioxidants in white asparagus spears (*Asparagus officinalis* L.) and are known to exert health beneficial effects. UV-B radiation induces stress mediated plant defence systems and is known to cause an acceleration of antioxidant compounds in plant tissue. Several key enzymes play an important role in phenol metabolism and are discussed for their free radical scavenging properties: phenylalanine ammonia lyase (PAL) and peroxidases (POD). The aim of the present study was to investigate the impact of UV-B irradiation on the PAL and POD activity and mediated changes in flavonoid composition and antioxidant activity. Asparagus spears cv. 'Gynlim' were exposed to UV-B irradiation dosages (0.075 Wh m^{-2} , 0.15 Wh m^{-2}) using an UV-B fluorescence light source (FL 20SE, 305-310 nm). Apical and basal sections of spears were used for further analysis: PAL and POD activity using photometric assays; flavonoid compound composition determined by HPLC-DAD, and antioxidant activity determined using the Trolox Equivalent Antioxidant Capacity assay.

Results showed that enzyme activity (PAL and POD) and flavonoids can be induced by moderate UV-B application and thus might be an interesting tool for postharvest application.

UV-B sensitivity and tolerance mechanisms in seedlings of three deciduous forest tree species

Réka Láposi, Szilvia Veres, Ilona Mészáros

University of Debrecen, Department of Botany, Debrecen Egyetem tér 1. H-4032,
Hungary

This study aimed at revealing the ecophysiological responses of 2-year old seedlings of three deciduous tree species occurring together in forest stands in mountainous regions of Hungary (beech, ash and hornbeam) to enhanced UV-B radiation (exceeding the ambient UV-B by 40%) in field experiments. For all three species, accumulation of flavonoids with UV-B filtering and antioxidant features was a sensitive response to the increased UV-B radiation, which appeared in correlation with specific leaf mass (SLM) referring to anatomical changes e.g. thickening of leaves. The three species differed in leaf flavonoid content. Leaves flavonoid content was high in hornbeam and ash and was very low in beech. Contrary to the extra metabolic cost, the large accumulation of flavonoids in leaves of hornbeam and ash might be a favourable trait against the effects of UV-B on photosynthetic apparatus. Enhanced UV-B radiation had no significant impact on photochemical efficiency of PSII of hornbeam and ash compared to beech which exhibited significant decrease in actual photochemical efficiency of PSII ($\Delta F/F_m'$), and relative fluorescence decrease (RFD) of illuminated leaves with a simultaneous chlorophyll degradation. In UV-B treated seedlings of hornbeam increase of β -carotene content per unit chlorophyll and DEPS (deepoxidation index) reflecting the activity of xanthophyll cycle was also observed. The studied ecophysiological traits showed that among the three species seedlings of beech was more susceptible to enhanced UV-B radiation which might influence the regeneration success in open habitats.

UV-B radiation on rice – Reactive oxygen species interaction with photoassimilates biosynthesis

Fernando José Cebola Lidon¹, Miguel Teixeira², José Cochicho Ramalho³, Maria Manuela Abreu da Silva⁴, António Eduardo Leitão³

¹GDEH, Faculdade de Ciências e Tecnologia, Univ. Nova de Lisboa, Caparica, Portugal;

²Instituto de Tecnologia Química e Biológica, Univ. Nova de Lisboa, Oeiras, Portugal;

³Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Instituto de Investigação Científica Tropical, Oeiras, Portugal; ⁴ESE Almeida Garrett, Grupo Universidade Lusófona, COFAC, Lisboa, Portugal

Triggering of oxidative stress by UV-B radiation (total biological effective UV-B of 20.825 kJ m⁻²) was studied in *Oryza sativa* L. cv. Safari plants irradiated between 8 and 14 days after germination (DAG). Until 21 DAG UV-B induced significant rises in superoxide, hydroxyl and hydrogen peroxide contents, whereas ascorbate and superoxide dismutase (Cu and Zn containing), glutathione and dehydroascorbate reductases, ascorbate peroxidase and catalase activities decreased. Also, photosynthetic pigments and galactolipid contents dropped in opposition to de-epoxidation state of xanthophylls and lipid peroxidation. Following higher grana disorganization and degradation, accumulation of chloroplast polypeptides 72/69, 33/32, 28/26, 22/20 and 18/16 kDa bands decreased, but the ratio of 49/46 kDa band increased. These changes paralleled a significant failure in both photosystems, particularly in Hill reactions coupled to water splitting complex. In 28 DAG plants, chloroplast lamellae of leaves grown after UV-B exposure showed a general recovery from oxidative burst, but a subsequent higher stacking of thylakoids was found. We concluded that UV-B mediated chloroplasts degradation coupled to oxidative stress is minimized by a modulated metabolism in leaves grown after UV-B exposure, which implicates the recover of arrays of detoxification mechanisms.

Micronutrients accumulation in rice after supplemental UV-B irradiation

Fernando José Cebola Lidon¹, Maria Manuela Abreu da Silva²

¹GDEH, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal; ²ESE Almeida Garrett, Grupo Universidade Lusófona, COFAC, Palácio de Santa Helena, Largo do Sequeira n° 7, Lisboa, Portugal

Rice plants (*Oryza sativa* L. cv Safari) were submitted to UV-B irradiation and, during the initial vegetative growth phase and in end of the life cycle, the accumulation of Mn, Cu, Zn and Fe, root and shoot electrolytic conductance, the activity of the roots ATPase proton extrusion pump and the photosynthetic performance of the light reactions were determined. It was found that the increasing membrane permeability of the UV-B treated leaves inhibits Fe accumulation in rice tissues, being developed an antagonistic interaction with Mn. An increasing passive Cu uptake is also pointed, whereas Zn contents are associated to a higher root acidification capacity. Considering the implications of the UV-B irradiation in the plant metabolism, the accumulation of micronutrients is further correlated with the inhibition and recover of pigments accumulation and the photosynthetic electron transport rates in the leaves of rice plants directly submitted to UV-B radiation and grown after this stress exposure, respectively. It is concluded that while, membrane permeability triggers leaves necrosis in directly UV-B exposed rice, micronutrients accumulation is associated to the recover of photosynthesis after high UV-B radiation.

