evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

An Enlightening Tale about UV-B Radiation

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University College Cork, Ireland

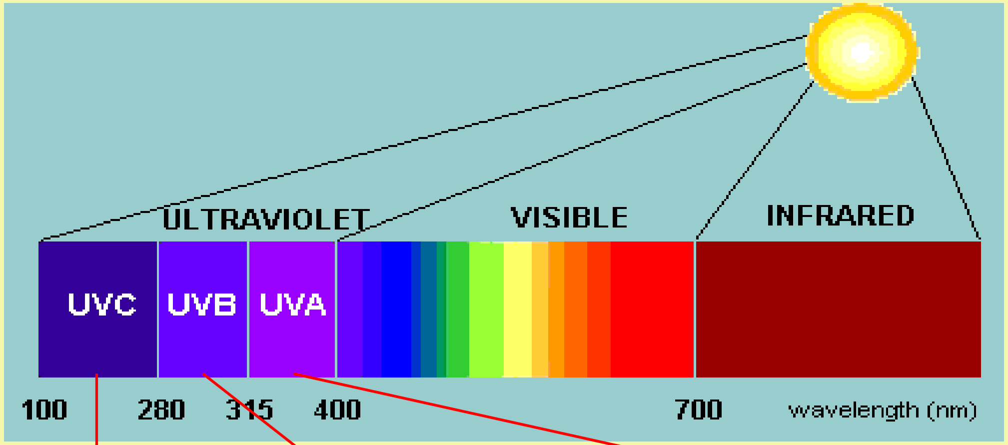
Tato akce se koná v rámci projektu:
Vybudování vědeckého týmu environmentální metabolomiky a ekofyziologie a jeho zapojení do mezinárodních sítí (ENVIMET; r.č. **CZ.1.07/2.3.00/20.0246**) realizovaného v rámci Operačního programu Vzdělávání pro konkurenceschopnost.

Topics

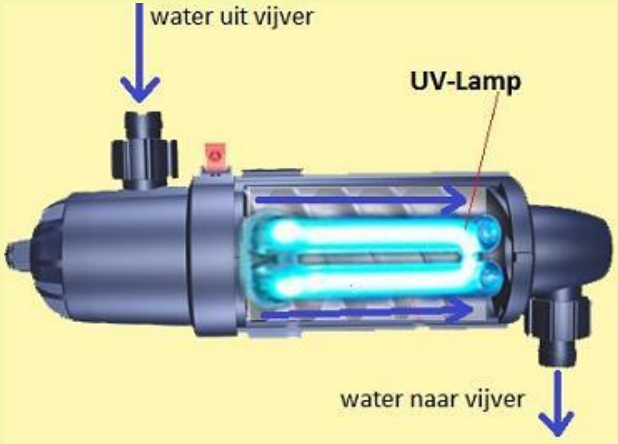
- What is UV-B radiation?
- What does UV-B do to living organisms?
- How can one study effects of UV-B radiation on plants?
- What does UV-B radiation do to plants?
- How do plants perceive UV-B radiation?
- Why study effects of UV-B radiation?

What is UV-B radiation?

What is UV-B radiation?



- UV = natural component sunlight
- UV; wavelengths between 100 – 400nm
- UV in biosphere >~295nm

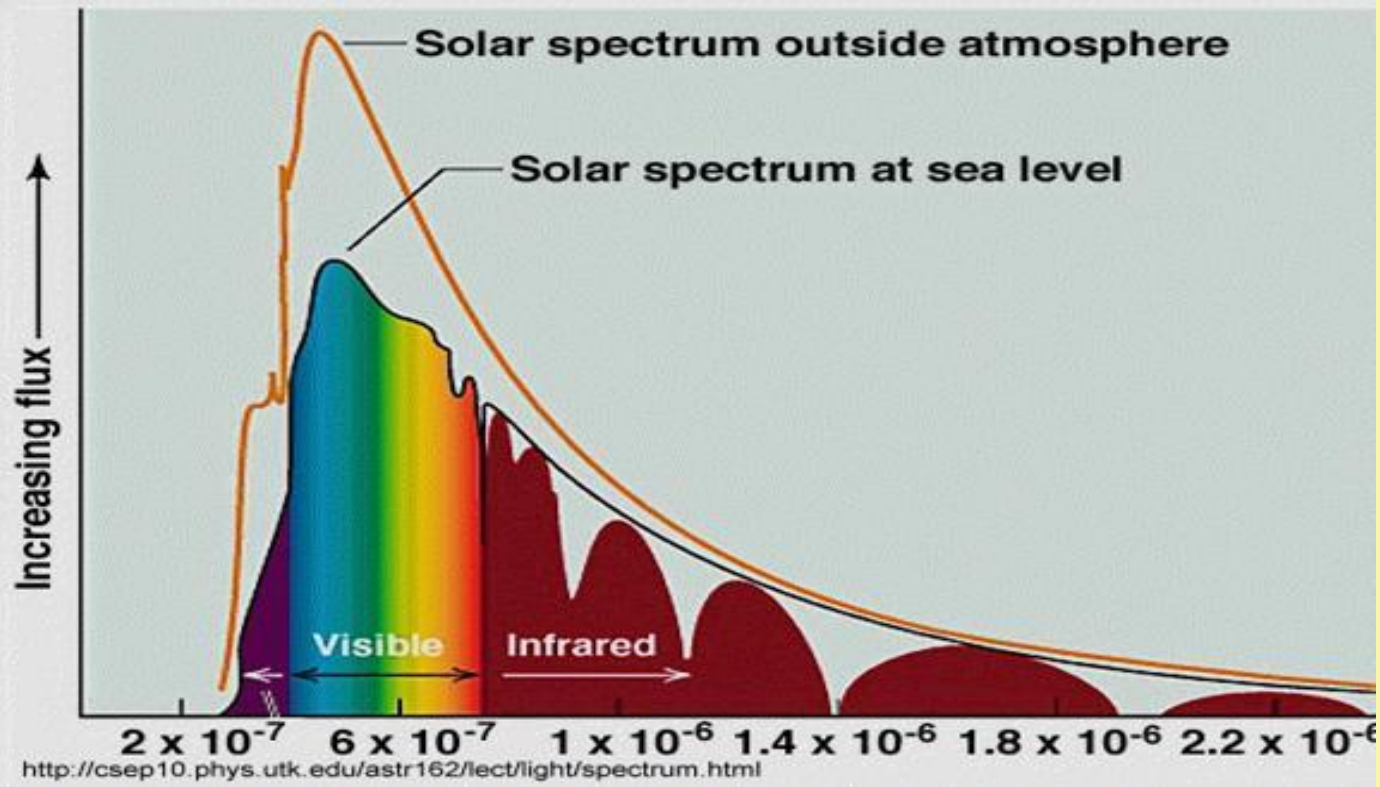


UV-C; 100 – 280 nm

UV-B;
280 – 315nm

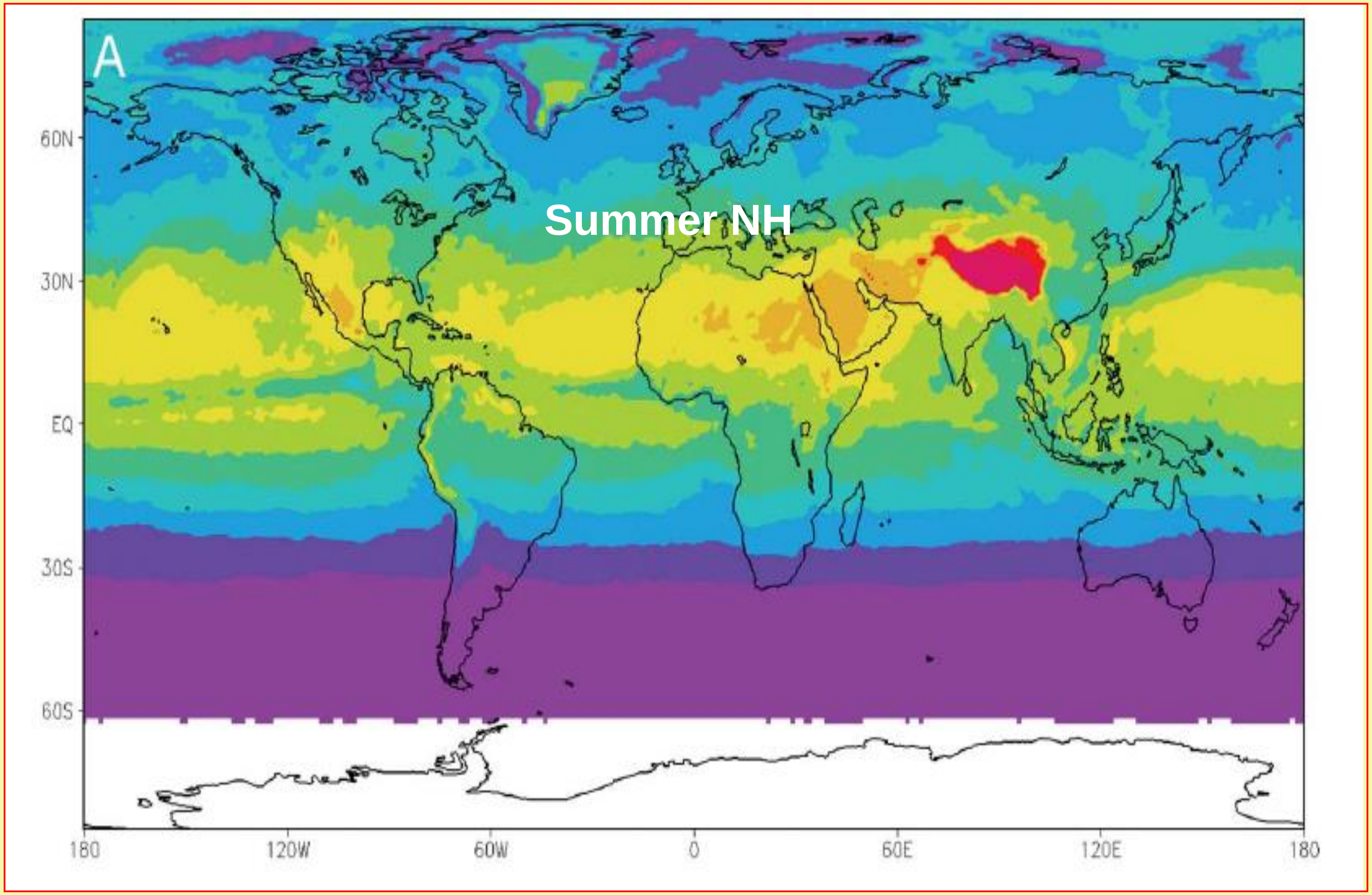
UV-A; 315 – 400 nm

Filtering of sunlight (O_3, O_2, H_2O and CO_2)



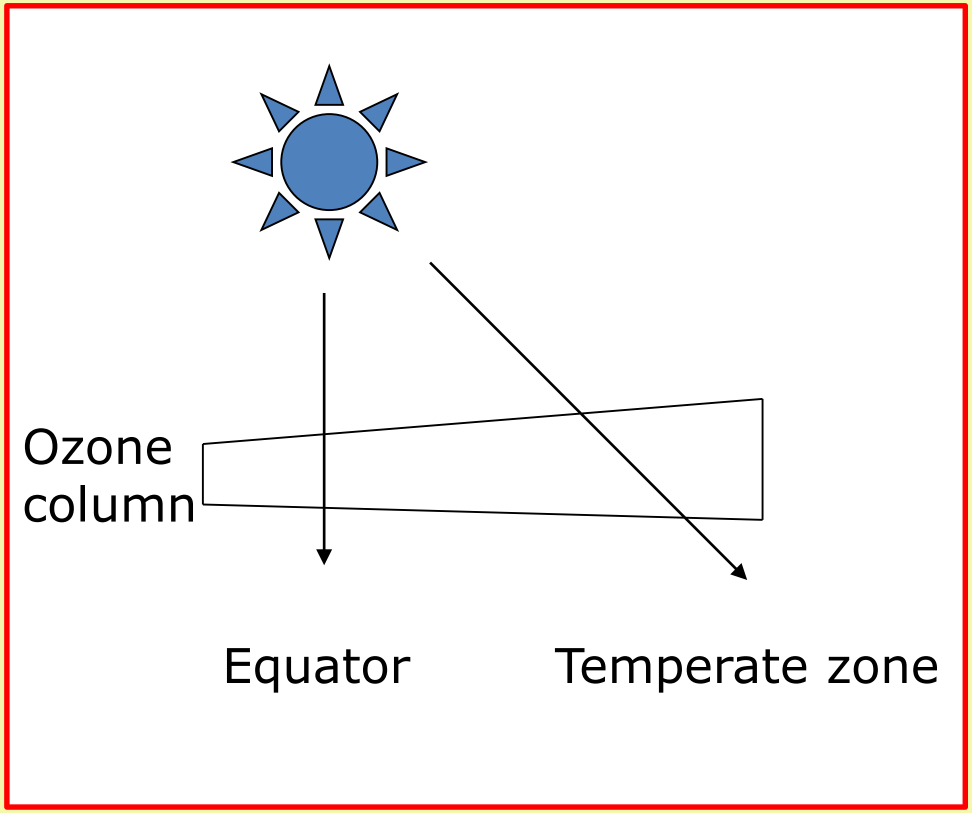
All solar UV-C, and part of solar UV-B are filtered out by the stratospheric (10-30km altitude) ozone layer

A ~6 fold latitudinal & altitudinal UV-B gradient



McKenzie, Aucamp, Bais, Björn, & Ilyas (2007)
Photochem. Photobiol. Sci 6, 218-231

A ~6 fold latitudinal & altitudinal UV-B gradient



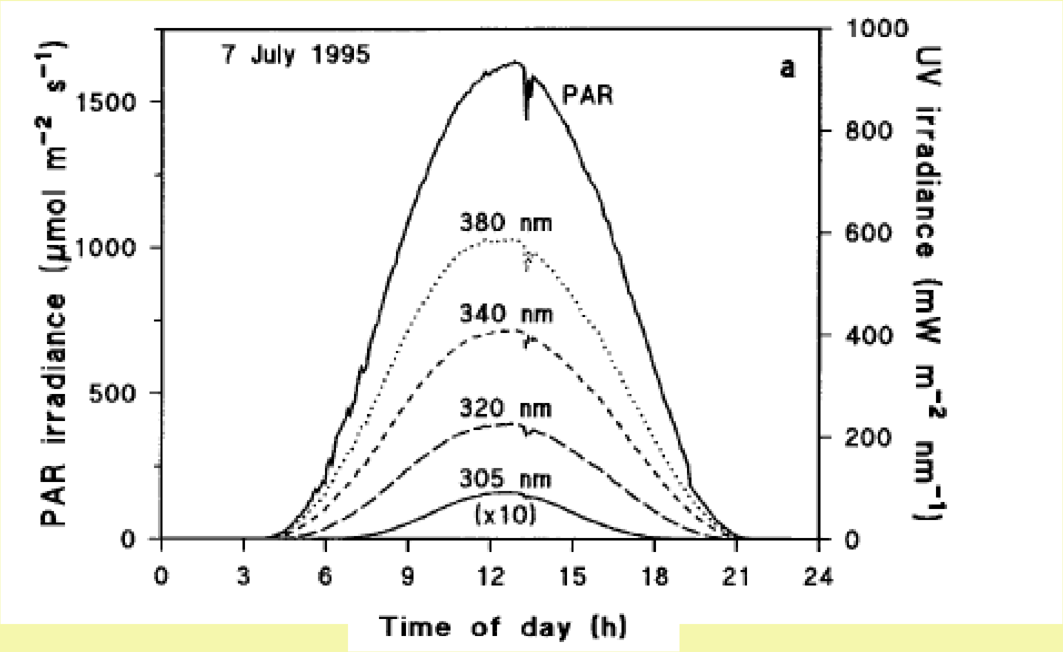
Most UV-B near equator

- Solar angle determines length path through ozone layer (i.e. shortest screening pathway nr equator)

- Stratosperic ozone layer thinnest near equator

- Hours of sunshine / meteorological conditions

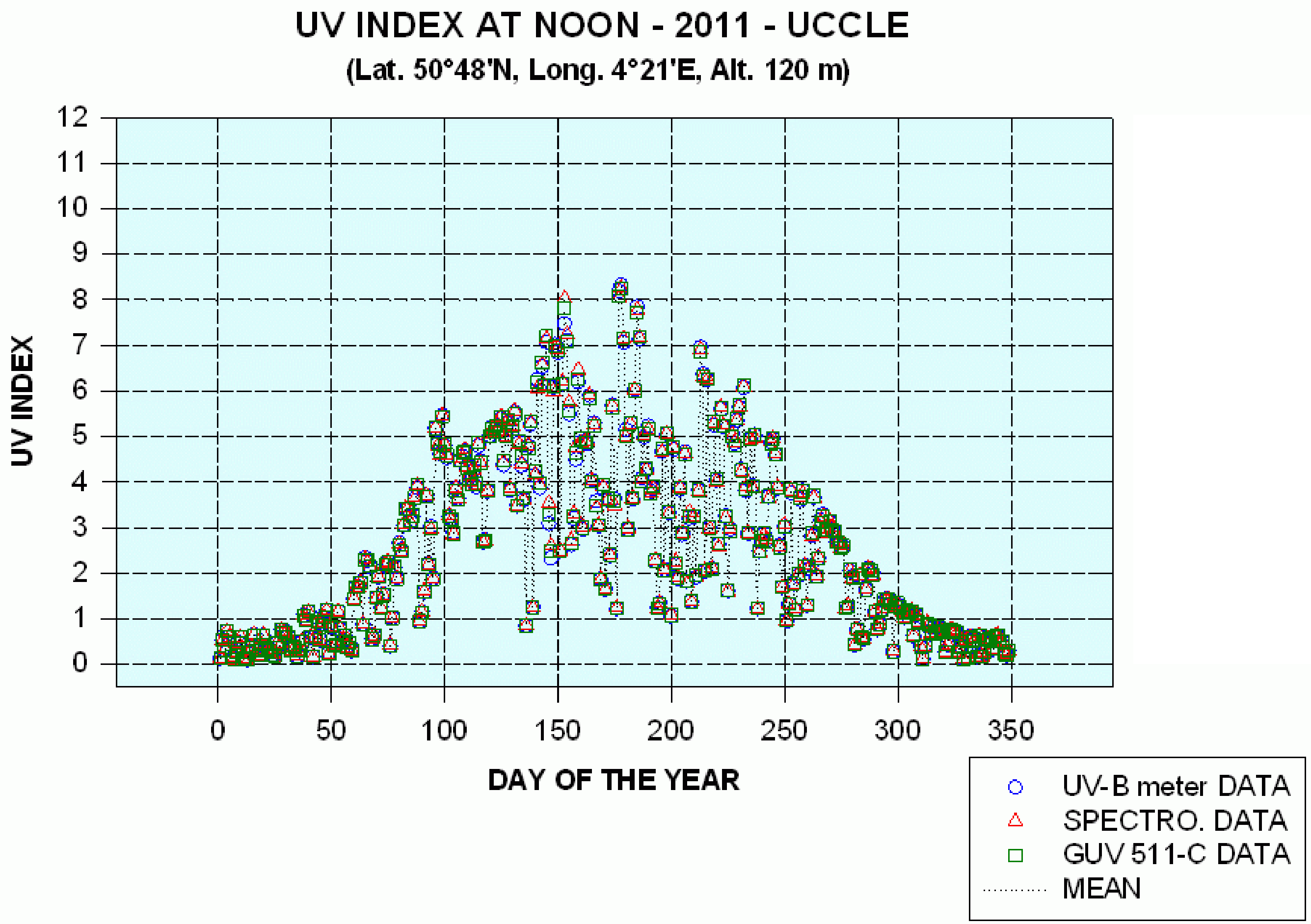
Daily/seasonal variations UV-B



Dring et al., Helgol Mar Res 2001

Solar angle (i.e. length pathway through ozone layer and atmosphere) is also responsible for relatively low levels of UV-B during dawn/dusk and winter.

