



Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

P3 – PLANT RESPONSES TO UVB

P3.1

09:00 Wednesday 1st July 2009

Specific effects of solar UV-B radiation on plant defense mediated by the jasmonate signaling pathway

Carlos L. Ballaré ((IFEVA) Institute for Agricultural Plant Physiology and Ecology and University of Buenos Aires), Patricia V. Demkura ((IFEVA) Institute for Agricultural Plant Physiology and Ecology and University of Buenos Aires), Guillermina Abdala (University of Rio Cuarto), Ian T. Baldwin (Max Planck Institute of Chemical Ecology)

Natural fluences of solar UV-B (c. $2 \mu\text{mol m}^{-2} \text{s}^{-1}$, or 0.1% of the total quantum flux between 285 and 700 nm) induce changes in plant secondary chemistry that have inordinately large effects on plant–insect interactions. The signal transduction pathways that mediate these highly specific effects of UV-B are not known. We investigated the role of jasmonates by measuring the responses to UV-B in wild-type (WT) and transgenic *Nicotiana attenuata* plants silenced in their lipoxygenase gene to block jasmonate production (*as-lox*). In WT plants, UV-B failed to increase the levels of jasmonic acid (JA) or JA-Ile conjugates, but increased the response to wounding of jasmonate-inducible genes and promoted the accumulation of several phenylpropanoid derivatives. Some of the phenylpropanoid responses were missing in *as-lox*, whereas the effect of UV-B on accumulation of flavonoids was similar in both genotypes. *As-lox* plants were more susceptible to high UV-B than WT plants, suggesting that the phenylpropanoid derivatives that are not produced in these jasmonate-deficient plants are important for UV-B protection. In an herbivory trial under field conditions, *as-lox* plants supported more insect damage than WT plants, as expected. More importantly, the effects of UV-B increasing plant resistance to herbivory, which were highly significant in WT plants, were not detected in the *as-lox* line. We conclude: (1) some effects of natural UV-B fluxes on plant secondary chemistry require jasmonate biosynthesis; (2) these effects are likely mediated by changes in jasmonate sensitivity, and play a critical role in both UV-B protection and UV-B-induced anti-herbivore defense.

Email Address for correspondence: ballare@ifeva.edu.ar

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P3.2

11:10 Wednesday 1st July 2009

Regulation of UV-B-induced photomorphogenesis in *Arabidopsis*

Henriette Gruber (University of Freiburg), Roman Ulm (University of Freiburg), Marc Heijde (University of Freiburg)

Organisms living on the surface of the earth are exposed to UV-B radiation intrinsic to sunlight. Being sessile photosynthetic organisms, plants necessarily have to deal with UV-B radiation and its impact. However, UV-B is not only a stress factor but also triggers specific and protective photomorphogenic responses mediated by a molecularly unknown UV-B photoreceptor. Concerning this latter aspect, we still have only limited knowledge on components mediating signal transduction and on how integration with other environmental factors is enabled. Recently several players of the regulatory UV-B signalling pathway were identified: the bZIP transcription factor ELONGATED HYPOCOTYL 5 (HY5), the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and the beta-propeller protein UV RESISTANCE LOCUS 8 (UVR8). However, negative regulators of the corresponding pathway are not yet described. We will present two novel factors of previously unknown function that we named REPRESSOR OF UV-B-INDUCED PHOTOMORPHOGENESIS (RUP) 1 and 2. These two proteins are highly similar to each other and act redundantly as negative regulators of the UVR8-/COP1-mediated UV-B pathway.