UV responses of Mediterranean plant species and its importance under low water availability conditions

Laura Llorens¹, Meritxell Bernal¹, Jordi Badosa² and Dolors Verdaguer¹

¹Environmental Sciences Department, Faculty of Sciences, University of Girona, Girona, Spain; ²Department of Physics, Faculty of Sciences, University of Girona, Girona, Spain

Models predict that, as a consequence of climate change, water availability, an important factor modulating plant responses to UV, will be reduced in the Mediterranean region. We developed 2 experiments to investigate: 1) Mediterranean plant mechanisms to cope with UV and 2) the effect of a reduction in water availability on these mechanisms. In the first experiment, we grew seedlings of 3 xerophytic (*Phillyrea angustifolia*, *Daphne gnidium*, *Pistacia lentiscus*) and 3 mesophytic (*Rosa sempervirens*, *Ilex aquifolium*, *Laurus nobilis*) Mediterranean species in a greenhouse under 3 UV treatments (without UV, with UVA, with UVA+UVB) and 2 irrigation levels (watered to saturation, low watered). In the second one, *Pistacia lentiscus* and *Laurus nobilis* seedlings were grown outdoors under 3 UV treatments (ambient UV, enhanced UVA, enhanced UVA+UVB) and 2 irrigation levels (watered to saturation, low watered). We measured treatment effects on several plant morphological, biochemical and physiological traits. In general, plants were more responsive to UV under drier conditions, with UVA radiation having an important effect on the studied traits. Our results suggest that some Mediterranean species might benefit from a cross-tolerance phenomenon between UVB radiation and low water availability.

Enhanced UV-B irradiation as the inducer of cytoskeletal instability and apoptosis in BY-2 cells

Dmytro I. Lytvyn, Yuliya A. Krasylenko, Alla I. Yemets and Yaroslav B. Blume

Institute of Food Biotechnology and Genomics, Natl. Acad. Sci. of Ukraine, Kiev, Ukraine

In spite of rising influence of UV-B on living organisms evidences about mechanisms and consequences of UV-B impact on plant cells remains insufficient. We investigated effects of 34, 81 and 135 kJ/m² of UV-B doses modified by different times of exposure (100, 240, 400 min, irradiation spectrum was a half-width of the range from 297 to 332 nm) on the microtubule dynamics and induction of apoptosis in tobacco BY-2 cells. Apoptosis in irradiated cells was determined using variable fluorescent staining and DNA laddering and TUNEL assays also. Microtubular changes were investigated by visualizing of fused GFP-MAP4 protein using confocal microscopy. As result, apoptotic morphology such as cytoplasm vacuolization, cell shrinkage, stretched nuclei, chromatin condensation and appearance of micronuclei were detected as consequences of UV-B influence. DNA fragmentation and acidification of cytoplasm were observed also in treated cells. Irradiated cells showed significant disorders of the microtubular cytoskeleton, namely dose-dependent fragmentation of cortical microtubular arrays coupled with further stabilization of their fragments. Mitotic microtubules as well as interphase microtubules of BY-2 cells were sensitive to the UV-B exposure and might be involved in apoptotic UV-B response.

Are young ones more vigorous? A comparative study of photosynthesis and antioxidant responses of younger and older Vitis leaves under UV-B radiation in a green-house experiment

Petra Majer and Éva Hideg

Institute of Plant Biology, Biological Research Center Szeged

In plant leaves, reactive oxygen species (ROS) are known to participate in the damage induced by high UV-B doses and are suspected to be involved in signals leading to acclimation to lower doses. While in the first scenario ROS are accessible to direct detection by selective fluorescent or EPR active probes, lower ROS levels assumed to be produced in response to low to slightly above ambient UV-B dosages can not be detected with presently available techniques. A traditional alternative is to follow changes in antioxidant capacities and use these as indicators of ROS levels. Our experiments, however, suggest that results of these indirect methods are to be interpreted with care. Grapevine (*Vitis vinifera* L. cv. Chardonnay) cuttings were grown in the green-house. Older (but not senescent) and younger leaves were exposed to daily (9AM – 3 PM) doses of supplemental UV-B (Q-Panel UVB-313EL tubes, $2.2 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Photosynthesis (carbon-dioxide uptake, photochemical yield) decreased to a larger extent in older leaves than in younger ones, and young leaves had more dynamic responses in pigment concentrations and in antioxidant capacities than older ones. Nevertheless, no direct correlation was found between any of these biochemical parameters and the UV-B sensitivity of photosynthesis.

Diel changes in the physiological and protective responses to enhanced UV-B radiation in an aquatic liverwort

Javier Martínez-Abaigar, Gabriel Fabón, Rafael Tomás, Laura Monforte and Encarnación Núñez-Olivera

Universidad de La Rioja, Logroño (La Rioja), Spain

Bryophytes are structurally simple and their protection against ultraviolet radiation (UVR) may be mainly based on the accumulation of ultraviolet-absorbing compounds (UVAC), whose diel changes are unknown in bryophytes. We studied the diel changes in the UVAC levels (in terms of the bulk UV absorbance of acidified methanol extracts) in the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia* under laboratory conditions. The samples were exposed to three different radiation regimes: P (only PAR), PA (PAR + UV-A) and PAB (PAR + UV-A + UV-B). A simple extraction with acidified methanol was firstly conducted, presumably extracting UVAC from vacuoles, and, after this, a NaOH digestion of the residuum and a second extraction was performed to extract the cell-wall-bound UVAC. At every moment of the day, UVAC levels were higher in PAB than in PA and P samples, and in PA than in P samples. In general, UVAC levels were higher at midday than in the rest of the day, and this occurred in both cell compartments (vacuoles and cell walls) and in the three radiation regimes. Thus, UVAC levels showed clear diel changes and, although responding more strongly to UVR, responded also to high PAR. Other physiological responses (chlorophyll fluorescence, DNA damage) were also measured.