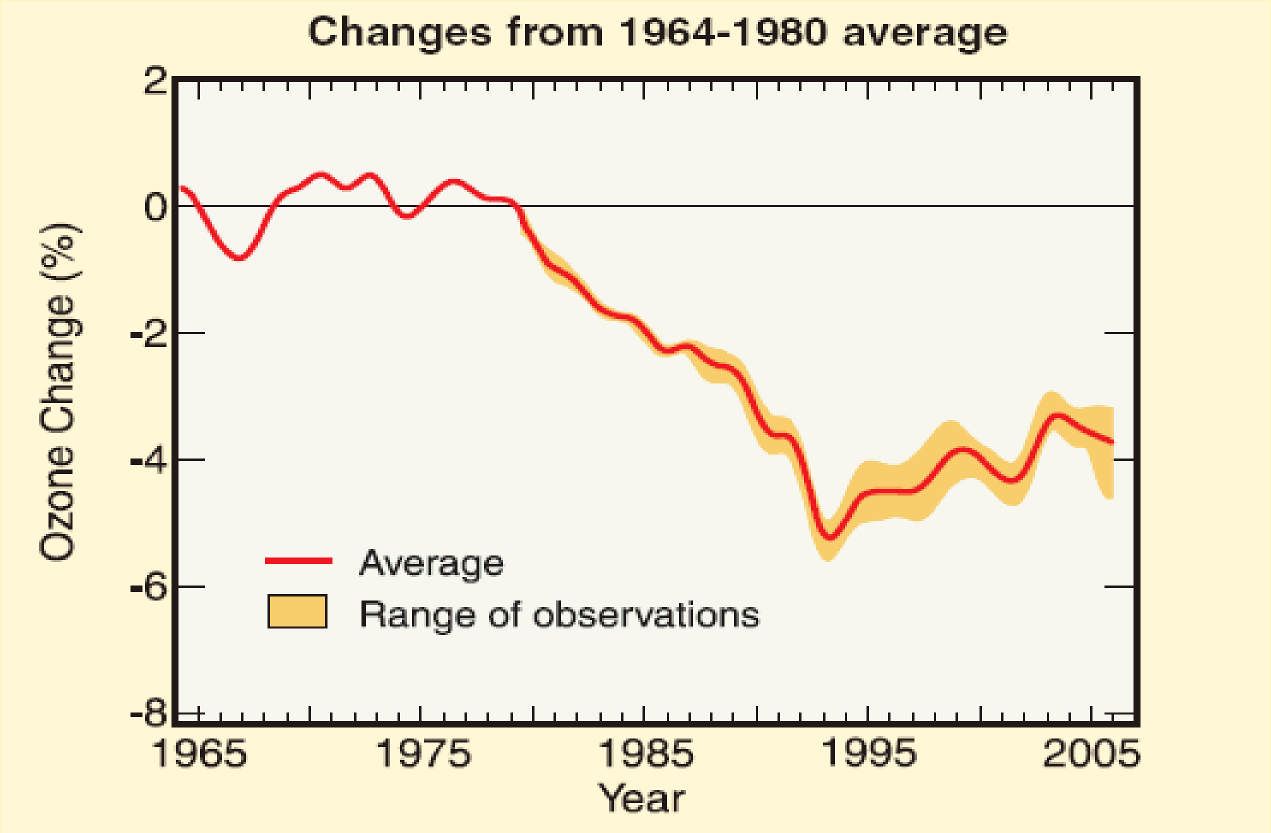
Daily/seasonal variations UV-B



UV-B doses in the environment are highly variable!

(experimental design?)

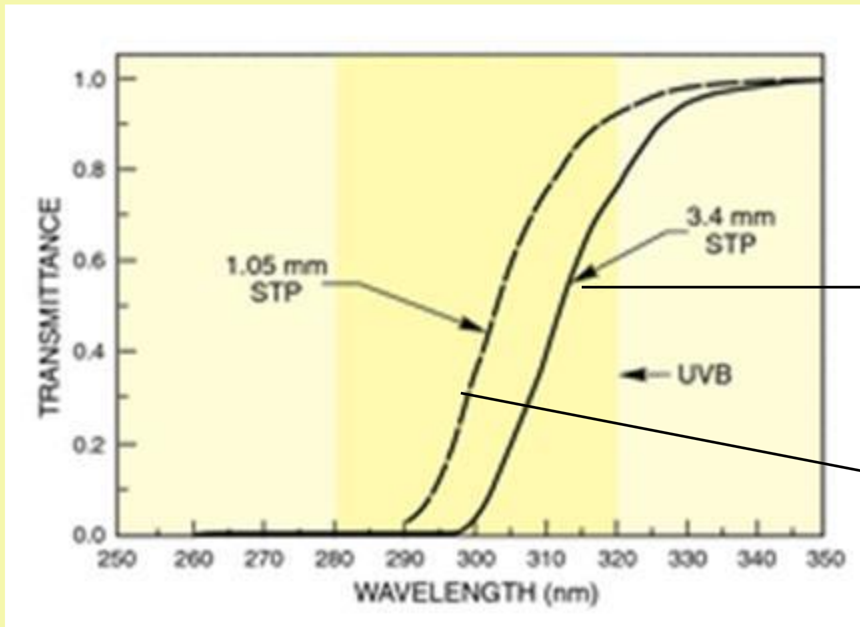
Stratospheric ozone layer depletion



Ground based measurements
60°S-60°N; WMO 2006

Ozone layer depletion

(worldwide 20-40% increases UV-B since 1980s)



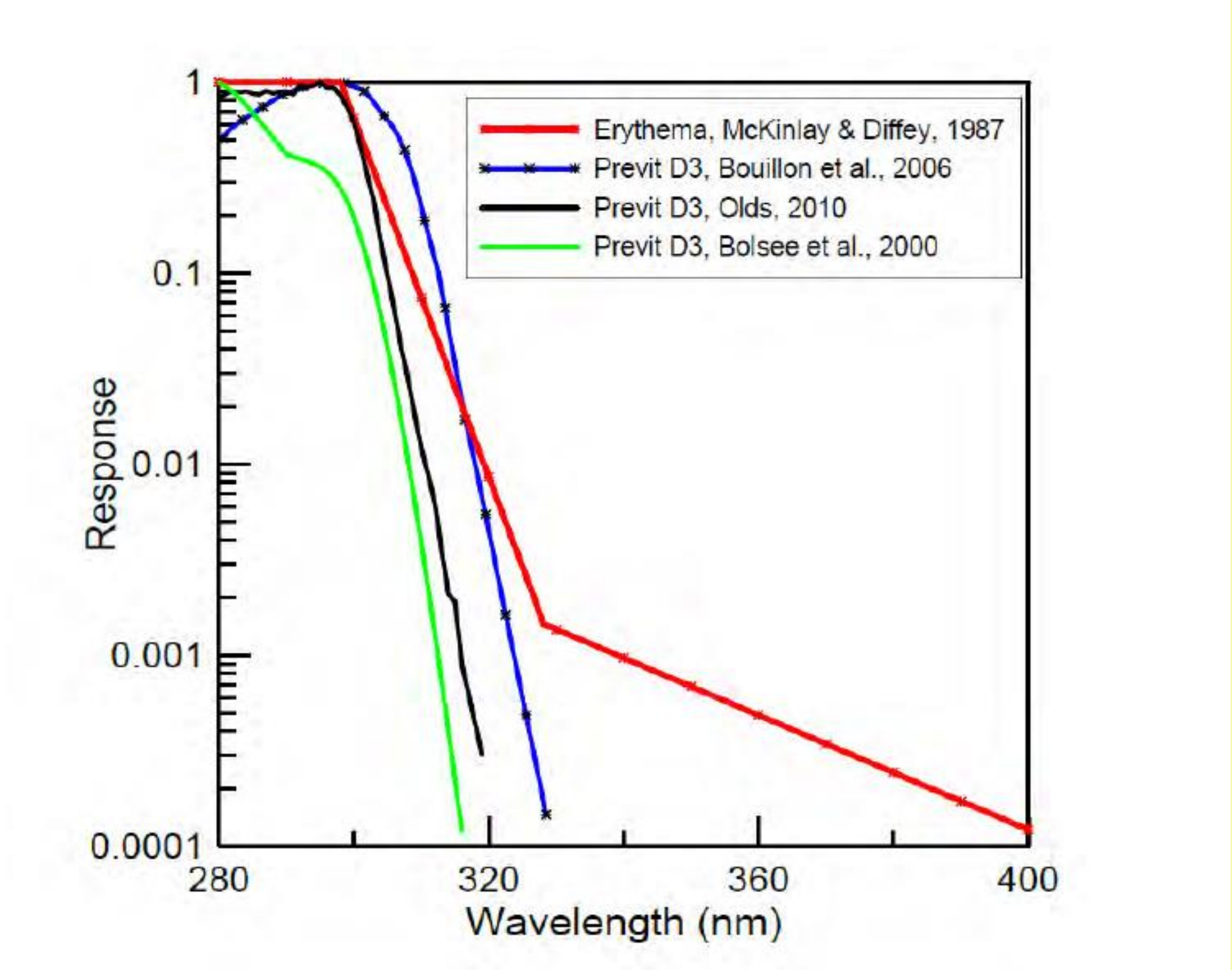
Full ozone

Depleted ozone

Consequences:

- Increase in total UV-B
- Shift spectrum towards shorter wavelengths

The shorter the wavelength, the more reactive the radiation



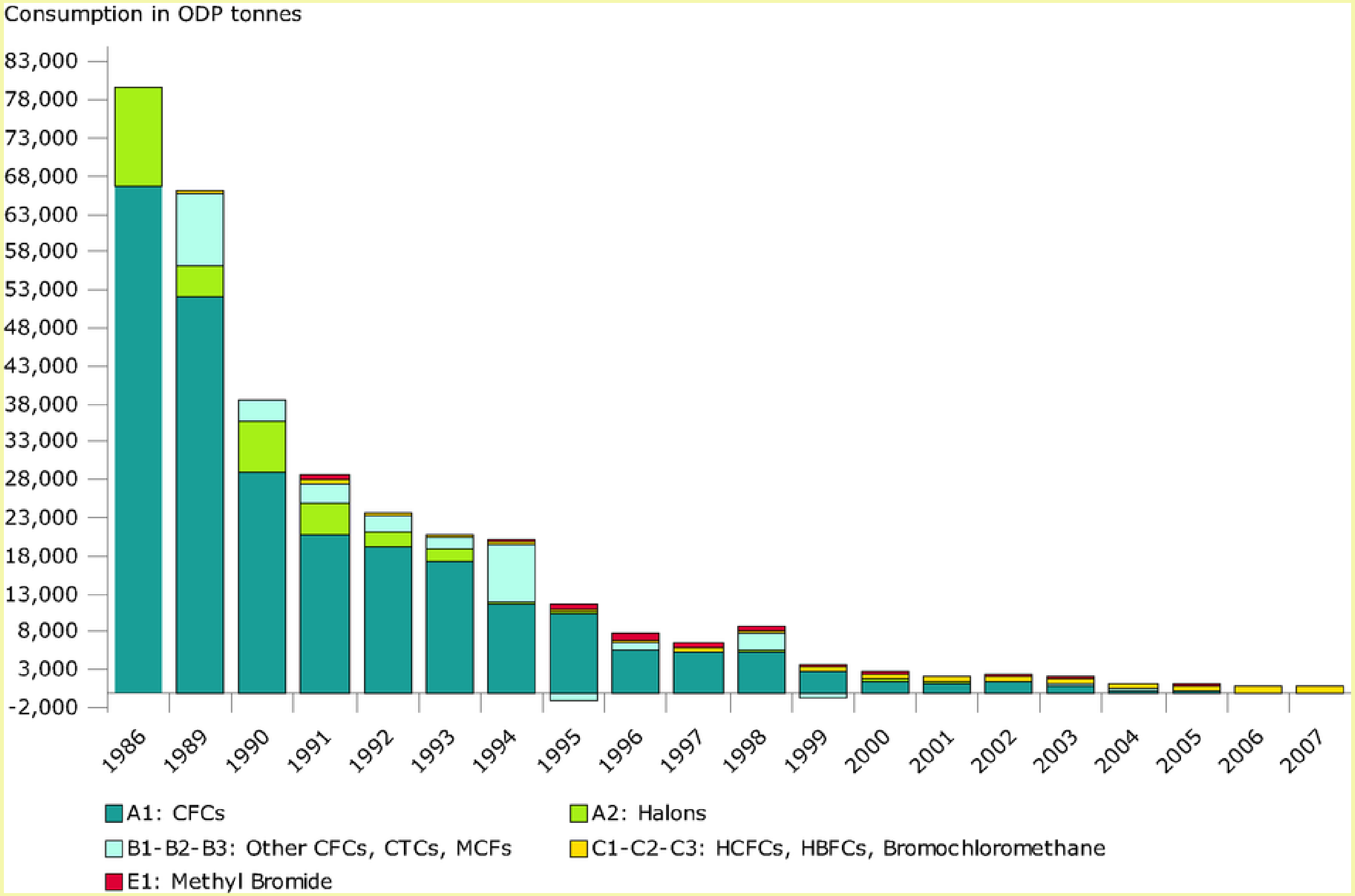
Biologically effects typically increase by orders of magnitude with decreasing UV-B wavelengths

Montreal protocol

- Montreal Protocol on Substances that Deplete the Ozone Layer (1987)
- Phases out production of CFCs and related compounds

The Montreal protocol works!!!

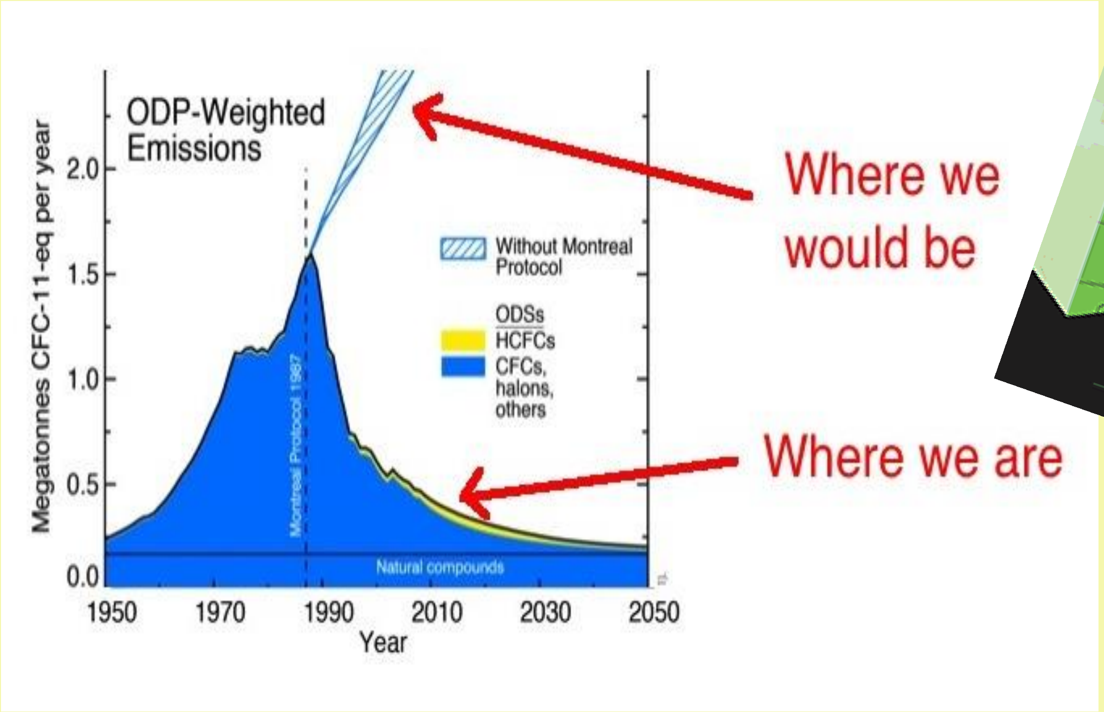
Rapidly decreasing use ozone depleting substances

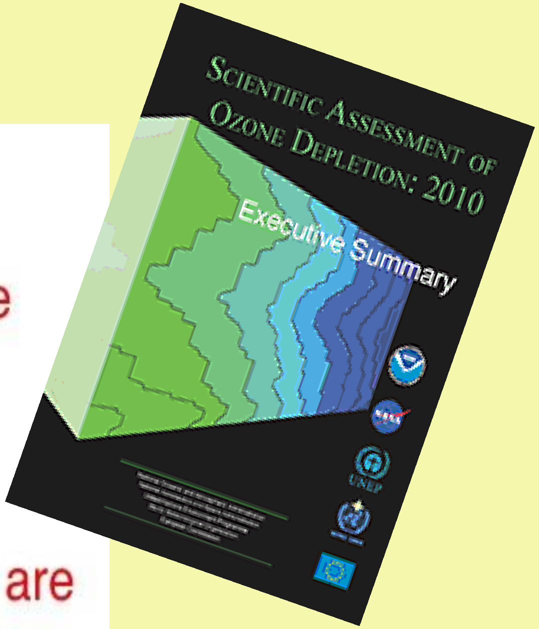


European Environment Agency (2007); Production and consumption of ozone depleting substances by EU-27 (CSI 006)

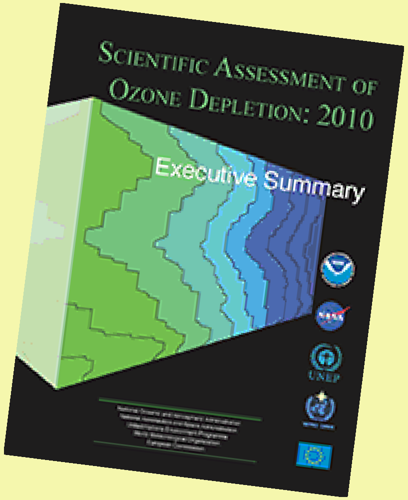
The Montreal protocol works!!!

Rapidly decreasing use ozone depleting substances





The Montreal protocol works!!!



UNEP-2014 report

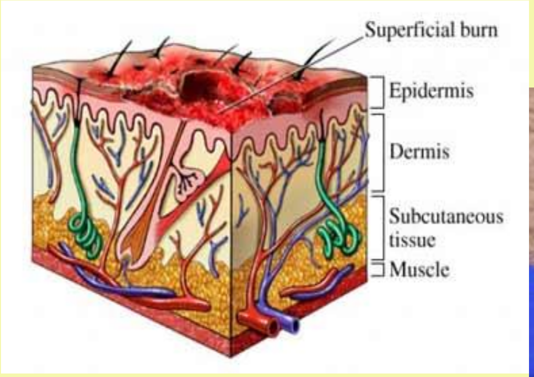
- Decrease statospheric ozone has been halted
- Slow decline levels ozone depleting substances

- **Some evidence recovery stratospheric ozone**
- No statistically significant decrease of UV in biosphere

- **Tropospheric ozone in northern hemisphere decreases impact stratospheric ozone layer depletion**

What does UV-B do to living organisms?

UV-B - Stressor

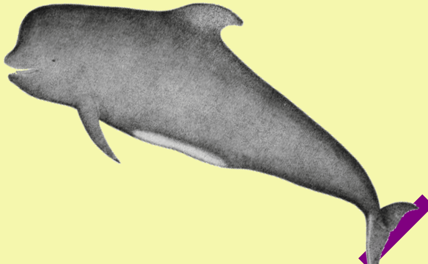


Cellular (DNA) damage and inflammatory response

UV-B - Stressor

Domestic pig with sunburn
Commercial problem for free range / organic farming

UV sensitivity shorn sheep



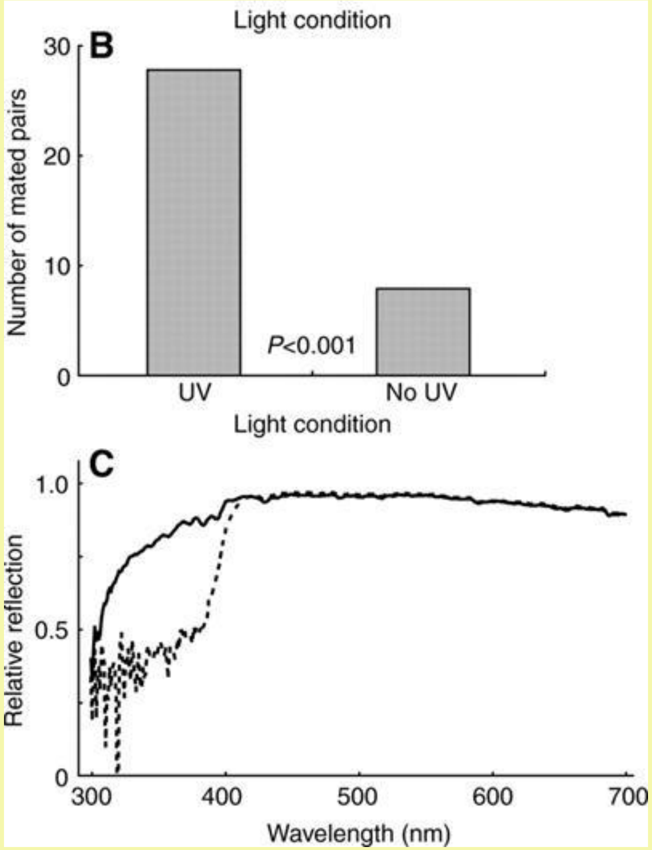
UV-B - environmental information

UV-vision based on UV-transmitting lens, and UV-sensitive cones

347 out of 968 surveyed bird species, displayed UV reflective patterns

MULLEN & POHLAND (2008), Ibis.