Email Address for correspondence: roman.ulm@biologie.uni-freiburg.de

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P3.3

11:50 Wednesday 1st July 2009

Evidence of physiological action of phototropin1 in response to UV-C illumination

Melissa Hamner (University of Florida), Erin H. Kowalik (Trinity University), Kevin M. Folta (University of Florida), James Shinkle (Trinity University)

Plants maintain a suite of sensitive photosensors that monitor the light environment and adjust plant form and function to best match ambient conditions. A previous study demonstrated that the blue-light sensing phototropin1 (phot1) receptor achieves an atypical phosphorylation state following irradiation with UV-C (250–300 nm). To date, no phot-dependent UV-C effect on physiology has been described. We applied genetic and physiological tools look for phot-1 mediated growth responses to UV-C. Using highly-sensitive monitoring systems stem growth kinetics were measured in cucumber and *Arabidopsis thaliana* responding to a short, single pulse of UV-C (254 nm) irradiation. The fluence-response kinetics and time course were remarkably similar to that observed following a blue pulse. In *Arabidopsis* the results were similar in cryptochrome and phytochrome mutants, suggesting these receptor classes do not contribute to this response. However, the phototropin mutants exhibited defects in stem growth rate inhibition, achieving only 50% of the wild type response. The remainder of the inhibition was present in all mutants tested, suggesting that it is under the direction of a novel sensor or redundant function. The work demonstrates that UV-C has an effect on a rapid plant photomorphogenic response and that the response is partially mediated by the phototropin1 photoreceptor. Both UVB and UVA cause responses with the same kinetics as UVC, with the implication that phototropins mediate photomorphogenic responses to radiation across the UV spectrum.

Email Address for correspondence: jshinkle@trinity.edu

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P3.4

13:30 Wednesday 1st July 2009

Dosage and time course analysis of maize responses to UV-B

Virginia Walbot (Stanford University), Darren Morrow (Stanford University), John Fernandes (Stanford University), Mabel Campi (CEFOBI, Argentina), Paula Casati (CEFOBI, Argentina)

Transcriptome, proteome, and metabolome data are being developed for two experiments. In protocol #1 a variable number of adult leaves are exposed to UV-B for a fixed time and responses measured in illuminated vs. shielded leaves and in immature ears. In protocol #2, two leaves are exposed for 1, 2, 4, or 6 h with measurements on illuminated and exposed leaves plus ears. Controls are fully exposed and no UV-B greenhouse plants and field plants plus supplemental UV-B and shielded organs. The talk will concentrate on the RNA profiling data. The ultimate goal is to identify the mobile UV-B stress signal molecule(s).

Email Address for correspondence: walbot@stanford.edu

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P3.5

14:30 Wednesday 1st July 2009

Participation of chromatin remodeling proteins in UV-B responses

Paula Casati (UNR, Argentina), Luciana D. Lario (UNR, Argentina), Julia Emiliani (UNR, Argentina), Mabel Campi (UNR, Argentina), Virginia Walbot (Stanford University), Julia Qüesta (UNR, Argentina)

Terrestrial life evolved after the ozone layer formed and could absorb most damaging UV-B in solar radiation. Chromatin remodeling in response to UV-B has been implicated in maize; in addition, several chromatin proteins are also UV-B regulated in *Arabidopsis*.

Therefore, chromatin remodeling capacity appears to be a key process for effective responses to UV-B. ChIP assays were done to monitor chromatin status at several UV-B responsive genes: the promoter and transcribed regions in tolerant maize lines showed different chromatin protein association after UV-B than sensitive lines. Chromatin status was also investigated at terminal inverted repeats of the mutator transposon in maize, both in active and silencing states: reactivation by UV-B is accompanied by histone and DNA demethylation, correlating with an increase in *MuRA* and *B* transcripts. On the other hand, UV-B causes the formation of photoproducts in DNA, which affects the structural and dynamic properties of chromatin. To investigate the role of different chromatin remodeling proteins in DNA repair, *Arabidopsis* T-DNA mutants, and maize and *Arabidopsis* RNAi plants were analyzed. Several chromatin deficient plants (maize *chc101*, *mbd101*, *sdg102* and *nfc102*; and *Arabidopsis* *sdg26*) showed increased UV-B-induced DNA damage; while others (*Arabidopsis* *chc1*) did not show significant differences with WT plants. On the contrary, *ros1* mutants showed increased tolerance to DNA damage, suggesting that chromatin proteins regulate different aspects of DNA repair in plants. Our results indicate that chromatin remodeling is a key process in acclimation to UV-B and that plants deficient in this process are more sensitive to UV-B.