UV radiation and plant methane emissions: estimating their contribution to global biogeochemistry

Andy R. McLeod¹, A. Anthony Bloom¹, Wesley T. Fraser¹, Julia Lee-Taylor², Sasha Madronich², David J. Messenger¹, Paul I. Palmer¹ and David S. Reay¹

¹School of GeoSciences, The University of Edinburgh, Edinburgh, United Kingdom

²National Center for Atmospheric Research (NCAR), Boulder, Colorado, USA

The production of volatile organic compounds by terrestrial vegetation is well known but there has been much controversy about reported emissions of the important greenhouse gas methane from plant foliage. This presentation will describe experiments demonstrating that UV radiation in sunlight and from experimental lamps does indeed drive an *in situ* emission of methane and other trace gases from plant leaves and from pectin, a major plant cell wall polysaccharide. Such effects may have been overlooked in the past because experimental leaf cuvettes and plant chambers do not transmit all UV wavelengths. Our experiments have demonstrated a role of UV-generated reactive oxygen species (ROS) in foliar emissions of trace gases which also include ethane, ethene, carbon monoxide and carbon dioxide. We will describe the determination of an action spectrum for the process, its use to create a global climatology for methane-effective UV radiation and how such information can be applied with satellite-derived leaf area and temperature data to estimate the contribution of UV-driven plant emissions in global-scale biogeochemistry.

Responses of sessile oak (*Quercus petraea* L.) seedlings to enhanced UVB radiation and water stress

Erzsébet Szöllősi, Viktor Oláh, Péter Kanalas, Réka Láposi and **Ilona Mészáros**

University of Debrecen, Department of Botany, Debrecen Egyetem tér 1. H-4032 Hungary

Availability of water and ultraviolet B radiation (UVB) are concomitant factors to affect the terrestrial vegetation in the future and their possible interactive effects are especially significant for the forestry in temperate regions. Enhanced UV-B radiation and water stress may have direct and indirect effects on physiology of plants. In the present study we investigated the responses of sessile oak seedlings grown from seeds to supplementary UV-B radiation and water stress. Seedlings of sessile oak showed rapidly developing susceptibility to the enhanced UV-B radiation which appeared in lowering the chlorophyll content and potential photochemical efficiency (Fv/Fm) and actual photochemical efficiency of PSII ($\Delta F/Fm'$). UV-B radiation caused slight changes in leaf water relations. Water withdrawal from the plants resulted in very low leaf water content and water potential. When plants were simultaneously exposed to UV-B and water stress leaf water relations of seedlings were improved and increase in flavonoid accumulation was observed. However, values of Fv/Fm, $\Delta F/Fm'$ and RFD were the lowest in these plants as compared to control and those exposed to separately to enhanced UV-B or water withdrawal. Both UV-B radiation and water stress increased non-photochemical quenching with a parallel enhancement of zeaxanthin formation.

Temporal variation of epidermal flavonoids in *Betula pendula* under altered solar UV radiation depends on leaf position

Luis Orlando Morales¹, Riitta Tegelberg^{1,2}, Mikael Brosché^{1,3}, Anders Lindfors^{4,5}, Sari Siipola¹ and Pedro José Aphalo¹

¹Department of Biosciences, University of Helsinki, Helsinki, Finland

²Faculty of Science and Forestry, University of East Finland, Joensuu, Finland

³Institute of Technology, University of Tartu, Tartu, Estonia

⁴University of Edinburgh, School of Geosciences, Edinburgh, UK

⁵Climate Change Research, Finnish Meteorological Institute, Helsinki, Finland

Betula pendula (silver birch) seedlings were grown in the greenhouse without UV and transferred outdoors under three UV treatments (UV-0, UV-A and UVA+B) created by plastic films. After seven and thirteen days, flavonoid contents of adaxial and abaxial epidermis of leaves at different positions in the seedling (L1, L2 and L3) were estimated *in vivo* with Dualex. After fourteen days, some of the seedlings were swapped amongst these treatments to study the time-course of the accumulation of epidermal flavonoids in the youngest unfolded leaves under changing solar UV-B and UV-A environments. Leaves at different positions differentially regulated UV absorbance in adaxial and abaxial epidermis according to the UV treatment. UV-B increased the flavonoid contents in the leaves independently of leaf position, except for the youngest leaves (L3) where no differences were detected between UV-0 and UV A+B. Already three days after swapping the seedlings, the youngest unfolded leaves had adjusted the contents of flavonoids in their epidermis according to the UV they received and these adjustments were affected by the previous UV exposure. Accumulation of flavonoids was enhanced by initial absence of UV-A+B or UV-A followed by exposure to UV A+B.