Male cabbage butterflies (*Pieris rapae crucivora)* detect UV-reflecting females easiest in a UV-rich environment



Number of mated pairs in closed arenas

Reflection of ventral side of a female hind wing in the UV-present (solid) and in the UV-absent (dotted) arenas.

Obara Y et al. J Exp Biol 2008; 211:3698-3702

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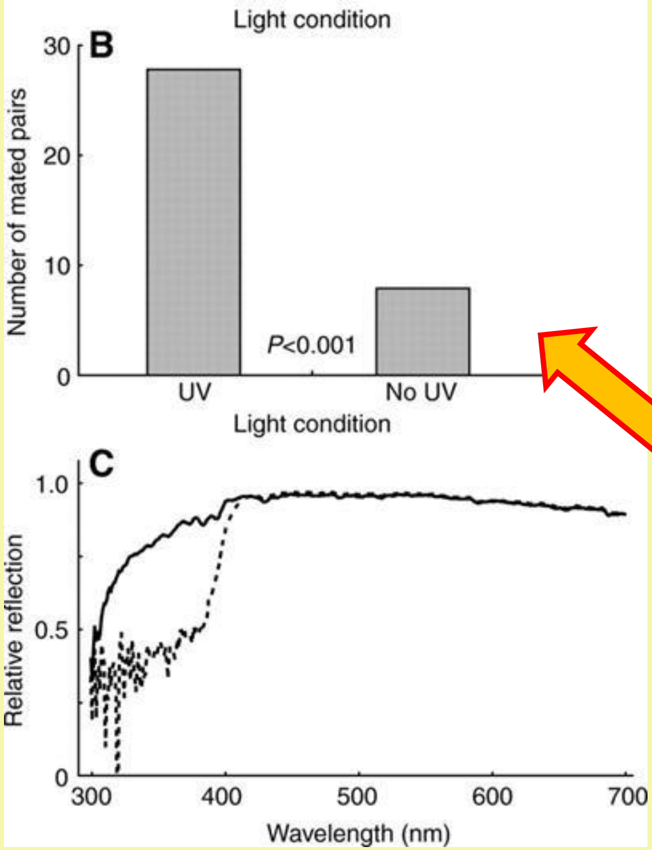


Number of mated pairs in closed arenas

Reflection of ventral side of a female hind wing in the UV-present (solid) and in the UV-absent (dotted) arenas.

Horticultural importance!!!

Obara Y et al. J Exp Biol 2008; 211:3698-3702

The two-faces of UV-B photobiology

Low UV doses
Specific information / regulator

High UV doses
Stressor

The two (or three)-faces of UV-B photobiology

Photochemistry

Low UV doses
Specific information / regulator

High UV doses
Stressor

Exposure to high UV-B; an unavoidable consequence of photosynthetic life

How can we study the effects of UV-B radiation on plants?

How to study the impact of UV-B?

Exploiting elevational UV-gradients

Rozema et al. (1997) Plant ecology

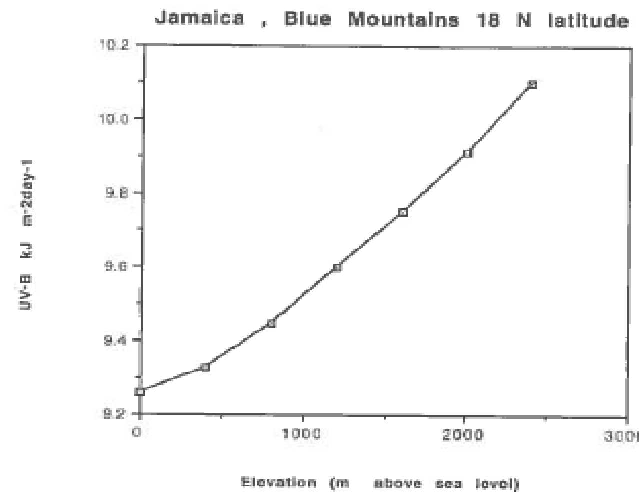
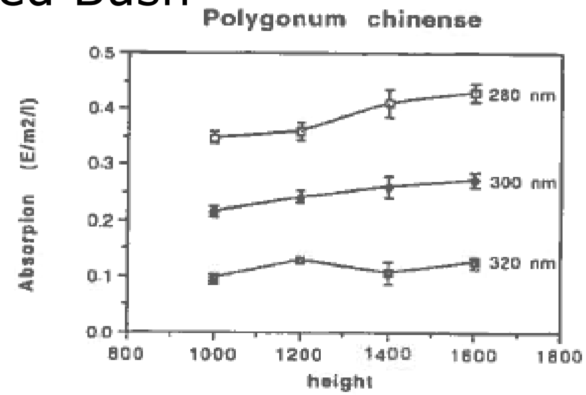


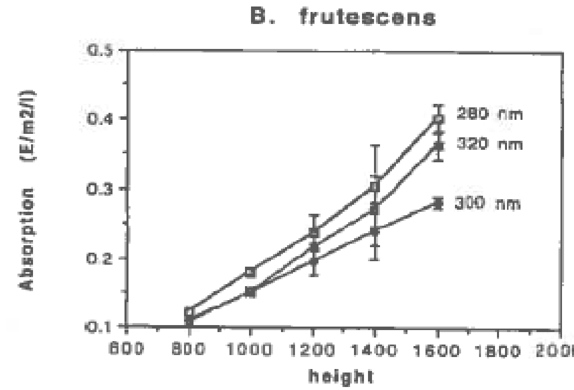
Figure 2. The relationship between the UV-B fluence rate (kJ m^{-2} day^{-1}) along an elevational transect 0–2400 m above sea-level in Jamaica (18° N) latitude. Fluence rate values have been calculated based on an empirical model (Green et al. 1980) and using the Generalized Plant Weighting function (Caldwell 1977) for May 1, 1995.

UV-B absorbing pigments along an elevational gradient

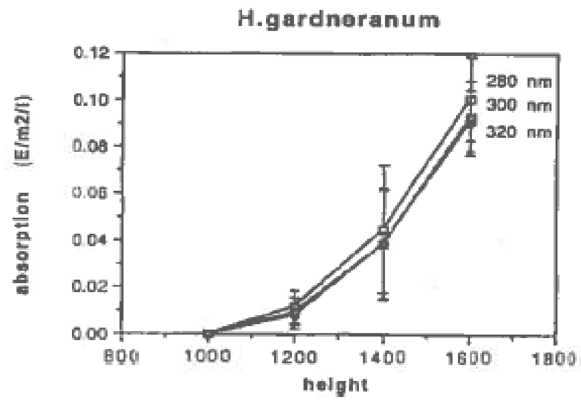
Red Bush



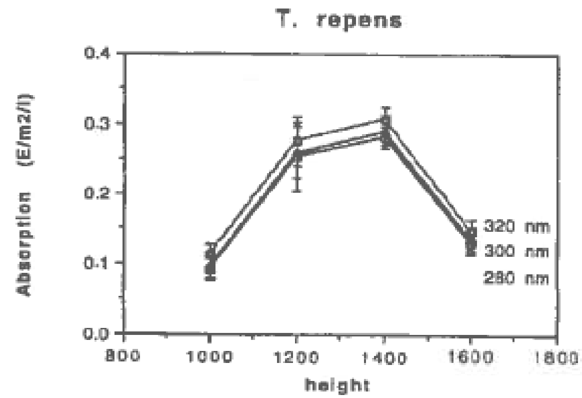
John Crow Bush



Wild Ginger



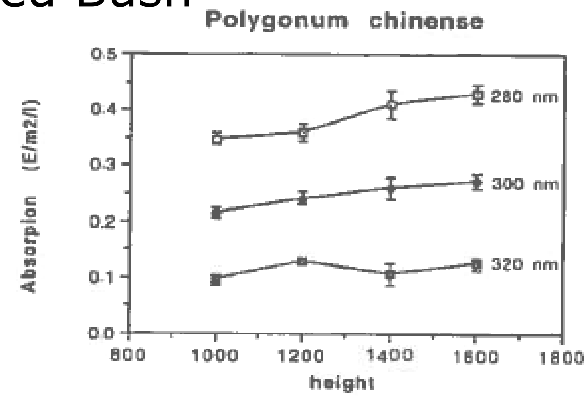
White Clover



Rozema et al. (1997) Plant ecology

UV-B absorbing pigments along an elevational gradient

Red Bush



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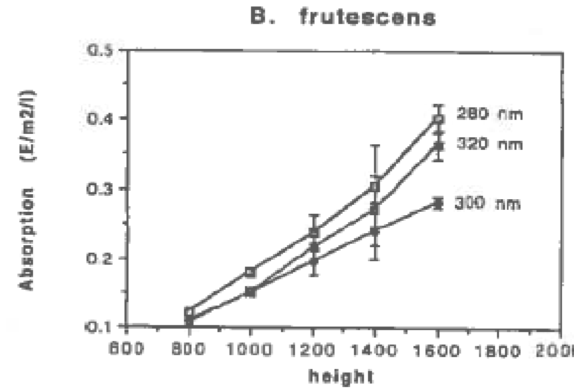
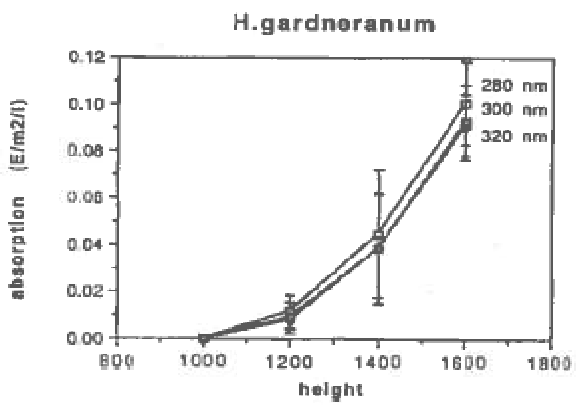
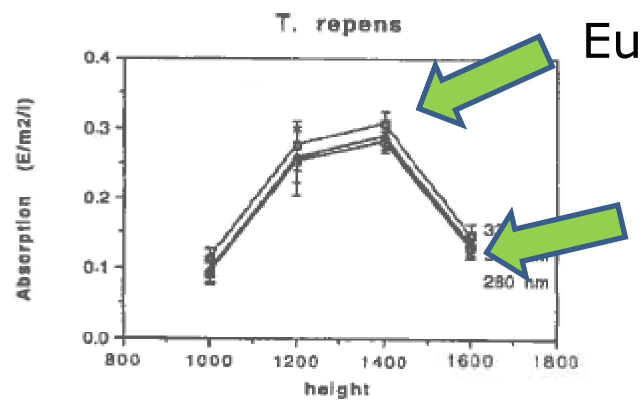


Wild Ginger



White Clover



Eustress?

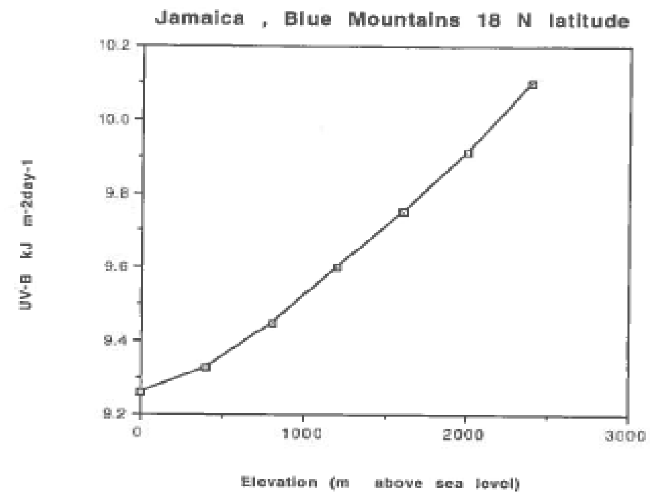
Distress?

Rozema et al. (1997) Plant ecology

How to study the impact of UV-B?

Exploiting elevational UV-gradients

- Difficult to pinpoint impact UV-B relative to other environmental factors
- Ecologically relevant
- **Plants grow in their natural habitat**



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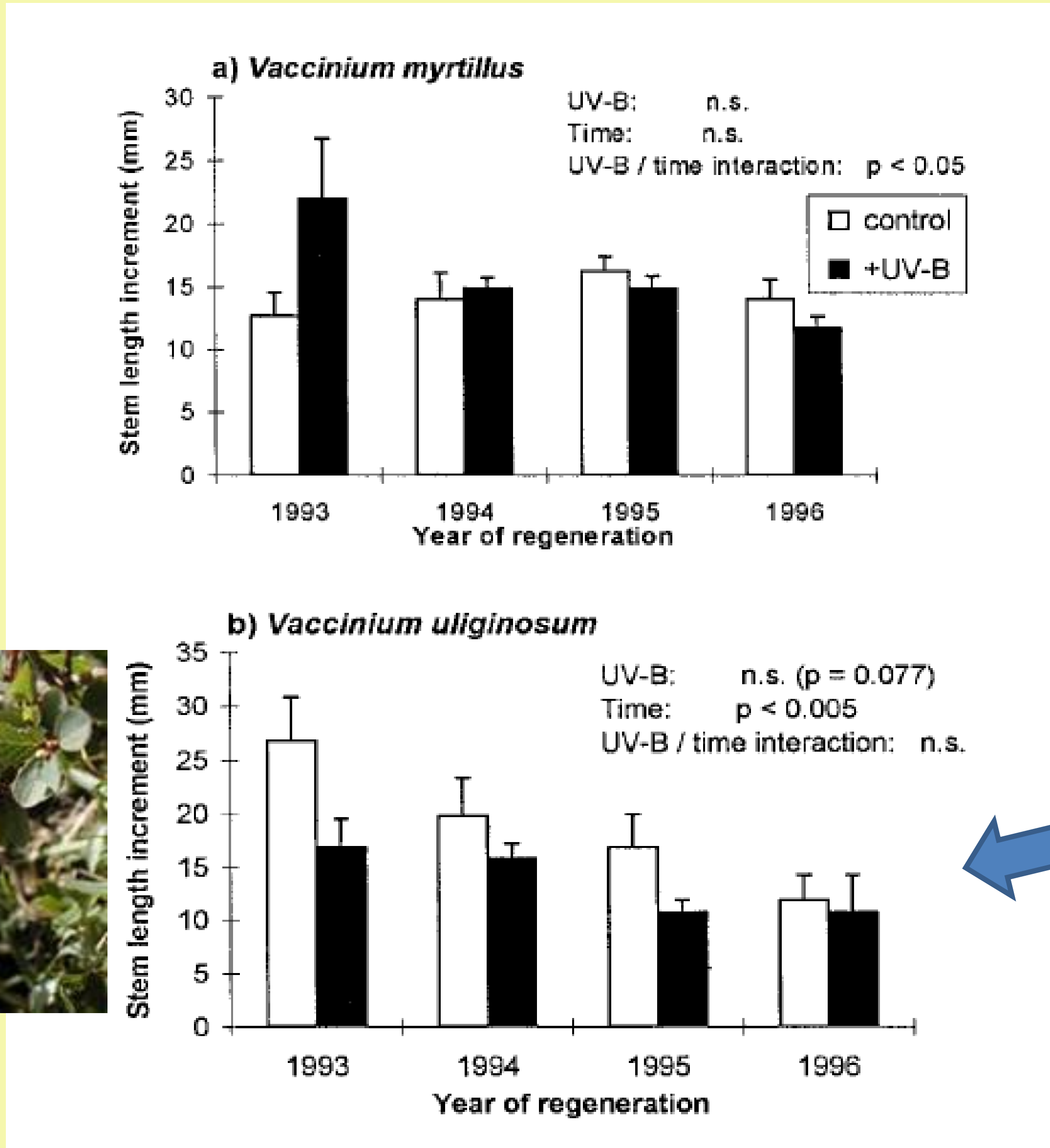
How to study the impact of UV-B?

- **Long term outdoor supplementation**
- Used to study consequences increases in UV
- Proportional UV-B increases
- Realistic – can show complexity ecological role UV

Abisko, Sweden

Real life scenario's; Abisko, Sweden



- Species specific effects
- Different effects in different years

UV-induced "dwarfing"; well established under laboratory conditions

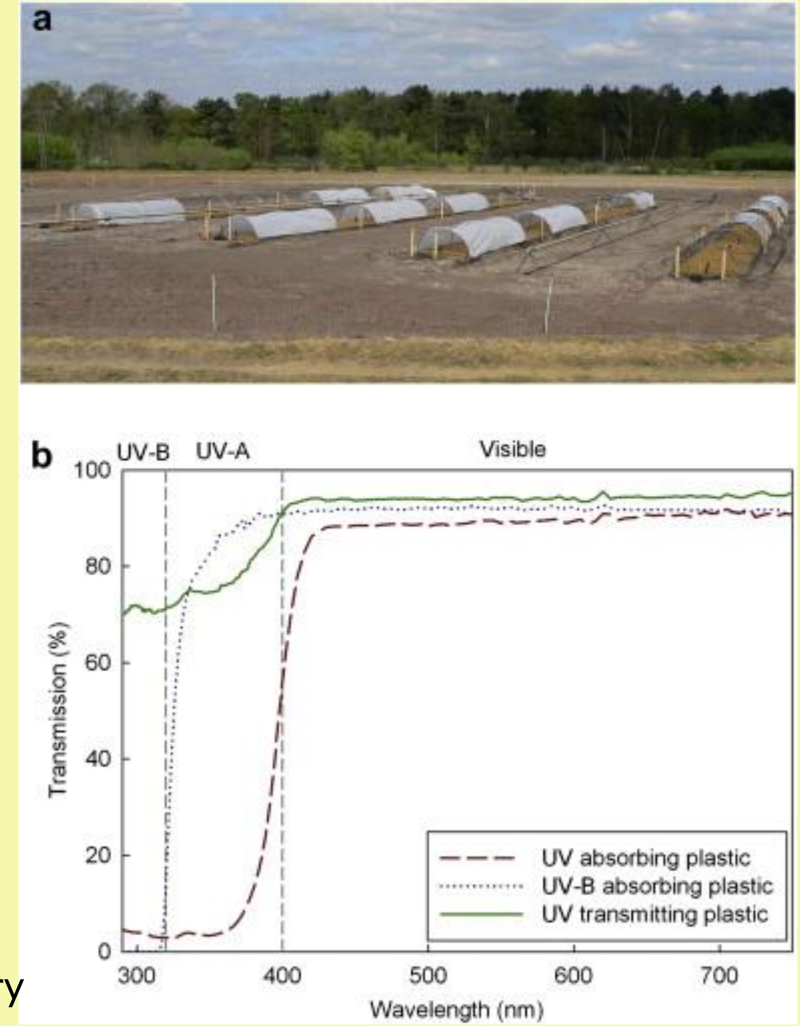
G. K. Phoenix, D. Gwynn-Jones, J. A. Lee & T. V. Callaghan *Plant Ecology* **146:** 67–75, 2000.

Field studies do not necessarily show the same as laboratory studies!

Interactions with other environmental factors modify UV-B responses

How to study the impact of UV-B?

- **Long term outdoor exclusion**
- Used to study consequences current UV
- Realistic – can show complexity ecological role UV
- Cheap!



Messenger et al; Soil Biology and Biochemistry 47, 2012, 133–141.