Email Address for correspondence: casati@cefobi-conicet.gov.ar

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P3.6

15:40 Wednesday 1st July 2009

Diurnal changes in UV-shielding in plants

Paul W. Barnes (Loyola University New Orleans), Stephan D. Flint (Utah State University), Ronald J. Ryel (Utah State University)

The accumulation of UV-absorbing compounds in leaf epidermal tissue and the associated decrease in epidermal UV transmittance (T_{uv}) has long been considered a primary protective response of plants to UV exposure. However, the degree and rapidity to which plants can modulate UV shielding is only poorly understood. We conducted non-destructive measurements of T_{uv} with a UVA-PAM fluorometer on several herbaceous plant species growing in various solar UV environments (sun/shade; tropical, subtropical and temperate habitats) to assess general patterns and drivers of diurnal changes in T_{uv} . Our results indicate that 1) T_{uv} varies on a diurnal basis in sun and shade leaves in many, but not all species tested; 2) under clear skies, T_{uv} is typically highest at predawn and sunset, and lowest near solar noon; 3) variation in the magnitude of diurnal changes in T_{uv} appears related to variation in the base-line (predawn) levels of UV shielding (i.e., greater diurnal changes in T_{uv} when predawn values of T_{uv} are low); 4) disruption of the solar radiation regime by clouds or experimental shading alters diurnal patterns in T_{uv} , but 5) under full sun, solar UV does not seem to be necessary to cause these diurnal changes. These findings, and others, indicate that diurnal variation in UV shielding is common in higher plants but variation among species exists in both the magnitude and extent of this response. To date, the significance of this interspecific variation and the mechanisms responsible for these diurnal changes in T_{uv} have yet to be elucidated.

Email Address for correspondence: pwbarnes@loyno.edu

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P3.7**16:00 Wednesday 1st July 2009****UV-B protection in *Arabidopsis thaliana***

Marcel Jansen (University College Cork), Kathleen Hectors (University of Antwerp), Benedicte Le Martret (National University of Ireland Maynooth), Yves Guisez (University of Antwerp), Els Prinsen (University of Antwerp), Maarten Koornneef (Wageningen University)

Plants from high altitudes and/or low latitudes will, typically, be exposed to relatively high doses of UV-B, therefore it might be hypothesised that these plants are particularly well protected from potentially harmful effects of such radiation. Analysis of the UV-susceptibility of 224 *Arabidopsis thaliana* accessions from across the northern hemisphere (15 and 67° N; and –6 and 300 m altitude) revealed a continuum of UV-protection levels. However, the relationship between the geographic origin of accessions and UV-tolerance is complex, and appears to be limited to altered acclimation induction thresholds, rather than UV-tolerance per se. Thus, our data emphasise the importance of (regulation of) inducible UV-B protection responses. Detailed molecular-physiological analysis of the UV-B acclimation response in *A. thaliana* shows parallel changes in gene-expression and plant architecture. Chronic UV-B treatment causes a re-distribution rather than a cessation of growth, and this comprises decreases in rosette diameter, decreases in inflorescence height but increases in the number of flowering stems. Gene expression profiling indicated that UV-induced morphogenesis is associated with subtle changes in phytohormone homeostasis, and this link is confirmed using physiological experiments.

Email Address for correspondence: m.jansen@ucc.ie

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P3.8**16:20 Wednesday 1st July 2009****Interactions between two UV-B protective responses: Morphogenesis and UV screening**

Kathleen Hectors (University of Antwerp), Els Prinsen (University of Antwerp), Sandra Van Oevelen (University of Antwerp), Yves Guisez (University of Antwerp), Marcel Jansen (University College Cork)

Ultraviolet-B (UV-B, 280–315 nm) radiation levels in the biosphere have increased due to the depletion of the stratospheric ozone layer. Frequently observed plant acclimation responses to UV-B irradiation are (1) an altered plant architecture showing shorter stems and hypocotyls, smaller and thicker leaves and increased axillary branching and (2) the accumulation of UV-B screening pigments. A possible relationship between these two parallel occurring acclimation responses is not described so far. Here we present new insights in UV-B induced morphogenesis, in UV-B mediated induction of flavonoids, as well as the potential interactions between these responses.