Effects of enhanced UV-B radiation and three orders of insects on glucosinolate induction in broccoli sprouts

Chau Nhi Nguyen^{1,2}, Monika Schreiner¹, Dietrich Knorr² and Inga Mewis¹

¹Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Quality Department, Großbeeren, Germany

²Berlin University of Technology, Department of Food Biotechnology and Food Process Engineering Berlin, Germany

In recent years the enhanced UV-B radiation on plants became of major interest. Application of UV-B can lead to changes in secondary metabolism of plants and is believed to be similar to the plant response caused by herbivores. In the present study, glucosinolate (GS) induction in broccoli sprouts (*Brassica oleraceae* var. *italica*) by UV-B radiation and by three orders of insects was investigated. After 5-times of UV-B exposure to 0.25 W h m² aliphatic GS increased significantly. A similar plant response was observed in the combination treatment of UV-B with *Myzus persicae* (Hemiptera), *Pieris brassicae* (Lepidoptera), or *Phaedon cochleariae* (Coleoptera) after 3 days of feeding. No significant changes within aliphatic GS levels were found after treatment with insects as biotic stressors. In contrast, a 2-fold increase of indole GS occurred after feeding of *P. cochleariae* compared to the control. Only the combination of UV-B and *P. cochleariae* showed an increase of indole GS in broccoli sprouts. Insect performance was conducted on UV-B treated and untreated sprouts, whereby larval weight gain of *P. brassicae* and population growth of *M. persicae* were negatively influenced by the UV-B treatment. This might be related to the change within the GS profile and concentration in the plant.

Combined enhancements of UVB and temperature influence growth and phenolics of field-grown dark-leaved willow (*Salix myrsinifolia*)

Line Nybakken, Riitta Julkunen-Tiitto and Riia Hörkkä

Natural Products Research Laboratories, Dep. of Biology, University of Eastern Finland, Box 111, FI-80101 Joensuu

The two sexes of dioecious species have been hypothesized to economize differently with their resources. It is believed that females will invest more in reproduction and defence, while males are thought to prioritize growth. We studied how climate change might influence on growth and defence of dark-leaved willows (*Salix myrsinifolia*) in a full-factorial field experiment with enhancements in UVA, UVB (lamps) and temperature (T) (infra-red heaters). Both factors were modulated to follow the ambient conditions.

Cuttings of eight clones, four of each sex, of dark-leaved willow were planted to the field in spring 2009. In both 2009 and 2010 total biomass increased significantly with T, and in 2010 there was an additive effect of UVB. The biomass also differed significantly between clones, but there was no difference between sexes. Height growth was also significantly increased under enhanced T. After the first growing season, most phenolic compounds in the leaves decreased under enhanced T, while quercetins increased under enhanced UVB. Only two compounds differed significantly between sexes; chlorogenic acid was highest in females, while one *p*-OH-cinnamic acid derivative was higher in males. These and further results will be discussed.

Natural variation of *Arabidopsis thaliana* in the response to UV-C irradiation

Thomas Piofczyk¹, Johannes Rungger², Ortrun Mittelsten Scheid² and **Ales Pecinka**^{1,2}

¹Max Planck Institute for Plant Breeding Research, Cologne, Germany

²Gregor Mendel Institute of Plant Molecular Biology, Vienna, Austria

On the Earth's surface, the amount of UV radiation is unequal and reaches maxima around equator and at the high altitudes. We are interested in understanding the molecular mechanisms of UV induced DNA damage repair and in identification of novel genes that are required for fine-tuning of the UV repair pathways on the latitudinal and altitudinal gradients.

To this end, large collection of *A. thaliana* accessions, originating from different geographical areas, was irradiated with UV light to induce specific type of DNA damage - pyrimidine dimers. UV-C, instead of UV-B, radiation was used to minimize physiological responses. Stress phenotypes were recorded and subjected to Genome Wide Association studies to identify causal genes. In addition, classical quantitative trait locus (QTL) mapping was performed and revealed two major QTLs responsible for an increased sensitivity towards UV-C. The position of QTLs suggests that at least one of them contains a novel gene associated with the repair of UV induced DNA damage. The causal genes are currently being fine mapped.

Dose dependence and action of modifying factors on UVB radiation effects in model plant *Crepis capillaris*

Vida Ranceliene and Regina Vysniauskiene

Institute of Botany of Nature Research Centre, Vilnius, Lithuania

The low number of *C. capillaris* chromosomes ($2n=6$) and relatively small plant habitus allow to compare action of UVB radiation on the whole plant and its chromosomes and investigate the action of modifying agents. A wide range of UVB doses (0; 0.75; 1.0; 1.125; 1.5; 2.0; 2.5; 3.0 kJm^{-2}) used for irradiation of root tip meristems allowed to determine the range doses producing negative effect on cell division (from 1.0 kJ) and increasing level of chromosome aberrations (CAs, from 1.13 kJ) as well as the ratio of chromosomal/chromatidial aberrations (from 1.125 kJ). In leaflet meristem (supplemental daily doses 0, 1, 3, 5, 7, 9 kJm^{-2} for 5 days) CA increased to significant value at 5 kJ UVB, while 3 kJ UVB and 5 kJ UVB activated cell division. The plant weight, leaf area, concentrations of chlorophylls a and b, carotenoids, proteins, SOD activity were determined. The most sensitive parameter was the leaf area. Protective effects of anthocyanin-rich extracts, ascorbic and salicylic acids on CA were observed. Photoreactivating light about two times reduced the CA level. Temperature regime (21/14 °C and 25/16 °C) and CO₂ (350 ppm and 700 ppm) action on UVB was investigated. At 25/16 °C MA activity increased in root and leaflet meristems. Irradiation of plants with UVB at 25/16 °C and increased CO₂ produced the opposite effect.