UV-exclusion studies

UV responses of *Lolium perenne* raised along a latitudinal gradient across Europe: a filtration study. David Comont, Javier Martinez Abaigar, Andreas Albert, Pedro Aphalo, David R. Causton, Felix Lopez Figueroa, Alenka Gaberscik, Laura Llorens, Marie-Theres Hauser, Marcel A. K. Jansen, Majlis Kardefelt, Paqui de la Coba Luque, Susanne Neubert, Encarnacion Nunez-Olivera, Jorunn Olsen, Matthew Robson, Monika Schreiner, Ruben Sommaruga, Ake Strid, Sissel Torre, Minna Turunen, Sonja Veljovic-Jovanovic, Dolors Verdaguer, Marija Vidovic, Johanna Wagner, Jana Barbro Winkler, Gaetano Zipoli and Dylan Gwynn-Jones

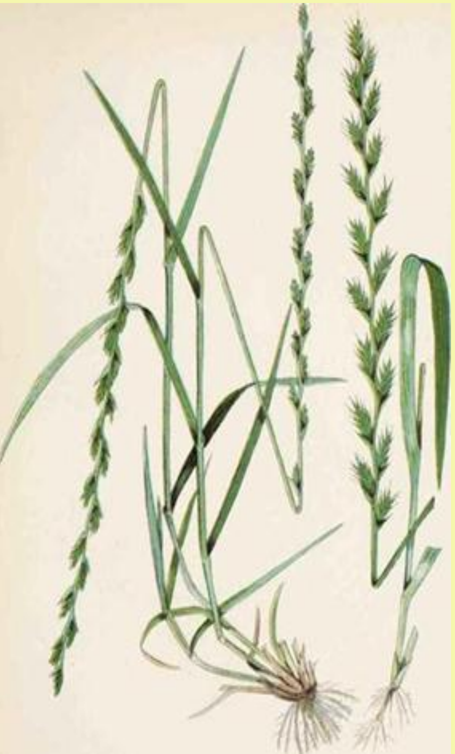
$2.1\ kJm^{-2}day^{-1}$

$4.7\ kJm^{-2}day^{-1}$

Comont et al., (2012) Physiol Plant

UV-exclusion studies

- UV-B reduces tiller length and increases UV-B absorbing pigments in *Lolium perenne*
- Other variations in growth due to combinations of climatic factors
- **Very significant effect of presence filter (vis a vis open control)**

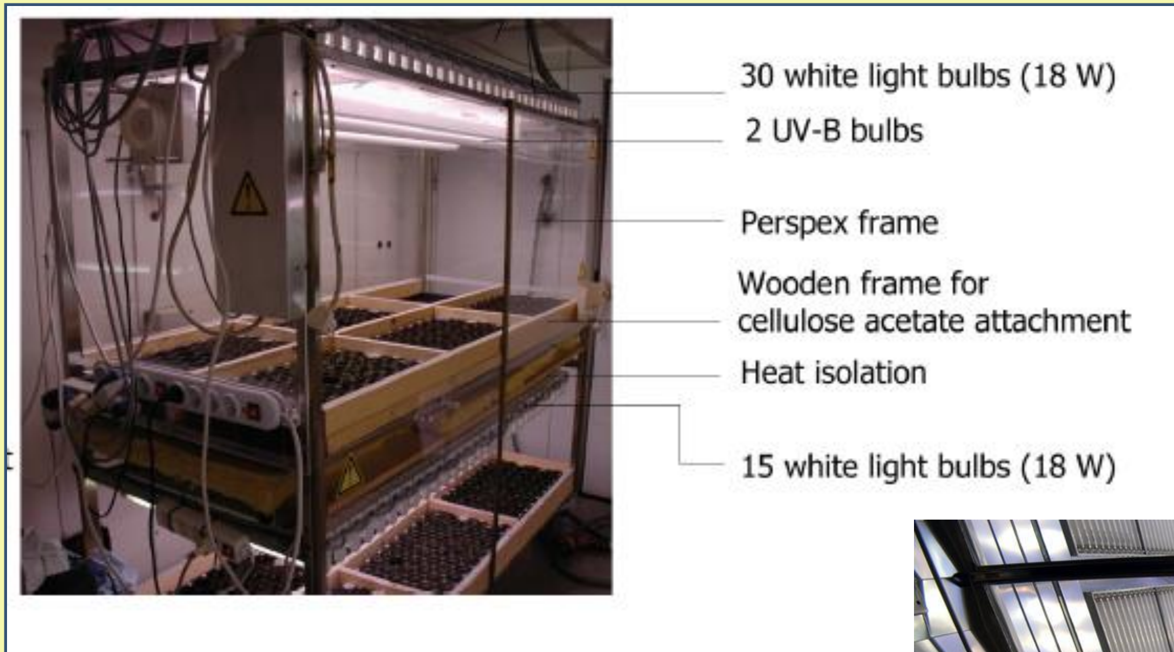


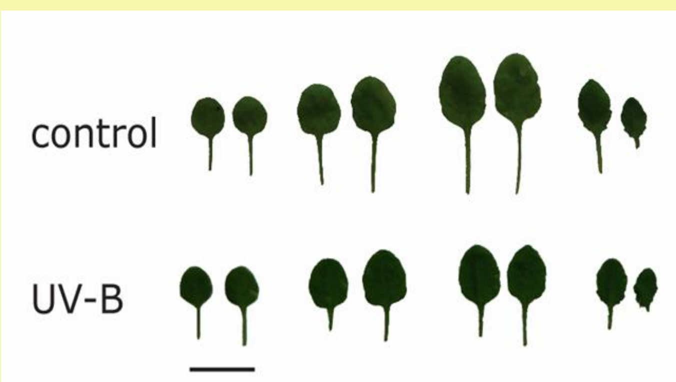
Comont et al., (2012) Physiol Plant

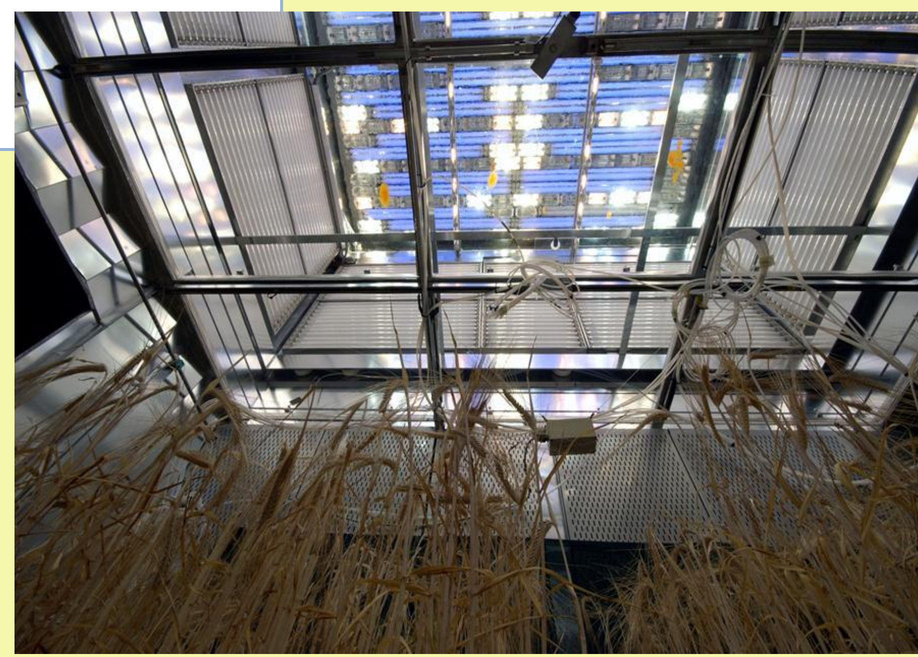
How to study the impact of UV-B?

- Indoor UV-B exposure studies
- Accurate/controlled
- Environmental relevance?

Controlled, indoor experiments







Helmholtz, Germany

What does UV-B radiation do to plants?

UV-distress in plants

Inactivation photosynthesis

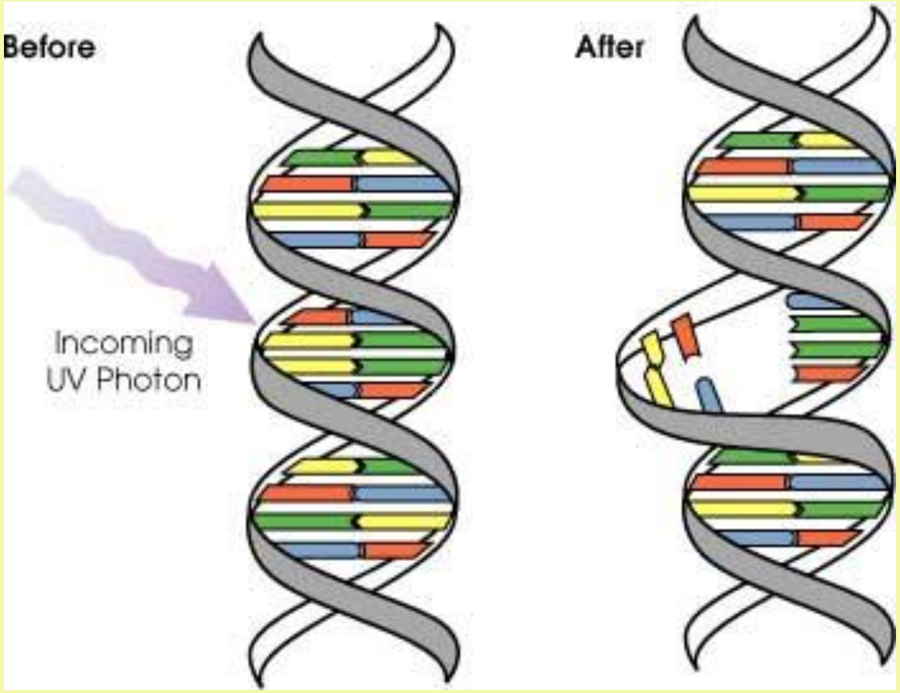
DNA damage

Oxidative stress (ROS) / membrane damage

↓

Resulting in impaired growth

UV-stress in plants – DNA damage



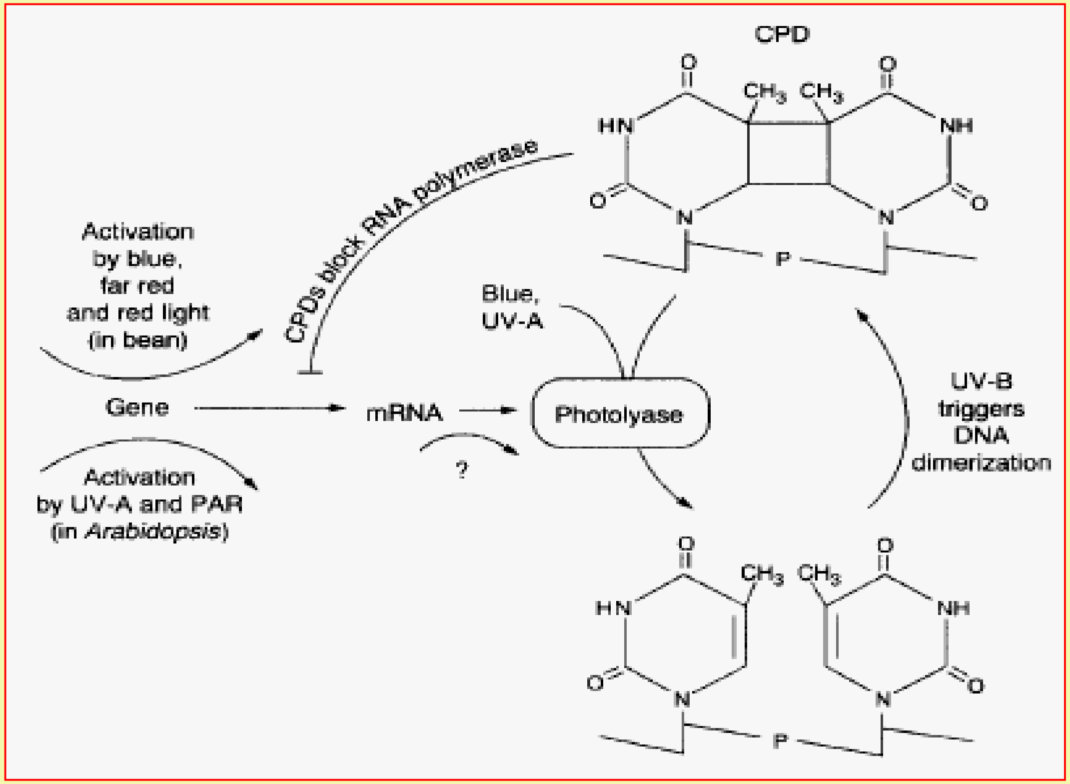
- DNA absorbs in the UV-wavelength band
- Formation Cyclobutane pyrimidine dimers (CPD) and pyrimidine (6, 4) pyrimidone dimers (6-4 PPs) through UV-driven photochemical reactions
- CPDs and 6-4 PPs block RNA and DNA polymerases

UV-stress in plants – DNA damage

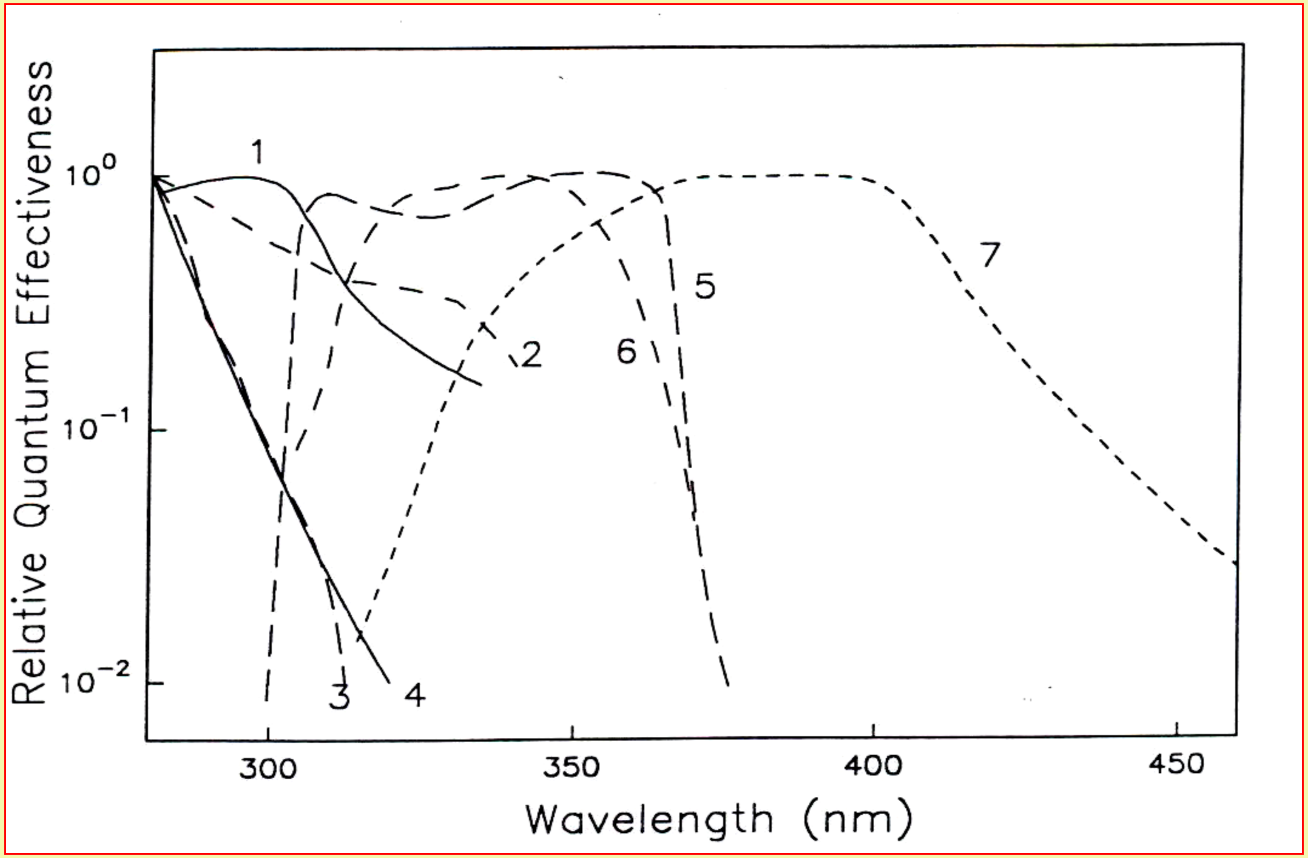
Dimers block RNA / DNA polymerases (no transcription and replication)

DNA repair by photolyases which require light (blue / UV-A) energy.

Damage and repair both driven by sunlight!



Jansen et al., 1998 TiPS



(1)flavonoid induction parsley cells
(2)inactivation photosystem II spinach
(3)DNA-dimer formation alfalfa seedlings
(4)inhibition net photosynthesis
(5)photoprotection *E. coli*
(6)carotenoid protection of UV damage *Sarcina lutea*
(7)photorepair of UV damage to DNA *E. coli*

M.M. Caldwell and S.D. Flint, 1994

UV-B damage depends on balance between UV-B and visible radiation

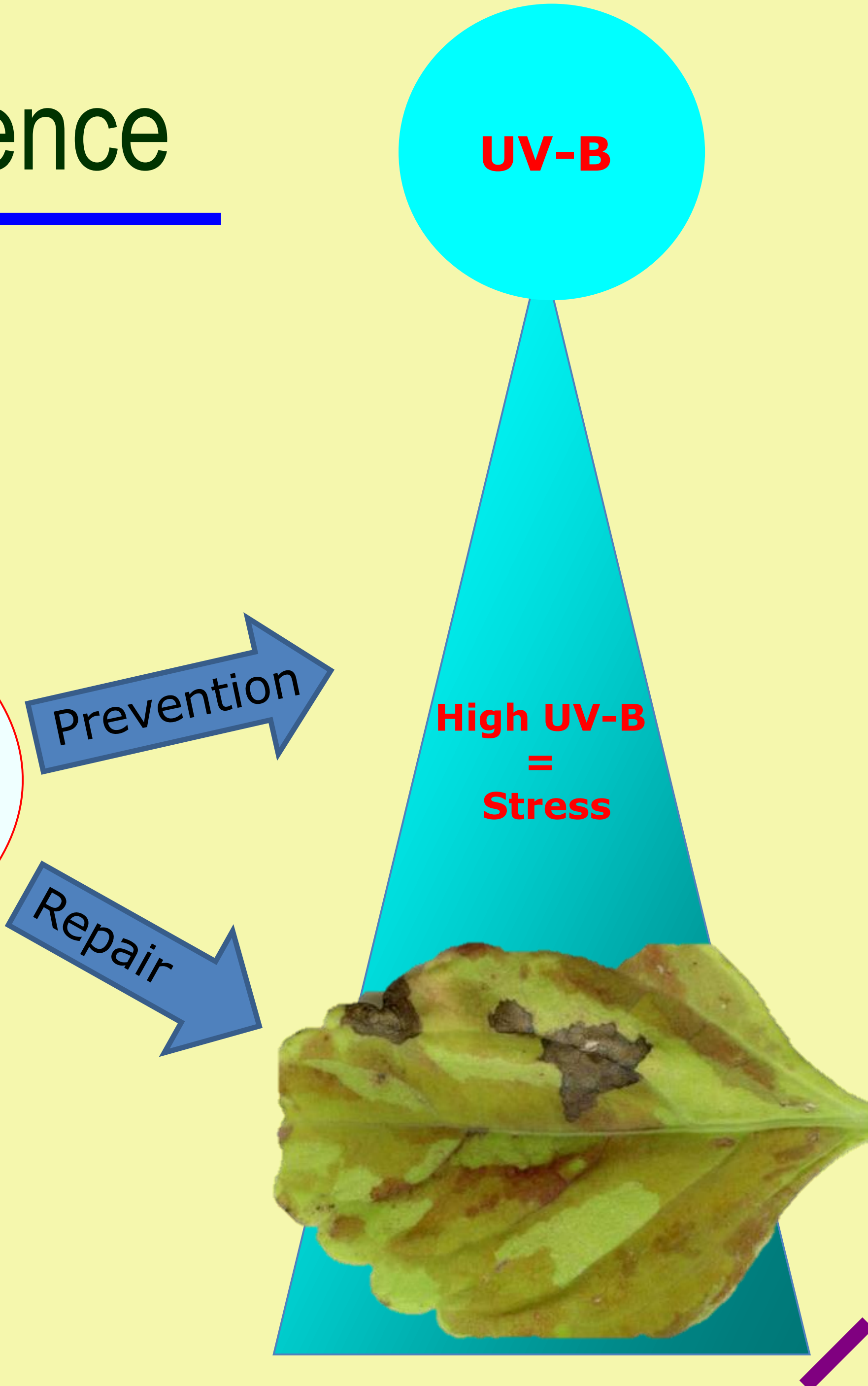
(level of visible radiation in growth room?)