Arabidopsis thaliana plants treated with chronic, low fluence-rate UV-B radiation showed a decreased petiole length, leaf blade length and width. Because auxin is one of the key regulators of leaf growth and development, we examined the impact of UV-B on auxin metabolism, by histochemical DR5-GUS staining, gene expression quantifications and endogenous IAA measurements in UV treated rosette leaves. In addition, we also monitored the acclimation responses of several ethylene and auxin mutants. Remarkably, a

detailed analysis revealed altered accumulations of kaempferol and quercetin derivatives in the leaves of IAA and ethylene mutants. A role for flavonoids as endogenous regulators of IAA transport in roots and stems is well-documented, while possible interactions in leaves are poorly examined. Here we propose a model describing the interaction between UV-B, auxin, ethylene and flavonoids to direct changes in morphology and plant growth.

Email Address for correspondence: kathleen.ectors@ua.ac.be

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P3.9**Poster Session – Tuesday 30th June 2009****Effects of solar UVA and UVB on gene expression and phenolic accumulation in *Betula pendula* leaves**

Luis O. Morales (University of Helsinki), Riitta Tegelberg (University of Helsinki), Brosche Mikael Brosché (University of Helsinki), Anders Lindfors (Finnish Meteorological Institute), Markku Keinänen (University of Joensuu), Pedro J. Aphalo (University of Helsinki)

The effects of solar UVA and UVB on phenylpropanoid gene expression and phenolic accumulation in *Betula pendula* leaves were studied in a field test during summer 2007. Plants were grown in the field under six radiation conditions (UV-B 100%, UV-A 100%; UV-B 0%, UV-A 100%; UV-A 0%, UV-B 0%; UV-B 50%, UV-A 100%; UV-B 50%, UV-A 50%; and UV-B 0%, UV-A 50%), all created by using plastic films that exclude the UV spectrum. Young and fully expanded leaves were sampled after 30 days of initiation of the experiment. A total of twenty-three individual compounds were identified by HPLC-MS. There was an overall treatment effect on the accumulation of six flavonoids at $P < 0.03$. The individual effects of UVA and UVB on these compounds was analyzed at $P < 0.10$. UVA regulated the accumulation of quercetin-3-galactoside $P < 0.0709$, quercetin-3-arabinyranoside $P < 0.0875$, both compounds were also affected by UVB ($P < 0.0662$, $P < 0.0980$ respectively). Myricetin-3-galactoside, quercetin-3-glucuronide, quercetin-3-rhamnoside, and kaempferol-3-rhamnoside were affected by UVB at $P < 0.005$. Transcript accumulation of nine phenylpropanoid genes and three transcription factors involved in plant response to UV was analysed by RT-PCR. Genes such as PAL, CHI, MYB4, TT2, F3H and HYH were clearly down-regulated when both UVA and UVB were completely excluded. Contrarily the expression of CHS, N3D, TT3, F5H, 4Cl, and OMT1 was indistinctly regulated by UVA and UVB.

Email Address for correspondence: luis.morales@helsinki.fi

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P3.10**Poster Session – Tuesday 30th June 2009****Waveband-dependence of UV effects on grape quality in New Zealand**

Rainer Hofmann (Lincoln University), James Shinkle (Trinity University), Chris Winefield (Lincoln University), Brian Jordan (Lincoln University)