Carotenoid profiling and biosynthetic gene expression in wild-type and *hp-1* tomato fruit under UV-B depletion

Valerio Lazzeri¹, Valentina Calvenzani², Katia Petroni², Chiara Tonelli², Antonella Castagna¹ and Annamaria Ranieri¹

¹Department of Crop Biology, University of Pisa, Italy

²Department of Biomolecular Sciences and Biotechnology, University of Milano, Italy

We investigated the molecular events underlying carotenoid accumulation in flesh and peel of wild-type and *hp-1* fruits in presence or absence of UV-B light. Gene expression analyses showed that in wild-type fruits solar UV-B radiation exerts a negative modulation mostly on the carotenoid biosynthetic genes encoding enzymes downstream of lycopene synthesis, both in flesh (*CrtL-b*, *CrtL-e*, *CrtRb1*) and in peel (*CrtL-b*, *CrtL-e*), suggesting that the down-regulation of *CrtL-b* and *CrtL-e* and the subsequent accumulation of lycopene during ripening are dependent on UV-B light. In wild-type peel, UV-B depletion determined an anticipated accumulation of lutein and a higher β -carotene content at the expense of phytoene and lycopene; in flesh, instead, determined a lower content of phytoene and phytopluene and a reduced lycopene accumulation compared to control conditions. In contrast to wild-type, UV-B depletion did not greatly affect carotenoid accumulation in *hp-1* and determined minor differences in gene expression between control and UV-B depleted conditions, but the carotenoid biosynthetic genes *CrtL-b*, *CrtL-e* and *CrtRb1* were also negatively modulated by UV-B light in *hp-1* flesh, suggesting that for these genes the response to UV-B radiation is independent from the *hp-1* mutation.

Eight years experimentation with UV-B exclusion in the High Arctic: What have we learned about plant responses?

Helge Ro-Poulsen¹, Kristian Rost Albert², Teis N. Mikkelsen², Anders Michelsen¹, Kristine Boesgaard², Marie F. Arndal³, Riikka Rinnan¹, Linda Bredahl², Kirsten B. Håkansson¹, Niels M. Schmidt⁴

¹Department of Biology, University of Copenhagen, Denmark

²Department of Biosystems, Research Center Risø, Technical University of Denmark

³Forest and Landscape Research Institute, University of Copenhagen, Denmark.

⁴National Environmental Research Institute, Aarhus University, Denmark

To evaluate how the prevailing UV-B radiation affect heath plants in high arctic Zackenberg (74°30'N, 20°30'W), exclusion experiments with various set-ups have been performed, starting in 2001. The filter types used were Mylar (UV-B reduction), Lexan (UV-AB reduction) and Teflon (control). The plants species studied were *Vaccinium uliginosum*, *Salix arctica* and *Betula nana*. Across all species and years significant effects on PSII performance were found, measured as e.g. a higher F_v/F_m and PI in the UV-B reduction treatments. High PAR levels increased the effects. Reduced UV-B caused a decreased production of UV-B absorbing compounds. *Salix* leaf photosynthesis was higher, and the exposure angle was shown to affect the effect. The leaves were thinner in reduced UV-B. In *Vaccinium*, an increased fine root growth was observed in reduced UV-B, and canopy photosynthesis measurements showed positive effects per leaf area basis, but the leaf architecture was changed to fewer but thicker leaves in reduced UV-B, in contrast to *Salix*. The PSII of *Betula* appeared to be the most sensitive to UV-B radiation, but application of the ozone protective chemical EDU seemed to reduce the effect. The conclusion is that the prevailing UV-B irradiance significantly impact high arctic plants.

Is response to solar UV-B consistent with differences in the general stress tolerance strategy of two *Nothofagus* species?

Matthew T. Robson and Pedro J. Aphalo

Dept. of Bioscience, University of Helsinki, Finland

Several species of *Nothofagus* co-occur in the south of South America where they are subject to an altered solar UV-B environment due to the annual break-up of the ozone hole during the Austral spring. These closely-related species inhabit sites of differing exposure, soil moisture retention, and precipitation patterns. Their ecophysiology has been largely unstudied, but niche differentiation among these closely-related species appears to reflect the extent of their stress tolerance. To discover whether the response of these species to solar UV-B is consistent with the differences in their general stress tolerance, we followed the response of adult *N. antarctica* and *N. pumilio* trees to solar UV-B in the field and compared this with the responses of seedlings to sun/shade and drought/well-watered treatments in a factorial experiment under controlled conditions. Plastic branch filters were used to produce near-ambient and reduced solar UV-B treatments over three growing seasons, allowing growth and leaf traits of these trees to be monitored. Leaf morphological and physiological traits of *N. antarctica* were unresponsive to solar UVB, whereas *N. pumilio* was somewhat affected. This difference corresponded with respective general stress responses and growth strategies of the species.

Post-harvest UV-B treatment and antioxidant potential of flesh and peel tomato fruits at different stages of ripening

Claudia Scattino¹, Esther Maragò², Antonella Castagna¹ and Annamaria Ranieri¹

¹Department of Crop Biology, University of Pisa, Italy

²Scuola Superiore S.Anna, Pisa, Italy

Recent studies have shown that UV-B radiation influences the synthesis of plant secondary metabolites such as phenols and carotenoids but there is little information about their effect in post-harvest. The aim of this study was to investigate if UV-B radiation in post-harvest affected the most representative carotenoids, phenolic compounds and ascorbic acid content in flesh and peel of tomato fruits, harvested at mature green and turning stages.

Two tomato genotypes, Money Maker and his mutant HP-1, were grown inside a greenhouse covered with polyethylene transparent to UV-B and put into two different controlled rooms with different light treatments, +UV-B (1,68 W/m²) and -UV-B, until red ripe stage.

Total phenols, flavonoids, flavonols and ascorbic acid were evaluated by spectrophotometric assays, whereas carotenoids by HPLC. The antioxidant properties of the two tissues were evaluated according to the ABTS method. The ethylene production was quantified by GC both before and after the post-harvest treatment on the whole fruits. The analyzed metabolites showed to be influenced by the UV-B treatments but different behaviors were described depending to both the ripening stage of harvest and the genotype. Ethylene production wasn't influenced by the post-harvest light treatment.