Balancing stress & defence

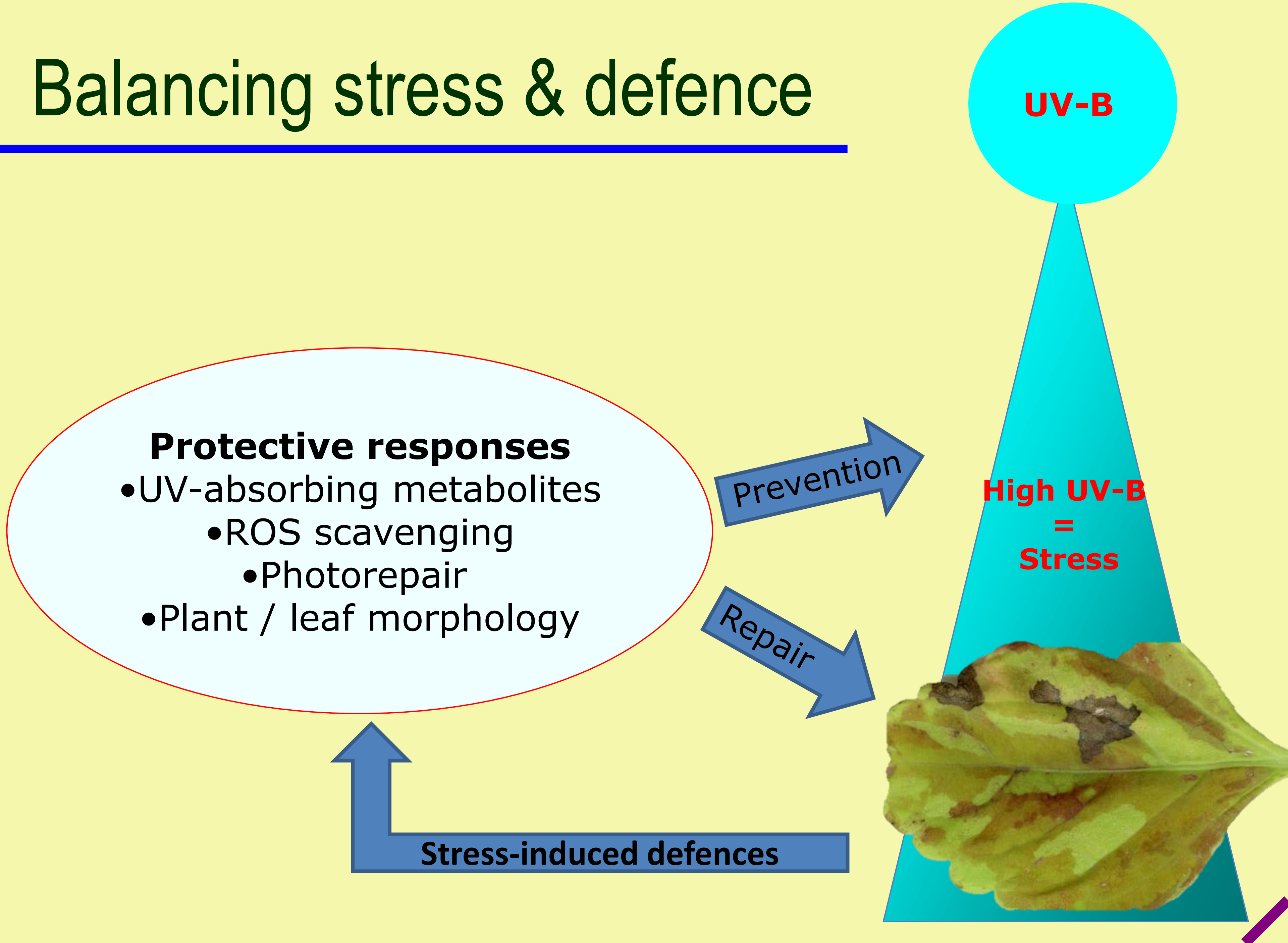
Balancing stress & defence

Protective responses

- UV-absorbing metabolites
- ROS scavenging
- Photorepair
- Plant / leaf morphology



Balancing stress & defence



Balancing stress & defence

UV-B

Low UV-B = Information

Protective responses

- UV-absorbing metabolites
- ROS scavenging
- Photorepair
- Plant / leaf morphology

Prevention

Repair

High UV-B = Stress

Stress-induced defences

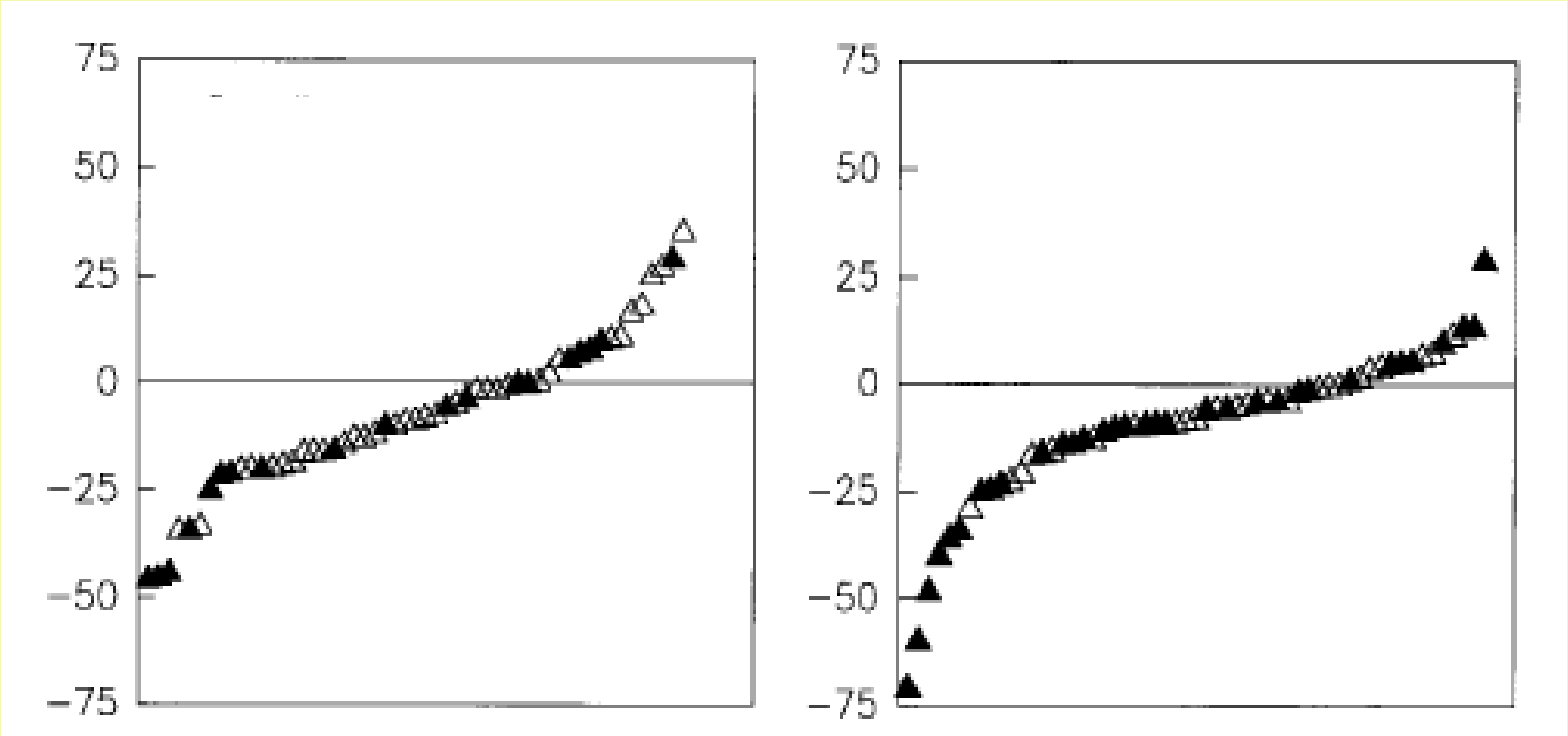
Due to activation defence responses plants are rarely (dis)stressed by UV-B

Meta-analysis data collected in 1990's; plant productivity under increased UV-B

Shoot biomass

Leaf area

% change with UV-B supplementation



Searles, Flint and Caldwell (2001) Oecologia 127, 1-10

UNITED NATIONS
ENVIRONMENT PROGRAMME 2011

…inhibitory UV effects on plant growth generally small…

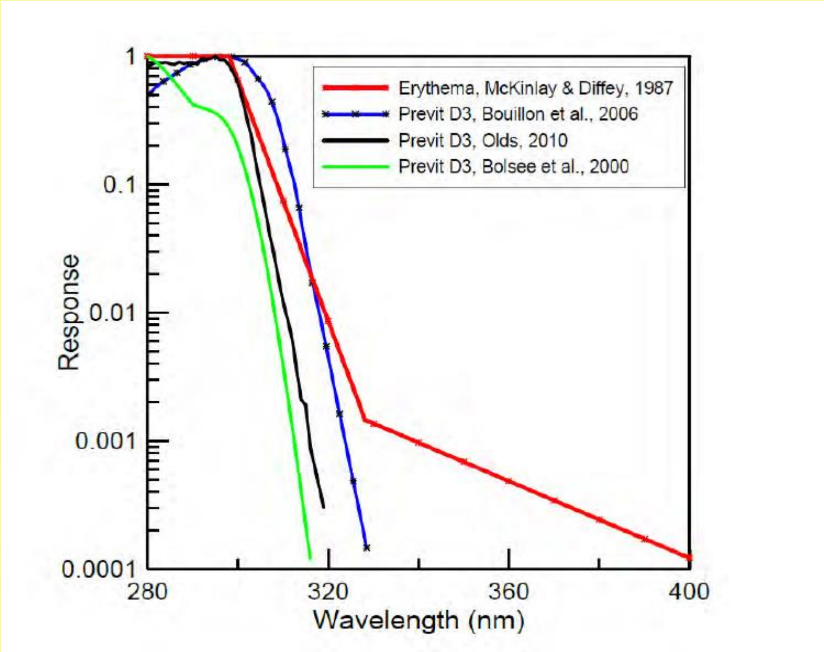
…UV-B radiation elicits plant acclimation responses…..

…UV-B frequently has large effects on interactions between plants and consumers …….

Radiation conditions decide the outcome of an experiment

Factors that determine the validity of an UV-B experiment;

- UV-dose
- UV-spectrum
- Visible light background
- UV-B acclimation plant
- UV-B adaptation plant



Many early UV-studies are now considered irrelevant

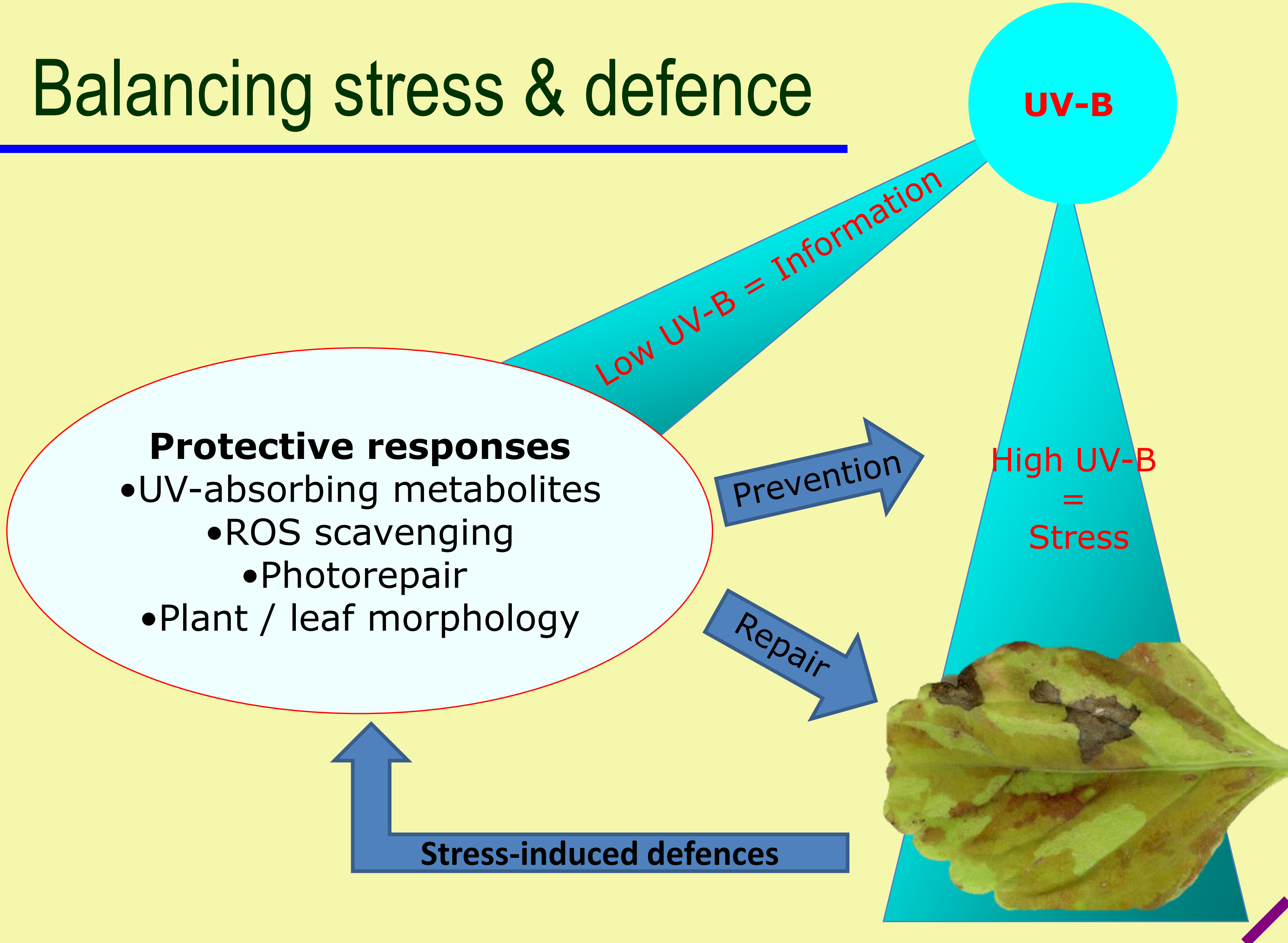
(for life on planet earth)

It took nearly 2 decades to develop effective UV-B exposure protocols

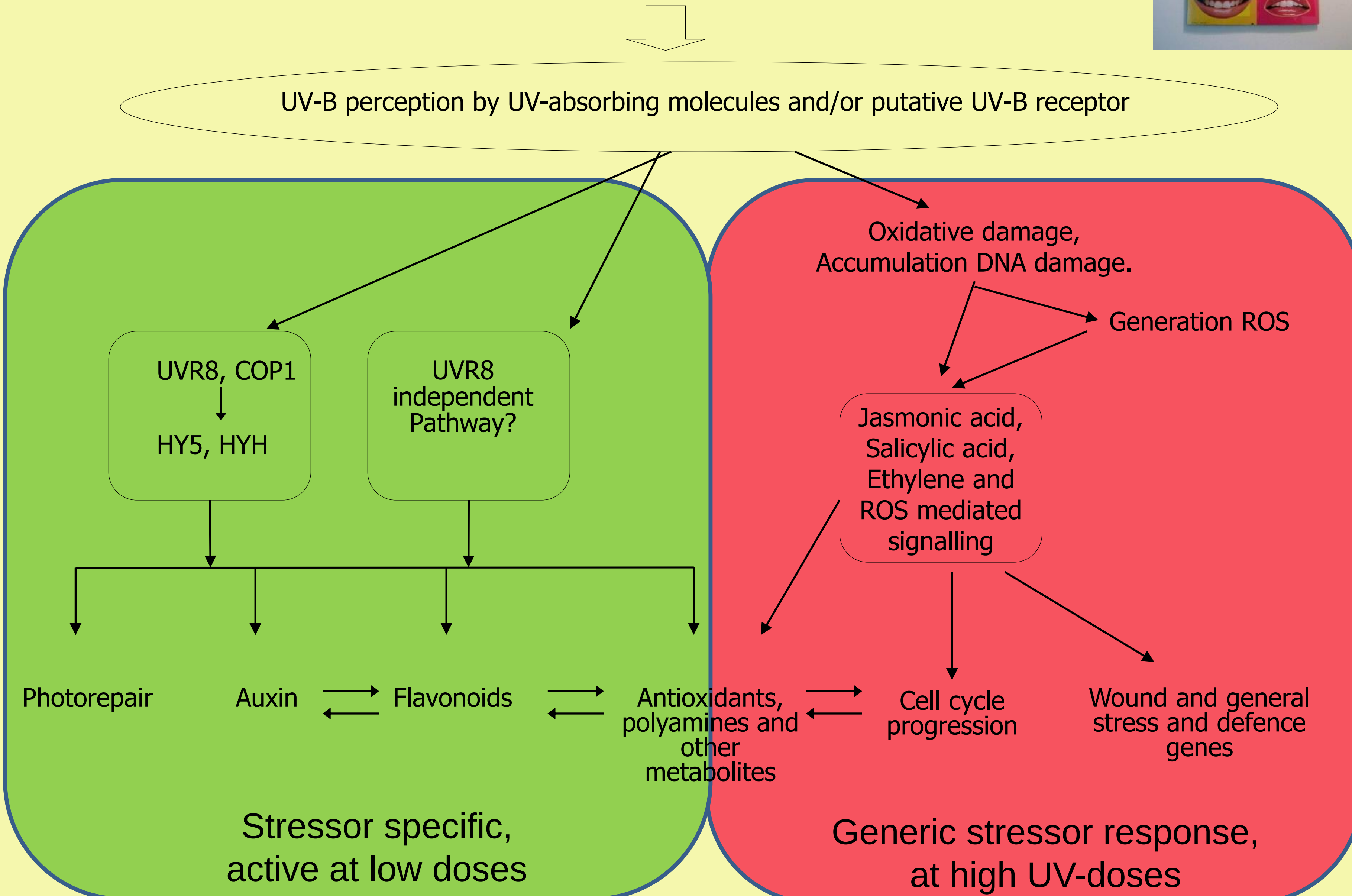
- Message for climate change studies?

How do plants perceive UV-B radiation?