New Zealand has relatively high levels of UV radiation, including UV-A and UV-B. While UV-B is recognised as a stress factor for plants, less is known about the roles of UV-A. The aim of this study was to investigate the effects of UV-A and UV-B on *Vitis vinifera* cv. Sauvignon Blanc grape chemistry under New Zealand field conditions. We hypothesised that

differential application of UV treatments to the fruit zone will affect grape quality. Using a variety of plastic filters, the fruit zone of Sauvignon Blanc vines was exposed to all ambient UV wavelengths, or to longer-wavelength UV-B and UV-A, or only to UV-A, or it was shielded from most UV. High performance liquid chromatography (HPLC) was used on pigment extracts and juice to examine UV effects on grape quality, including accumulation of total UV-absorbing compounds, flavonoids and amino acids. The results showed that effects of ambient UV radiation in NZ on grape quality are highly waveband-specific. While grape morphology was affected only by UV-B, levels of total UV-absorbing compounds in grape skins increased with decreasing UV wavelengths. UV-induced accumulation of flavonol glycosides were largely due to UV-A, increasing UV-absorbance and antioxidant potential of the grape skin tissue. UV-A also increased amino acid levels, potentially altering yeast-available N levels for wine production. The implications of these findings for vineyard management will be discussed.

Email Address for correspondence: Rainer.Hofmann@lincoln.ac.nz

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P3.11

Poster Session – Tuesday 30th June 2009

Leaf and cell development during UV-B acclimation in *Arabidopsis thaliana*

Eveline Jacques (University of Antwerp), Kathleen Hectors (University of Antwerp), Yves Guisez (University of Antwerp), Jean-Pierre Verbelen (University of Antwerp), Kris Vissenberg (University of Antwerp), Els Prinsen (University of Antwerp), Marcel Jansen (University College Cork)

Plant growth and development is controlled by a wide range of endogenous and exogenous clues. Plants developed a range of protection mechanisms against suboptimal environmental conditions. One of these generic responses is an altered plant architecture, often resulting in a reduction in leaf and stem growth. The mechanisms behind these environmentally controlled morphogenetic traits are, however, poorly understood.

In this study, the effects of low dose, chronic ultraviolet-B radiation on *Arabidopsis* leaf development are monitored during the acclimation period (after 7, 12 and 19 days). Different leaf parameters are analyzed such as leaf and petiole length, leaf width and blade area, showing that UV-B radiation impacts on leaf elongation. This triggers the question if UV-B affects cell division or cell elongation. In order to answer this question, cell size measurements are performed on the adaxial epidermal cell layer. Expanding leaves of *Arabidopsis* show a strong gradient in cell size and cell developmental status along the proximal-distal axis. Therefore three distinct regions are

considered (base, middle and top). The results indicate that UV-B affects only cell growth and not cell division of the pavement cells. The typical cell shape is also unaltered.

These observations reveal new insights in UV-B induced changes in epidermal cell development.

Email Address for correspondence: eveline.jacques@ua.ac.be

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P3.12

10.30 Wednesday 1st July

Photomorphogenic UV-B perception and signal transduction in *Arabidopsis*

Gareth I. Jenkins (University of Glasgow)

Plants are constantly exposed to ultraviolet-B (280–315 nm) radiation in sunlight. UV-B has the potential to cause damage to macromolecules and consequently plants need to protect against and repair UV damage to survive. Acute exposure of plants to high fluence rates of UV-B may elicit stress responses via signalling pathways that overlap with DNA damage, defence and wound signalling pathways. In contrast, low fluence rates of UV-B stimulate the expression of genes involved in UV-protective responses. Hence the low fluence 'photomorphogenic' UV-B signalling pathway(s) promotes plant survival in UV-B. 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 signalling pathway that orchestrates expression of a range of genes with vital UV-protective functions (Brown et al., 2005, PNAS 102, 18225–30). *Arabidopsis* *uvr8* mutants are therefore highly susceptible to damage by UV-B. UVR8 regulates expression of the transcription factor HY5 specifically in response to UV-B. HY5, acting in conjunction with the related HYH transcription factor is a key effector of the UVR8 pathway. UVR8 is a 7-bladed propeller protein that binds to chromatin via histones. Chromatin immunoprecipitation showed that UVR8 associates with chromatin at the HY5 locus, providing a mechanistic basis for its involvement in regulating transcription. UV-B exposure promotes the rapid nuclear accumulation of UVR8. Furthermore, there is evidence that UV-B stimulates an interaction between UVR8 and the COP1 protein. The role of UVR8 in photomorphogenic UV-B responses will be discussed.

Email address for correspondence: G.Jenkins@bio.gla.ac.uk

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