Influence of UV-B on flavonol aglycones and main flavonol glycosides in kale (*Brassica oleracea* var. *sabellica*)

Susanne Schmidt¹, Michaela Zietz², Monika Schreiner¹, Sascha Rohn³, Lothar W. Kroh² and Angelika Krumbein¹

¹Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V., Grossbeeren, Germany; ²Technische Universität Berlin, Institute of Food Technology and Food Chemistry, Berlin, Germany; ³Universität Hamburg, Institute of Food Chemistry, Hamburg, Germany

Kale has a wide spectrum of complex flavonol glycosides. A total of 71 flavonol glycosides (44 hydroxycinnamic acid acylated) based on the aglycones quercetin, kaempferol and isorhamnetin were detected by HPLC-DAD-MSⁿ. The aim of this work was to investigate the modifying influence of UV-B on structurally different flavonol glycosides.

In experiment I juvenile kale plants cv. Winterbor were exposed to a single UV-B dosage from 0.07 Wh m⁻² up to 0.56 Wh m⁻² and analyzed after a 24 h adaptation period. In experiment II multiple UV-B dosages were applied as 4 subsequent treatments of 0.28 Wh m⁻² (total dosage 1.12 Wh m⁻²) with a 24 h adaptation in between.

The flavonol aglycone quercetin (quercetin glycosides total) remained unchanged after a single UV-B dosage of 0.28 Wh m⁻² and a multiple UV-B exposure. Surprisingly, sinapic acid mono-acylated quercetin tri- and tetraglycosides decreased after a single UV-B dosage of 0.28 Wh m⁻², whereas a multiple UV-B dosage had no influence. In contrast, the corresponding kaempferol glycosides were not influenced by a single UV-B dosage of 0.28 Wh m⁻², but decreased at a multiple UV-B dosage. Summarising, structurally different flavonol glycosides respond differently to moderate UV-B application suggesting a variable UV-B protection mechanism.

Effect of UV-B and Asahi SL on antioxidant capacity of maize leaves

Elżbieta Skórska¹, Barbara Wójcik-Stopczyńska², Magdalena Witczak¹ and Monika Grzeszczuk²

¹ Department of Physics and Agrophysics, ² Department of Horticulture, West Pomeranian University of Technology, Szczecin, Poland

The aim of the experiment was study if long wave UV-B (305-320 nm, 2 kJ m⁻² d⁻¹) modifies the antioxidant capacity of *Zea mays* L. cv. Landmark (F1) plants treated with a biostimulator Asahi SL recommended for use on many agricultural crops, mainly to increase yielding and improve yield quality, and to reduce the adverse effects of stress-inducing factors. UV-B radiation increased ultraviolet absorbing compounds in the maize leaves plants cv. Landmark (F1) by 118% in comparison with the control plants. The plants treated with the Asahi SL had more these compounds by 78%. UV-B increased of DPPH radical scavenging activity by 42% in the leaves, and Asahi caused decrease by 25% in comparison with the untreated plants. These results showed significant effect of the applied dose of UV-B as well on antioxidant activity as ultraviolet compounds synthesis, while the applied biostimulator did not turn out to be a positive influence on the maize plants.

Kinetics of flavonoid synthesis in lettuce induced by low levels of UV-B from commercial cool-white fluorescent tubes

Christopher Rodriguez¹, Sissel Torre¹ and Knut Asbjørn Solhaug²

¹Norwegian University of Life Science, Department of Plant Science, Ås, Norway

² Norwegian University of Life Science, Department of Ecology and Natural Resource Management, Ås, Norway

Plants grown in greenhouses in Norway during winter synthesize low amounts of flavonoids. Higher content of flavonoids is wanted because flavonoids may be health beneficial due to their antioxidant and anticarcinogenic effects. The effects of UV-B given by UV-B tubes (Q-panel UV 313) and by standard cool-white fluorescent tubes (Philips Master TL-D 36W/840) on flavonoid and anthocyanin synthesis in lettuce cv. Lollo rosso (carmoli) were studied. The aim was to increase the content of flavonoids without reducing the plant growth. The cool-white fluorescent tubes have much lower biological effective UV-B (UV-B_{BE}) compared with absolute UV-B levels. Although the cool-white fluorescent tubes had very low UV-B_{BE} quite high amounts of flavonoids were synthesized. The highest doses of UV-B from the UV-B tubes gave high flavonoid synthesis but plant growth was reduced. The kinetics of flavonoid accumulation was measured with the chlorophyll fluorescence excitation ratio method with the new instrument Multiplex 3. Anthocyanins were estimated with the ratio between UV-A and red light excited fluorescence and colourless flavonoids were estimated with the ratio between UV-A and green light excited fluorescence.

Dynamics of acclimation of the assimilation apparatus to excessive UV-B radiation in barley grown under low and high PAR

Michal Štroch, Jakub Nezval, Václav Karlický, Martina Volfová, Hana Benešová, Ladislav Šigut, Irena Kurasová and Vladimír Špunda

Department of Physics, Faculty of Science, Ostrava University, Ostrava, Czech Republic

We examined the dynamics of the acclimation response after transfer of barley plants grown under low and high PAR to conditions with high UV-B dose. Our goal was to evaluate, how the level of PAR during growth of plants will affect the ability of protection against exposure to excessive UV-B radiation. Spring barley (*Hordeum vulgare* L. cv. Bonus) was grown under low and high PAR (LL – 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$; HL – 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in the absence of UV radiation for 8 days. Then the plants were exposed to UV-B radiation (2 W m^{-2} for 16 h per day; unweighted) for the next 6 days. The presentation will be focused especially on the changes of epidermal UV-shielding, blue-green fluorescence emission, functional state of photosystem II (PSII) and composition of photosynthetic pigments and phenolic compounds. In contrast to LL-acclimated plants, PSII function of HL-acclimated barley was not significantly disrupted by UV-B treatment. We suppose that the critical factor in the protection of the photosynthetic apparatus of HL barley was the ability to induce a considerable degree of epidermal UV-shielding only by high PAR. In addition, both HL- and UV-B-induced increase of the amount of unbound xanthophylls in HL barley could contribute to the resistance of PSII against UV-B radiation.