Balancing stress & defence



UV-B


UV-B perception by UV-absorbing molecules and/or putative UV-B receptor
UVR8, COP1
HY5, HYH
UVR8 independent Pathway?
Oxidative damage, Accumulation DNA damage.
Generation ROS
Jasmonic acid, Salicylic acid, Ethylene and ROS mediated signalling
Photorepair
Auxin
Flavonoids
Antioxidants, polyamines and other metabolites
Cell cycle progression
Wound and general stress and defence genes
Stressor specific, active at low doses
Generic stressor response, at high UV-doses


Specific UV-B perception
Holy Grail plant UV-research

www.sciencemag.org SCIENCE VOL 332 1 APRIL 2011

Perception of UV-B by the *Arabidopsis* UVR8 Protein

Luca Rizzini,[1*] Jean-Jacques Favory,[1*] Catherine Cloix,[2] Davide Faggionato,[3] Andrew O'Hara,[2] Eirini Kaiserli,[2†] Ralf Baumeister,[3,4] Eberhard Schäfer,[1,4] Ferenc Nagy,[5,6] Gareth I. Jenkins,[2] Roman Ulm[1,4,7‡]

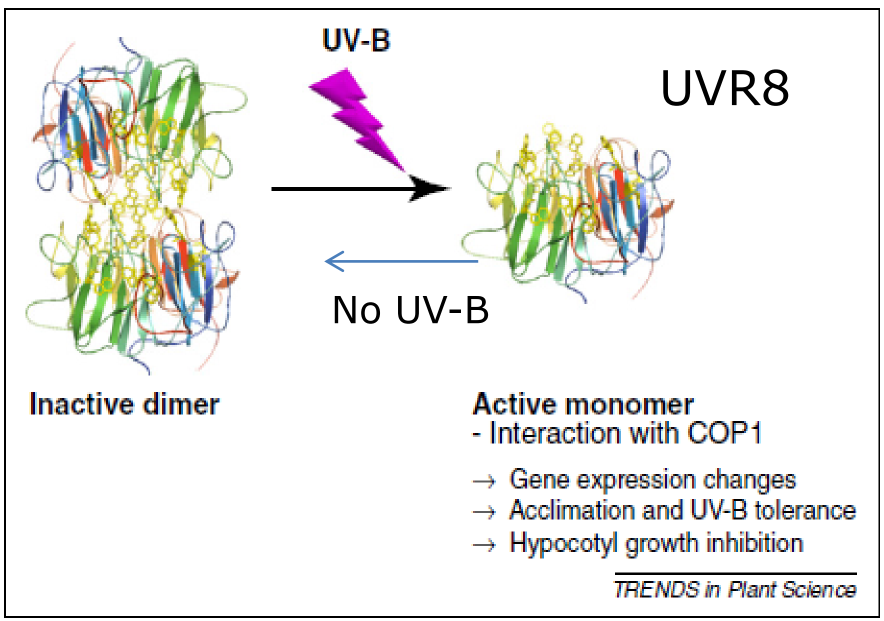
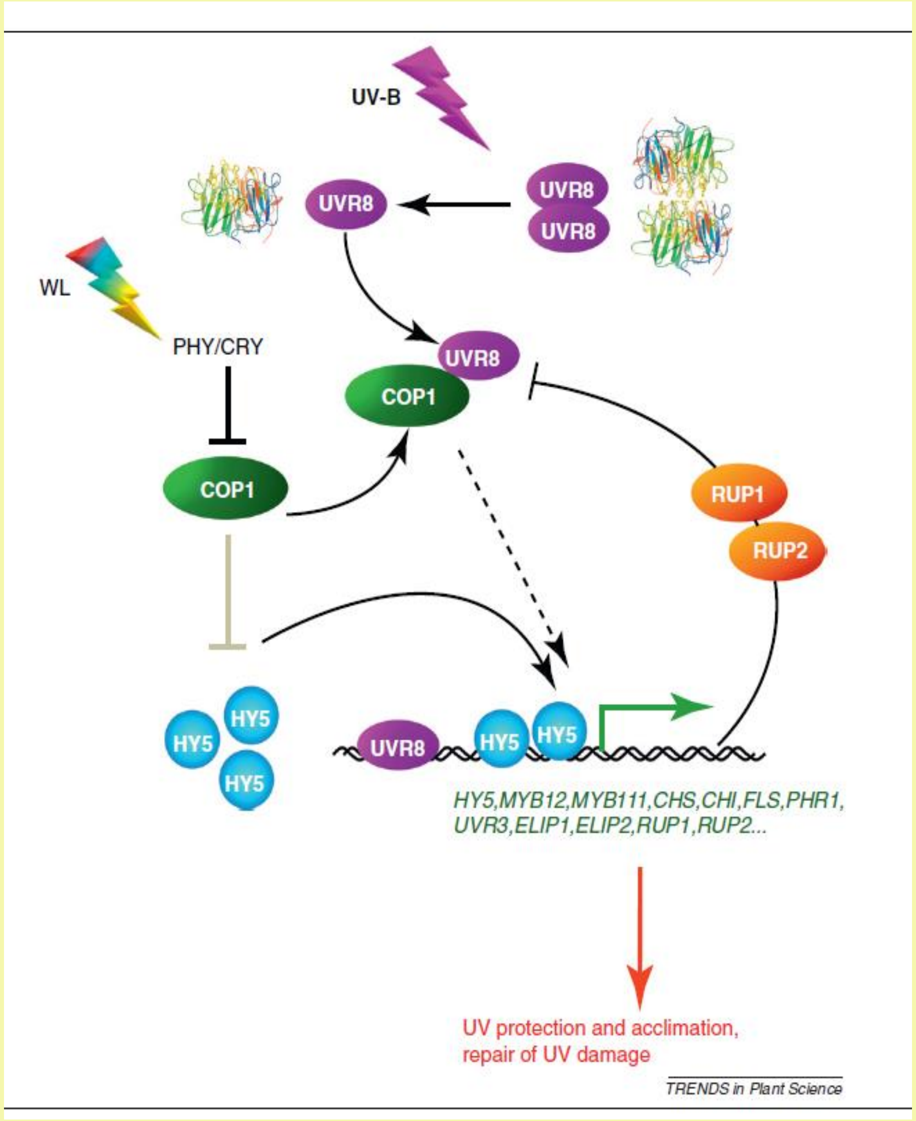


Figure 2. Model of UV-B perception by the UVR8 protein. UVR8 homodimerises in the absence of UV-B via a hydrophobic surface containing clustered tryptophans.

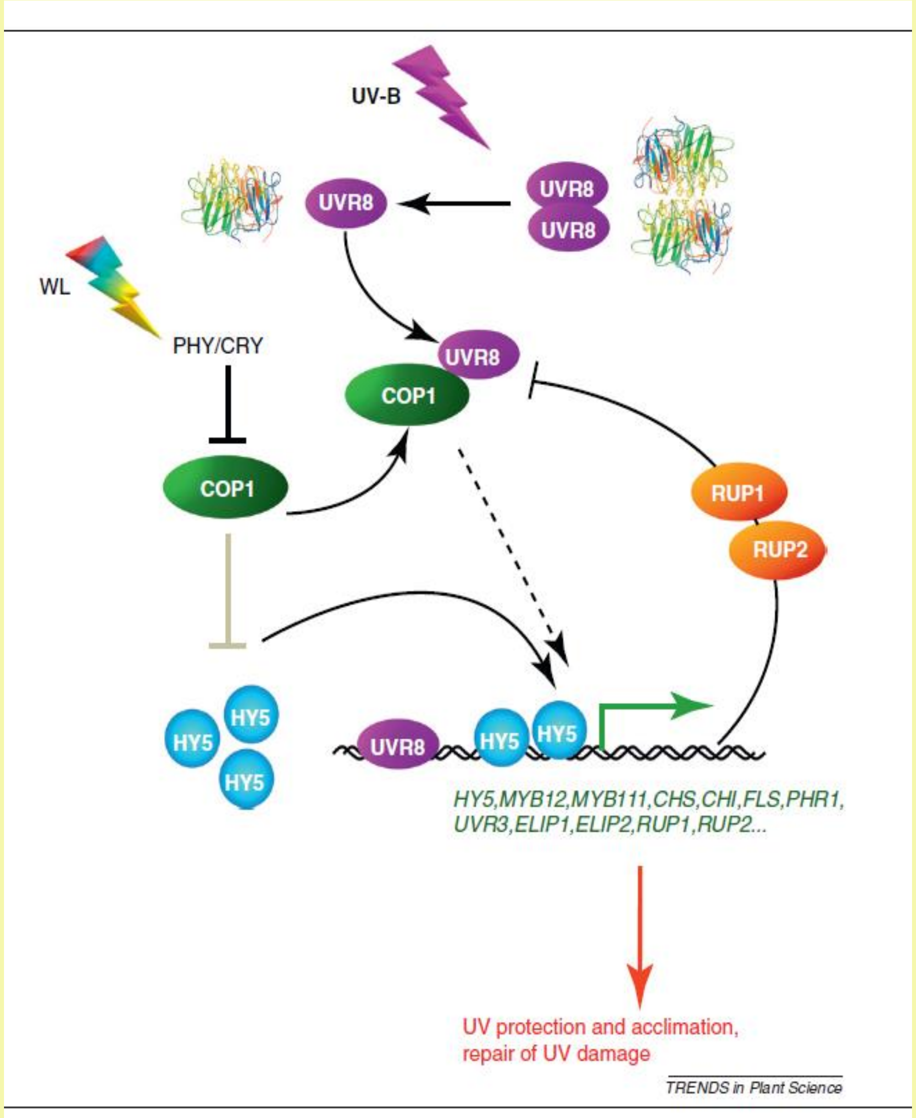
Heijde and Ulm, 2012 TiPS

- UV-B causes monomerisation UVR8
- No external chromophore, but cluster of tryptophans
- UVR8 monomer interacts with COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC1)
- Altered gene-expression



- UV-B causes monomerisation UVR8
- COP1 normally blocks HY5 (ELONGATED HYPOCOTYL5) mediated genexpression
- UVR8 monomer binds COP1 facilitating HY5 mediated gene expression

Heijde and Ulm, 2012 TiPS



- Negative feedback control via RUP1 and RUP2 (REPRESSOR OF PHOTOMORPHOGENESIS1 or 2)
- COP1 also involved in controlling R/FR and blue gene expression

Heijde and Ulm, 2012 TiPS

Analysis of UVR8 mutants under UV shows that UVR8 is important for regulation of:

- flavonoid biosynthesis
- other secondary metabolic pathways, such as alkaloid biosynthesis
- photolyase PHR1 (DNA repair)
- protection against oxidative stress
- genes encoding signaling components, transcription factors, transporters, and proteases,
- chloroplast proteins

Brown et al., Proc Natl Acad Sci USA 102 (2005)

Low UV-B ≠ oxidative stress

Low UV-B induced

Oxidative stress-induced

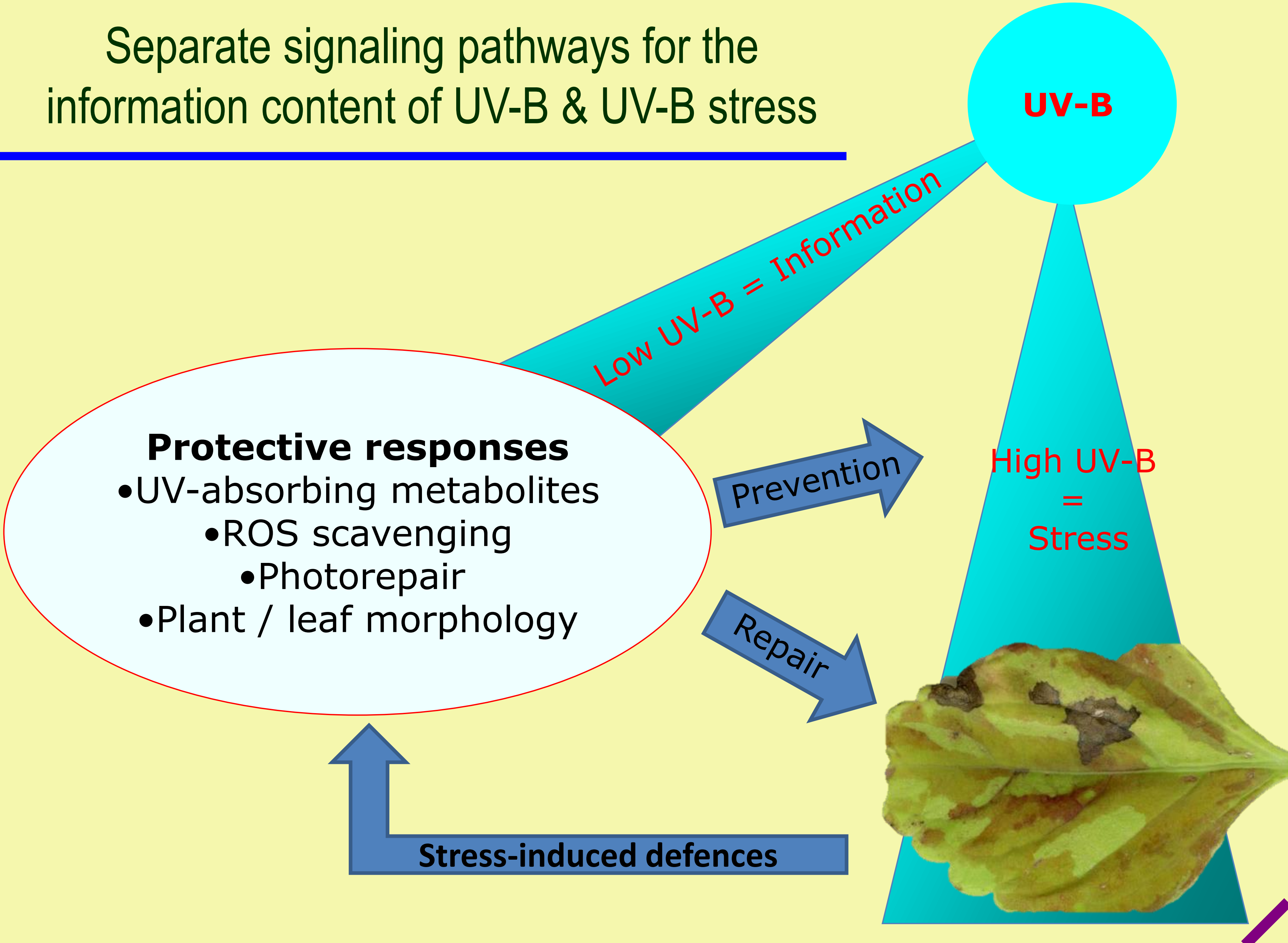
		UV-B	Ozone	Methylviologen	Norflurazon	flu mutant	H_2O_2
At3g24170	Glutathione reductase (GR1)	YES	YES	No	YES	No	No
At4g31870	Glutathione peroxidase (GPX7)	YES	No	No	No	No	No
At1g28480	Glutaredoxin (GRX480)	YES	YES	YES	No	YES	YES
At4g15680	Glutaredoxin/Thioredoxin	YES	No	No	YES	YES	No
At5g17220	Glutathione transferase (GST26)	YES	No	No	YES	No	No
At2g23910	Cinnamoyl-CoA reductase	YES	YES	No	No	No	No
At1g65060	4-Coumarate-CoA ligase 3 (4CL3)	YES	No	No	YES	No	No
At5g13930	Chalcone synthase (CHS; TT4)	YES	No	No	YES	No	No
At3g55120	Chalcone isomerase (CHI; TT5)	YES	No	No	No	No	No
At5g05270	Chalcone isomerase (CHI)	YES	No	No	No	No	No
At3g51240	Flavanone 3-hydroxylase (F3H; TT6)	YES	No	No	No	No	No
At5g08640	Flavonol synthase (FLS1)	YES	No	No	No	No	No
At5g01410	Pyridoxal synthase (PDX1.3; Pyro A)	YES	No	No	No	No	No
At1g78510	Solanesyl diphosphate synthase (SPS1)	YES	no	no	no	no	no

Low UV-B (through UVR8) induces different anti-oxidant genes than ROS themselves!

Low UV-B doses cause specific acclimation, inducing a state of low alert (eustress)

Hideg, Jansen, Strid, TiPS 2013

Separate signaling pathways for the information content of UV-B & UV-B stress



Responses to low, chronic UV-B doses are functionally uncoupled from stress responses to high UV-B

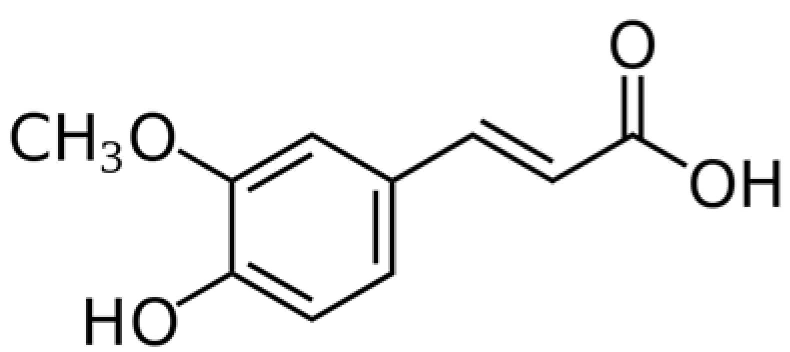
Due to activation defence responses by low UV-B, plants are rarely stressed by UV-B

If there is no stress, why study effects of UV-B radiation?

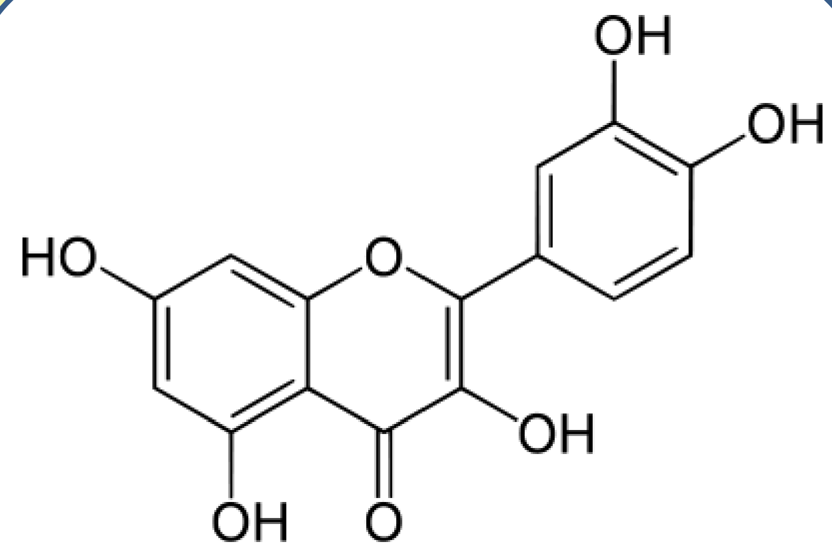
Part I

UV-B protection via phytochemicals

- Accumulation of simple phenolics and more complex flavonoids
  - Strong antioxidants
  - UV-B absorbers



Ferulic acid



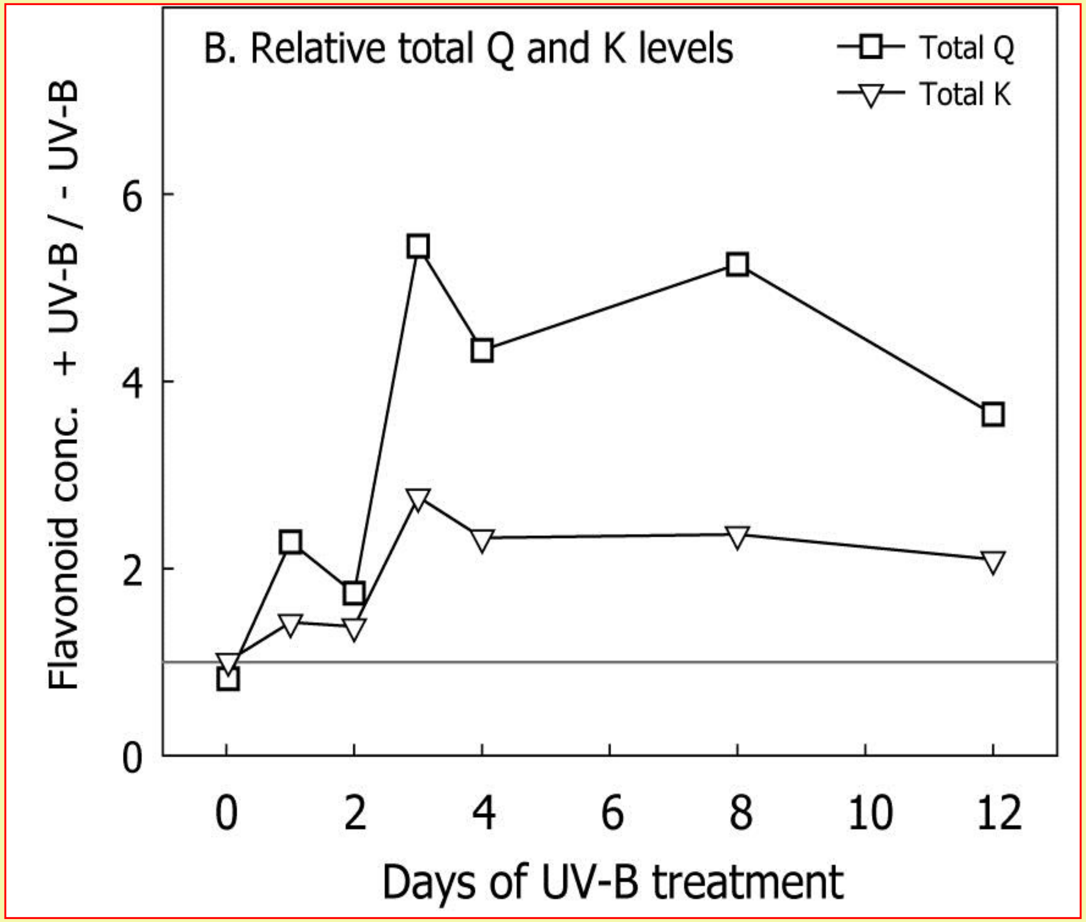
Quercetin



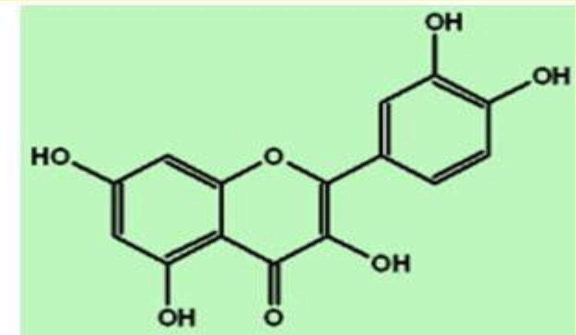
Flavonoids and related compounds are reportedly good for human consumers

Reported anti-allergic, anti-inflammatory, anti-microbial and anti-cancer effects

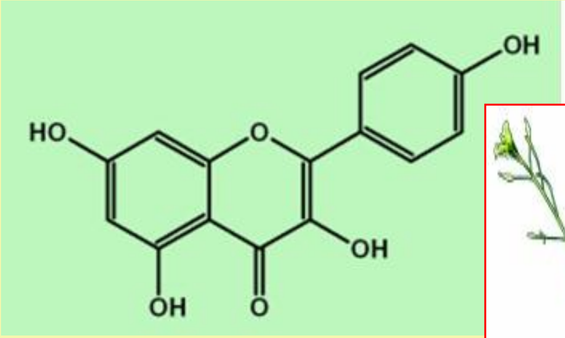
UV-B induced accumulation of flavonoids



Hectors et al., Physiol Plant 2014

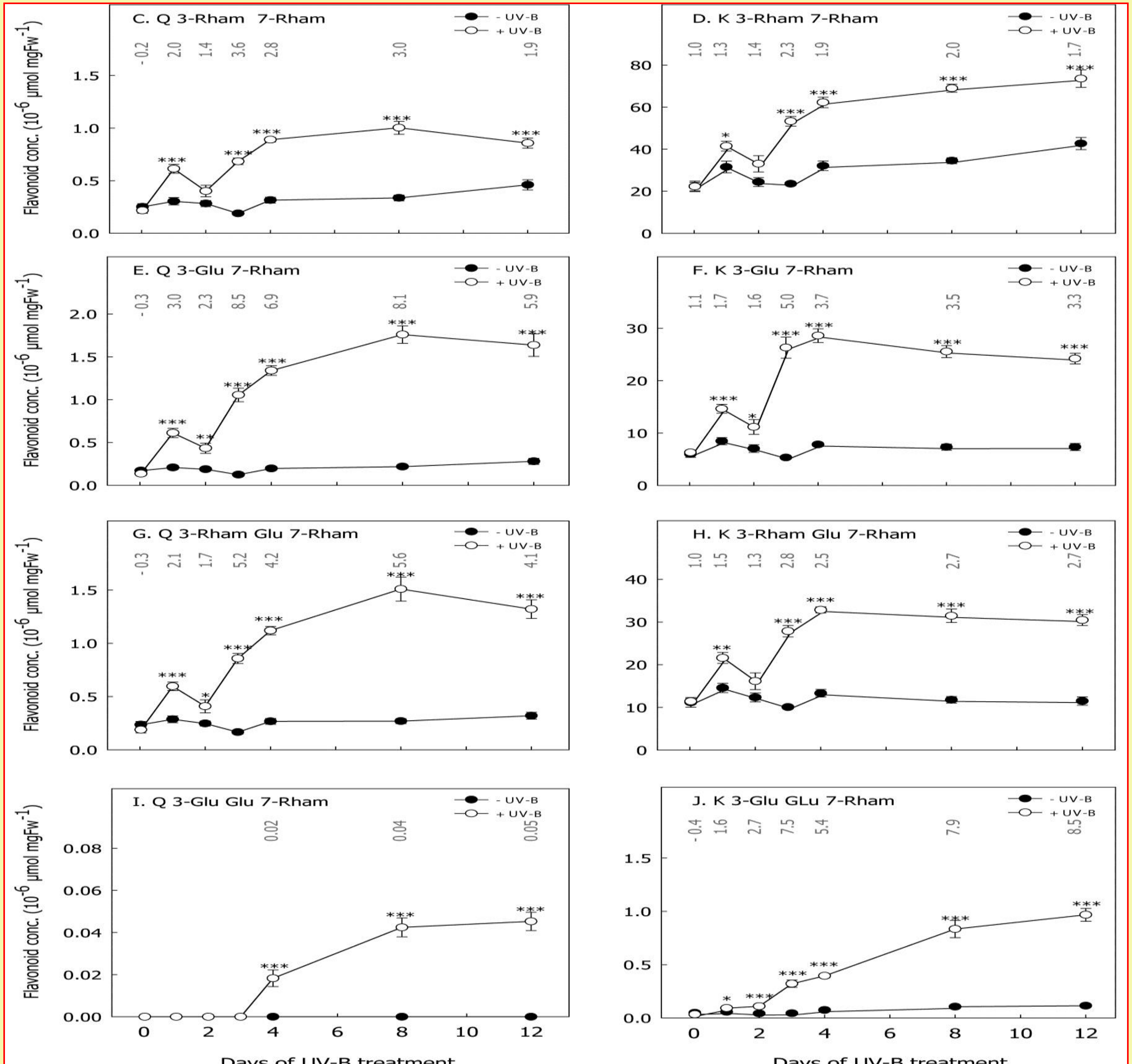


Q-Quercetin



K-Kaempferol

Acclimation: UV-B induced accumulation of flavonoids



Q-Quercetin
K-Kaempferol

Arabidopsis

Grown indoors

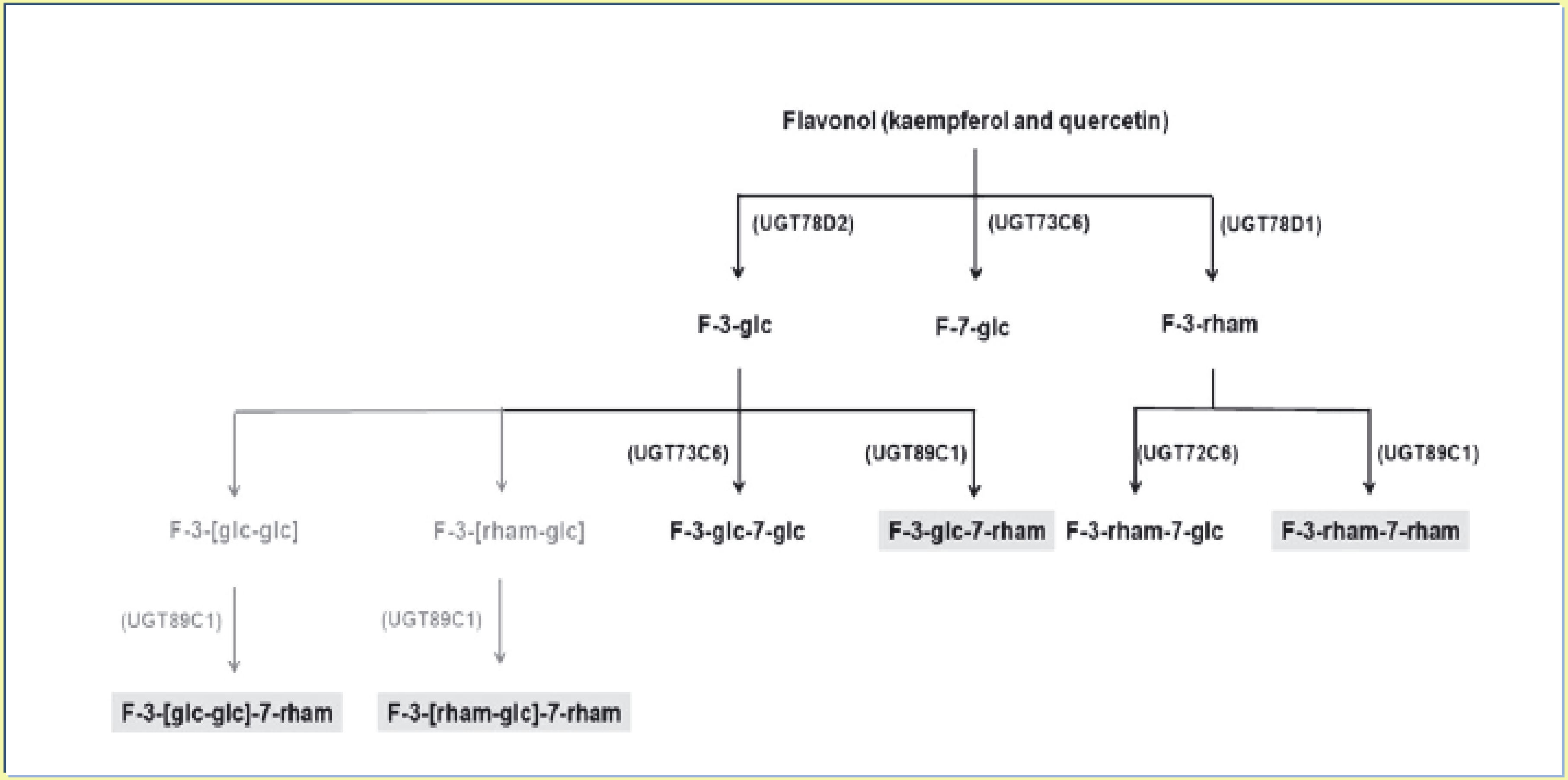
Upto 12 days under 0.59 kJ m^{-2} UV_{be}

Hectors et al (2008) New Phytol

Accumulated flavonols, all products 7-O-Rhamnosyltransferase activity by UGT89C1 gene-product

UGT89C1 expression is induced by UV-B in a UVR8 dependent manner

Functional role: stability? cellular location? activity?



Hectors et al., Physiol plant 2014

Lettuce with more intense colour
Grown under UV-transparent plastics

UV-transparent cladding

UV-blocking cladding

UV-A transparent cladding

Lettuce Cos grown outdoors under different cladding materials (Cork Autumn 2013)

Data Paul Carlos and Aoife Coffey

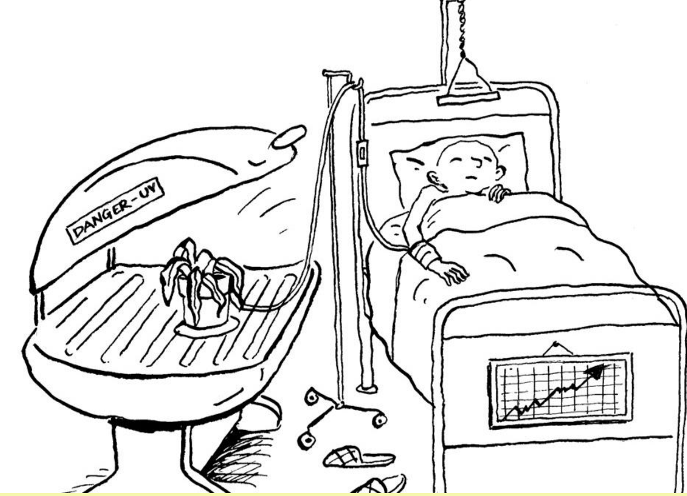
Not just phenolics........!

Parameters	PAR	PAR+UV-A	PAR+UV-A+B
Carotenoids (μg mg^{-1} Total chls)			
Vialoxanthin	13.66 ± 2.41^{b}	17.24 ± 2.20^{ab}	25.44 ± 2.69^{a}
Antheraxanthin	2.88 ± 0.55	1.46 ± 0.50	3.70 ± 0.61
Neoxanthin	5.18 ± 2.41^{b}	9.94 ± 2.20^{ab}	16.23 ± 2.70^{a}
Lutein	194.55 ± 17.33^{b}	251.10 ± 15.82^{b}	334.97 ± 19.37^{a}
9-cis ß-carotene	10.76 ± 0.73^{b}	10.40 ± 0.67^{b}	13.30 ± 0.82^{a}
Total ß-carotene	50.64 ± 4.75^{b}	51.89 ± 4.34^{b}	67.12 ± 5.31^{a}

Carotenoids in *Arabidopsis thaliana* grown under PAR, PAR+UV-A and PAR+UV-A+B for 10 days.

Jansen en Biswas; Unpublished

Not just phenolics……..!



Jansen et al. (2008) Plant Science

UV-B induces accumulation alkaloids such as nicotin and cannabinoids (UV-screening?)

UV-B also induces:

- carotenoids (lipid soluble antioxidant)
- tocopherols (lipid soluble antioxidant)
- waxes (reflectance)
- polyamines (stress antioxidants)
- Glucosinolates (defence compounds in Brassicaceae)

UV-B protection: opportunities?

Using UV-B radiation to increase flavour, colour and smell of potted herbs

UV-acclimation & herbivory

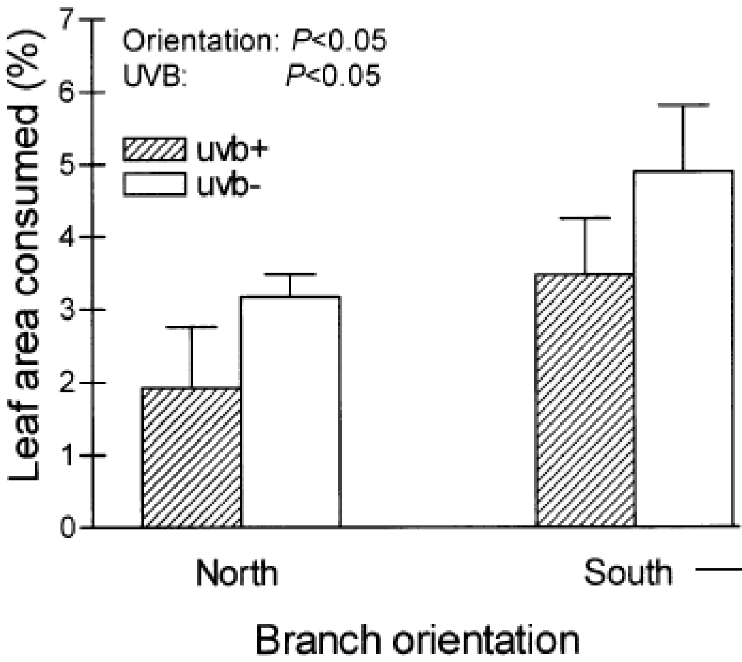


Fig. 3 Percentage of leaf area consumed by insects on north- and south-facing *N. antarctica* branches under near-ambient (uvb+) or attenuated UV-B (uvb−) radiation. Data are from the solar UV-B attenuation experiment (1998–99). Plastic filters were used to obtain the near-ambient and attenuated UV-B levels. Values represent the mean (n =10) ± SE

Nothofagus herbivory is affected by UV-B exposure

Lowest UV-B in Southern hemisphere

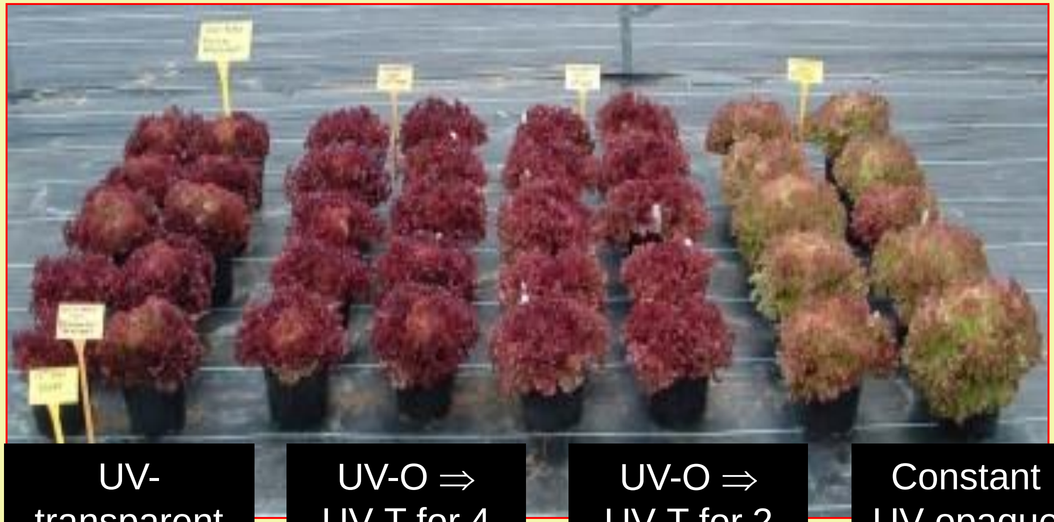
Rousseaux et al. (2004) Oecologia

UV-B induced changes in plant phytochemicals; a novel tool for horticulture!

If there is no stress, why study effects of UV-B radiation?

Part II

The UV-B phenotype



UV-transparent

UV-O ⇒ UV-T for 4 weeks

UV-O ⇒ UV-T for 2 weeks

Constant UV-opaque

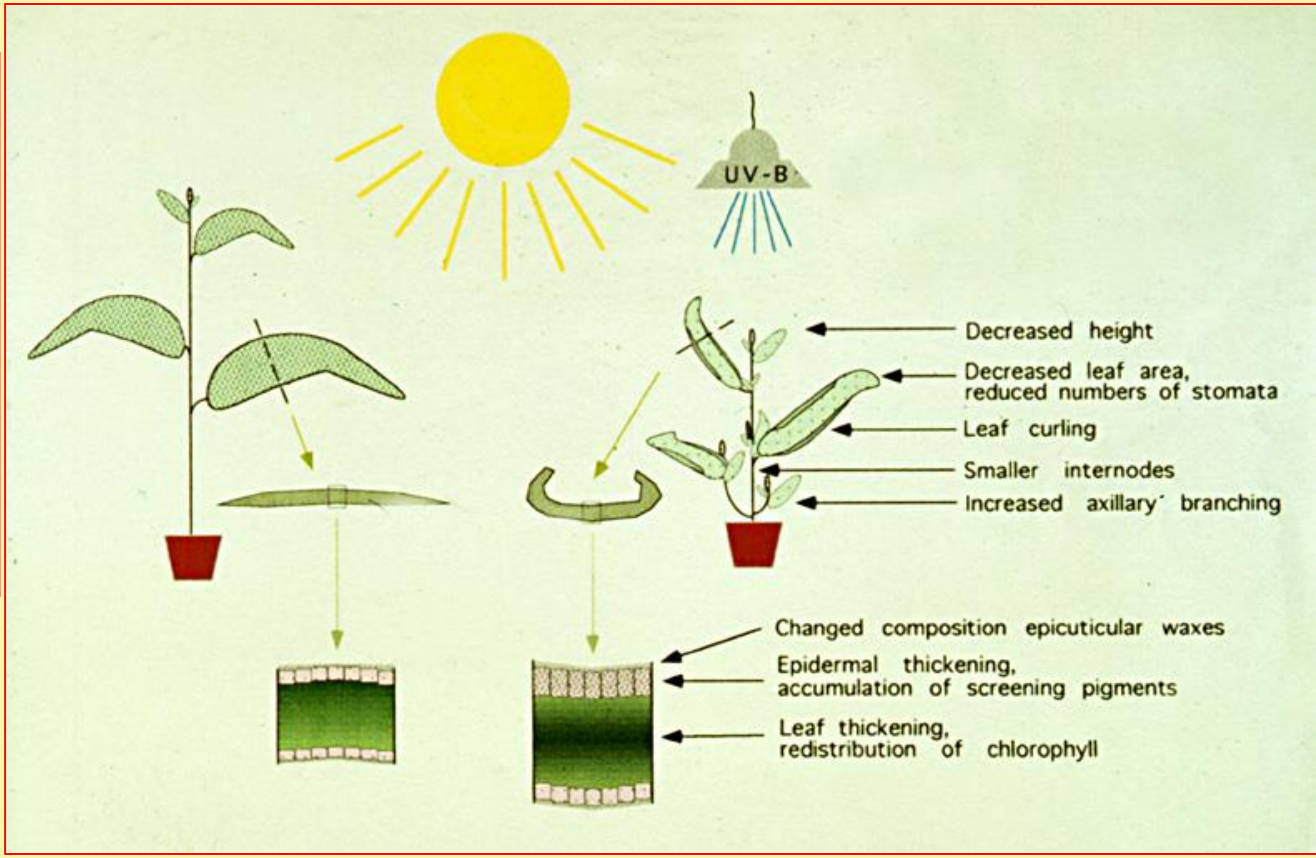
Data Nigel Paul, Lancaster, UK

Morphology
Dwarfed phenotype

Pigmentation
Flavonoids and other pigments

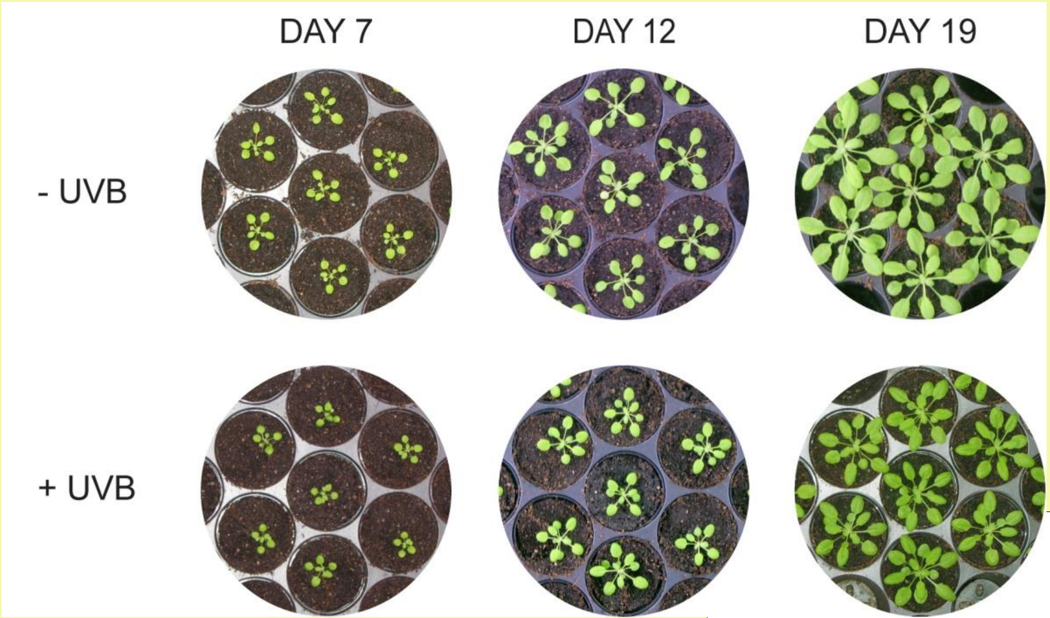
Re-interpreting UV-responses; the UV-B phenotype