Control of plant morphology by UV-B radiation in greenhouse produced pot and bedding plants

Sissel Torre¹, Staffan Bengtsson², Hans Ragnar Gislørød¹ and Jorunn E. Olsen¹

¹Norwegian University of Life Science, Department of Plant and Environmental Sciences, Ås, Norway

²Andersens Gartneri AS, Ringstad, Råde, Norway

Chemical plant growth retardants (PGRs) are used for controlling height of greenhouse grown ornamental pot- and bedding plants. Increasing environmental awareness has strongly promoted interest in alternative methods of regulating shoot elongation. Since UV-B is known to reduce shoot elongation in a number of species, the use of UV-B and UV-A- transmitting cladding material F-clean® (ETFE film, ethylene tetrafluoroethylene) in commercial greenhouses appear highly interesting. Substantially reduced shoot elongation was observed in a variety of bedding and pot plants produced in the spring in Norway at 59°N under F-clean compared to polyethylene. Furthermore, although natural UV- levels are low in the autumn at such high northern latitudes, a 40 % reduction in the use of PGRs as a consequence of reduced shoot elongation, higher chlorophyll content, reduced apical dominance and reduced leaf area were then observed in poinsettia under F-clean. To further evaluate UV-B radiation as a tool to control morphology, greenhouse grown poinsettias were exposed to short diurnal periods of UV-B by UV-B tubes (Q-panel UV 313). Effects on stem elongation, growth and amount of flavonoids of young and old leaves will be discussed.

Norway spruce and European beech activity over three seasons of enhanced UV-B exposure

Tadeja Trošt Sedej, Dušan Rupar and Alenka Gaberščik

University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia

Chosen responses of spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.) to enhanced UV-B radiation during three years treatment performed outdoors were monitored three times a year on three needle age classes and leaves, respectively. The tree seedlings were exposed to UV-B radiation, simulating 17 % ozone depletion. Both species exhibited great variability in the amounts of photosynthetic pigments, methanol-soluble UV-B absorbing compounds and light use efficiency. The effects of UV-B radiation depended on needle and leaf development stage and interaction with environmental conditions. Enhanced UV-B radiation triggered little influence to monitored responses of both trees. A reduced negative effect of UV-B radiation on spruce was observed during prolonged drought, which was hypothesised as alleviating effect. The current year needles but not leaves exhibited a tendency to increased production of UV-B absorbing compounds under enhanced UV-B radiation. Despite the hypothesis that high elevation evergreen conifers develop higher tolerance to enhanced UV-B in comparison to deciduous trees, our results suggested high UV-B tolerance of both species. Furthermore, the outdoor study performed under variable environmental conditions showed great complexity of spruce and beech responses to enhanced UV-B.

Synergic effect of photosynthetically active radiation on acclimation to UV radiation in two barley varieties

Karel Klem, Alexander Ač, Petr Holub and Otmar Urban

CzechGlobe – Centre for Global Change Impact Studies, Academy of Sciences of the Czech Republic, Czech Republic

The interactions of ultraviolet (UV) and photosynthetically active radiation (PAR) were studied in two barley varieties (Barke, Bonus). Two levels of UV (enhancement of UV-B and UV-A to ca 200% of ambient ~ UV+; exclusion of UV radiation by plastic filters ~ UV-) and PAR (ambient PAR intensity ~ PAR+; reduction to ca 25% of ambient PAR by plastic filters ~ PAR-) were combined in totally four treatments.

Short-term acclimation (7 days) of plants was studied in terms of changes in flavonols content in vivo (Dualox, ForceA, Fr), chlorophyll fluorescence (FluorPen, PSI, Cz), and gas-exchange parameters (Li-6400, Li-Cor, USA).

The UV+PAR- treatment caused significant reduction of photosynthetic activity and necrotic damage of barley leaves which was more pronounced in older than young leaves and in Barke than Bonus variety. PAR thus plays an important photo-protective role against negative effects of UV radiation, e.g. via accumulation of flavonols. However, the acclimation to both UV+ and PAR+ is necessary for induction of adequate protection mechanisms against following exposure to high intensities of UV. Both PAR+ and UV+ reduced the elongation growth of plants and individual leaves; however, UV radiation reduced only the growth of newly developed leaves.

Acknowledgement: This work is part of the research supported by grant 522/09/0468.

Influence of DNA damage and repair on the ability of cyanobacterial cells to repair UV-B radiation induced damage to the Photosystem II complex

István-Zoltán Vass, Péter B Kós and Imre Vass

Institute of Plant Biology, Biological Research Center, Szeged, Hungary

Two of the most significant primary effects of UV-B irradiation in cells of photosynthetic organisms are the damage to DNA and the impairment of active protein complexes, of which the most pronounced one is the inactivation of Photosystem II mainly due to damaging the D1 protein. We have investigated the correlation of Photosystem II protein damage and its repair, with the concomitant DNA damage and its repair. As model organisms the cyanobacterium *Synechocystis* PCC6803 wild type (WT), as well as its photolyase lacking mutant (Δ phrA) were used for this purpose. We found that during exposure to UV-B radiation the Δ phrA cells accumulated a significant number of DNA damages concomitant with a radical decrease in Photosystem II activity, and D1 protein levels. After terminating the UV-B illumination the Δ phrA cells showed no repair of damaged DNA, and only a limited capacity to repair the damaged Photosystem II centers. The WT cells, however, didn't suffer significant damages to their DNA. In these cells PSII activity as well as repair capacity, including effective turnover of the D1 protein pool, was maintained under the same UV-B irradiation conditions. These data show that the repair capacity of Photosystem II is directly influenced by the ability of cells to repair UV-B damaged DNA.

Impact of UVB radiation on growth characteristics and antioxidative enzymes' systems in potato (*Solanum tuberosum*)

Regina Vysniauskiene¹, V. Ranceliene¹, I. Kemeziene¹ and J. Jundulas²

¹Institute of Botany of Nature Research Centre, Vilnius, Lithuania

²Voke Branch of Lithuanian Research Centre for Agriculture and Forest, Lithuania

Adaptive response of many crops to stress induced by UVB exposure varies due to different sensitivity of plant characters to this factor, which is often influencing plant growth and development. The aim of this work to compare the effects of UVB on different potato cultivars and to determine the role of antioxidant enzymes in response mechanisms towards the UVB-induced stress. Lithuanian potato cultivars were studied: three early cultivars 'Venta', 'Goda', 'Liepa', medium early cultivar Nr. 2946-7 and late cultivar 'Aista'. The potatoes were treated with $6 \text{ kJ m}^{-2} \text{ d}^{-1}$ UVB radiation dose for 8 days. The research has shown that after the UVB exposure leaf area, fresh and dry biomass as well as plant height reduced. However, concentrations of chlorophyll a, b and carotenoids increased in early potato varieties 'Goda' and 'Liepa', while in late cultivar 'Aista' they remained unchanged. Increased activity of antioxidant SOD, CAT and POD enzymes in all tested potato cultivars after the UVB radiation treatment suggests an active plant response to UVB-induced stress.

What has isoprene emission to do with the UV-B induced accumulation of phenolic compounds?

Jana Barbro Winkler¹, Andreas Kaiser², Andreas Albert¹, Werner Heller³, Philippe Schmitt-Kopplin⁴, Francesco Loreto⁵ and Jörg-Peter Schnitzler^{1,2}

¹Helmholtz Zentrum München, Institute of Biochemical Plant Pathology, Department of Environmental Engineering, Neuherberg, Germany; ²Research Centre Karlsruhe, Institute for Meteorology and Climate Research (IMK-IFU), Garmisch-Partenkirchen, Germany; ³Helmholtz Zentrum München, Institute of Biochemical Plant Pathology, Neuherberg, Germany; ⁴Helmholtz Zentrum München, Institute of Ecological Chemistry, Department of Molecular BioGeoChemistry and Analytics, Neuherberg, Germany; ⁵Istituto per la Protezione delle Piante (IPP), Consiglio Nazionale delle Ricerche (CNR), Area della Ricerca del CNR di Firenze, Firenze, Italy

Isoprene plays a dual role in plants as thermo-protective volatile agent preventing degradation of photosynthetic structures and as reactive molecule reducing abiotic oxidative stress. High temperature and light intensities causes a down-regulation of genes and metabolites of condensed tannins and anthocyanins in transgenic non-isoprene emitting poplars. Visualization of hydrogen peroxide (H₂O₂) in poplar leaves indicates that isoprene has a third function as leaf internal quencher of ROS signals thereby modulating accumulation of phenolic compounds. Application of different UV-B intensities on WT and non-isoprene emitting poplar (R) lines in a sun simulator study demonstrated a diminished induction of phenolic compound accumulation in non-isoprene emitting lines. In particular the accumulation of anthocyanins became reduced and delayed. Non-target metabolome analysis by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) confirms a concerted down-regulation of phenolic compounds in isoprene-free mutants. Among all photosynthetic gas exchange parameters measured only net CO₂ assimilation in R-lines was effected by UV-B radiation, probably due to the higher penetration capacity of short wave UV radiation into the mesophyll leaf tissue.

Comparison of UV-B induced transcriptional changes in Arabidopsis thaliana and Broccoli sprouts

Rita Zrenner, Inga Mewis, Angelika Krumbain and Monika Schreiner

Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V.,
Department Quality, Grossbeeren, Germany

UV-B radiation is known as a specific regulator of molecular and metabolic processes in plants. Using *Arabidopsis thaliana* as a model we are studying several eco-physiological aspects of UV-B mediated changes in non-volatile secondary plant products and compare the differences with UV-B mediated changes in other plants of the *Brassicaceae* that are more relevant for human nutrition. Besides biochemical alterations of short term UV-B application in *Arabidopsis thaliana* and Broccoli sprouts (*Brassica oleracea* var. *italica*) we are mainly interested in the causal molecular processes. As expected for *Arabidopsis thaliana*, UV-B treatment leads to a decrease of most genes associated with photosynthesis with a concomitant activation of plant defensive genes and others involved in specific secondary compound synthesis. Agilent One-Color Gene Expression Microarray analysis using the Brassica Array with UV-B treated Broccoli sprouts reveal conformity with *Arabidopsis thaliana* but also shows distinct differences. Alterations in gene expression of candidates involved in secondary compound synthesis of Brassica vegetables are currently verified by real-time RT-PCR analysis.

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Published by the Biological Research Center of the Hungarian Academy of Sciences
address: P.O.Box 521, H-6701 Szeged, Hungary
www.brc.hu
Title: Abstracts of the 1st Annual Meeting of COST Action FA0906 UV4growth,
Szeged, Hungary, 7-9 February 2011
2011
ISBN 978-963-508-606-1