- Leaf area ↓& thickness ↑
- Leaf curling ↑
- Hypocotyl & stem length ↓
- Branching & tillering ↑
- Flower number & size
- Root/shoot ratio



(Jansen et al., TiPS 1998)

UV-B protection – III - Morphology

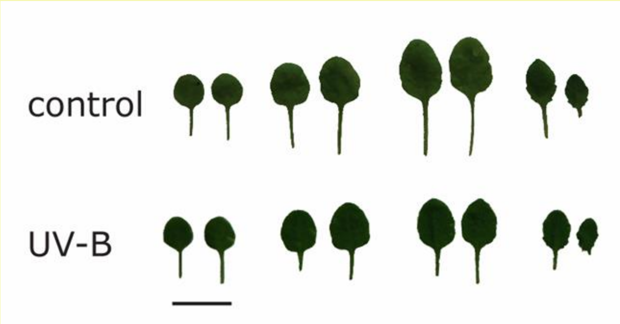


Trade off? (LAR↓→RGR↓)

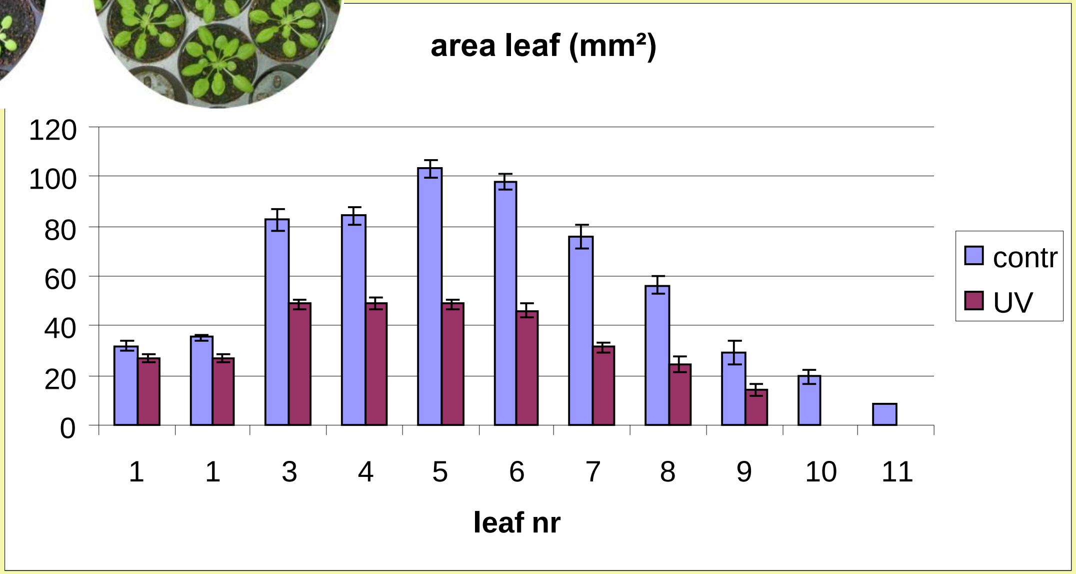
← Evolutionary pressure?
Functional Role?

↓

Self-shading???????



12 day ↑→



Hectors and Jansen (2012) Physiol Plant

A cellular perspective

- Hectors & Jansen; inhibition cell expansion (J Exp Bot 2010)
- Lake; inhibition cell division (PCE 2009)
- Wargent; Inhibition cell division, but stimulation cell expansion (New Phytol 2009)
- Staxen and Bornman; increased cell division (Physiol Plant 1994)

One response or mixture multiple responses?

Arabidopsis phenotype; complex dose-response

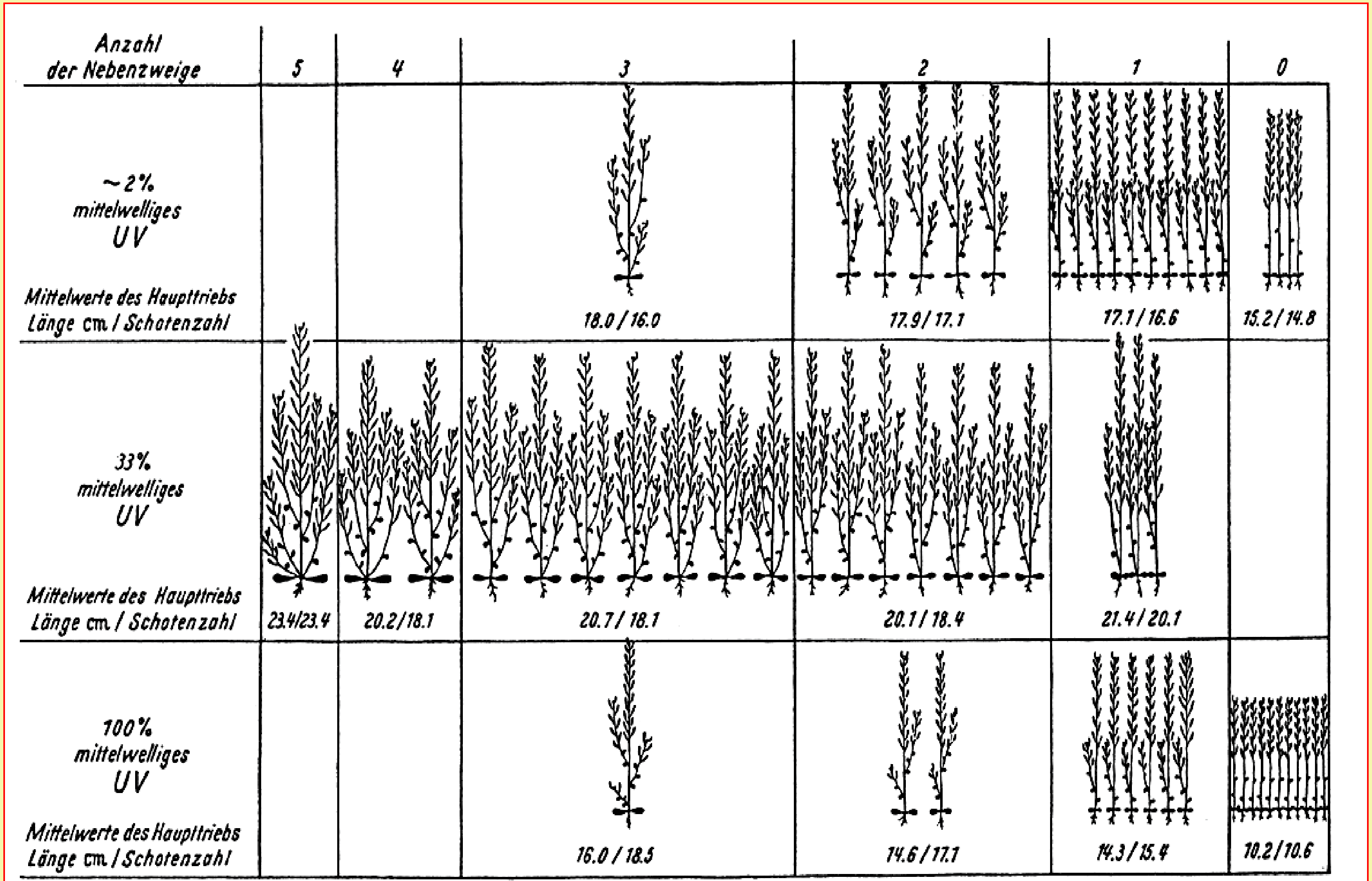


Abb. 14. *Arabidopsis thaliana*, Rasse Catania.

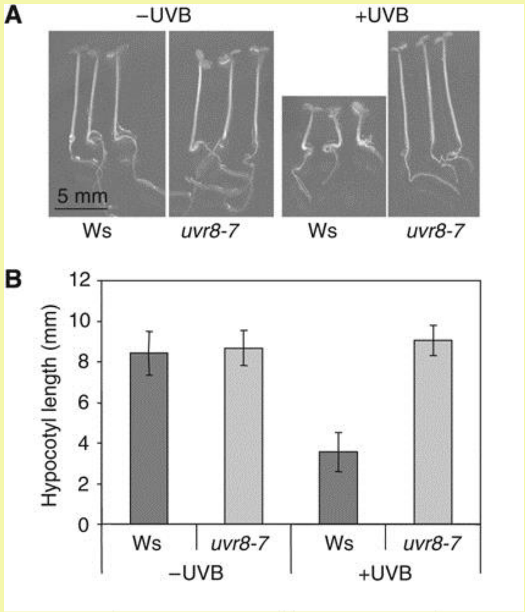
(Brodführer Planta 1955)

Flowering in *Silene vulgaris*; complex dose-response

	UV-B dose (kJm^{-2} $UV\text{-}B_{be}$)	Number of flowers
Lowland population	0	197
Netherlands	6	65
	16.2	127
Highland population	0	95
Austria	6	35
	16.2	105

Van de Staaij (Rozema) et al., (1997) *Plant Ecology*, **128**, 173-179.

Phenotypic variation & molecular mechanisms?



Inhibition elongation mediated by *uvr8*

Plants in sunlight simulators

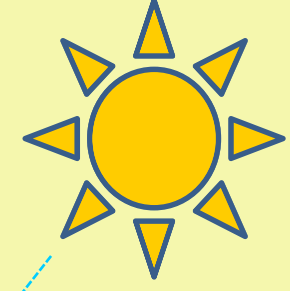
Favory, Jenkins, Ulm (2009). *The EMBO journal*

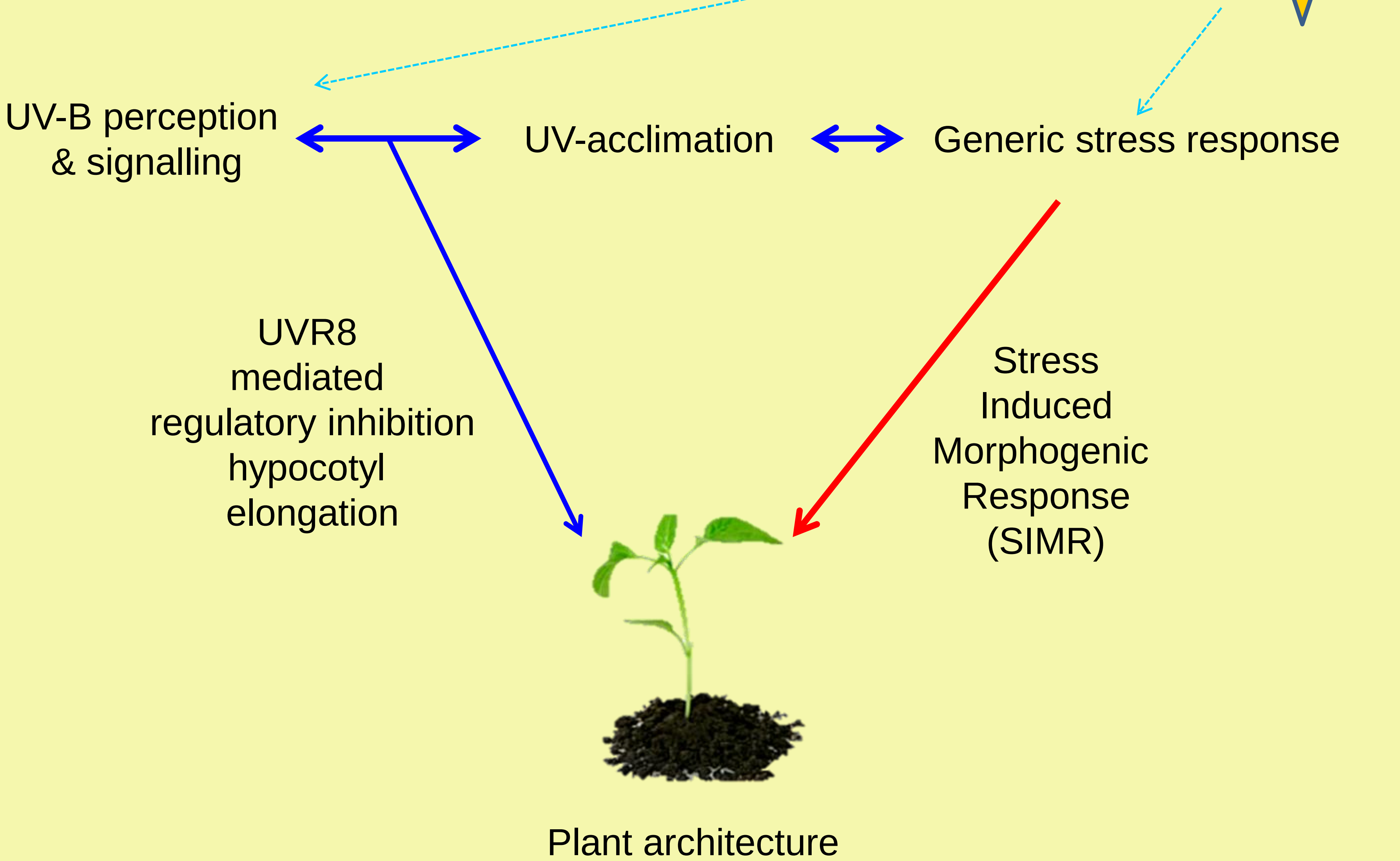
UVR8 mutants are *both* hypo- & hyper

UV-B sensitive

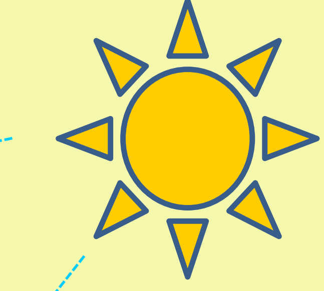
(Heijde & Ulm (2012) TiPS17, 230-237)

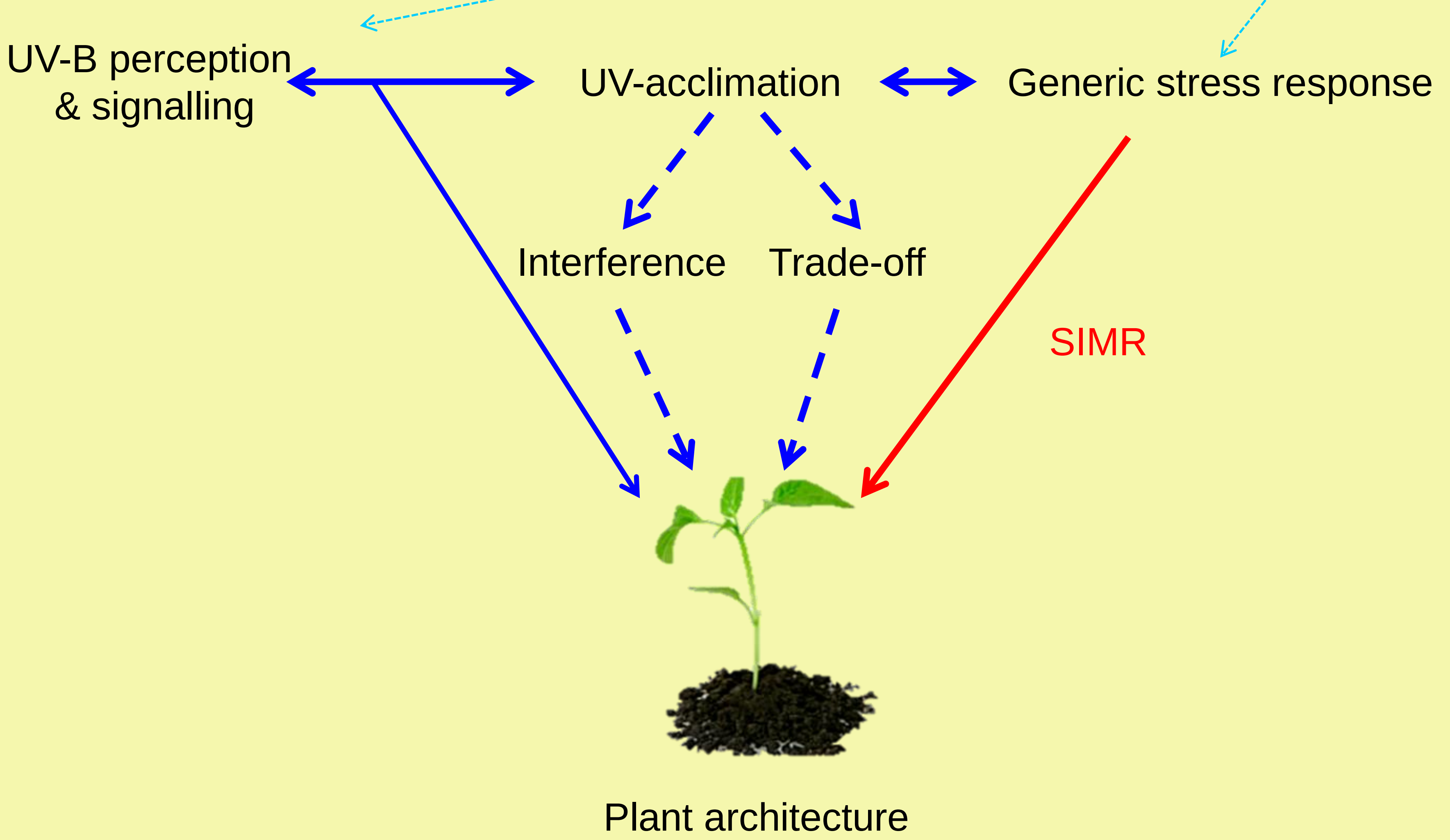
UV-B responses & morphology





UV-B responses & morphology





Size, shape and sturdiness matter!

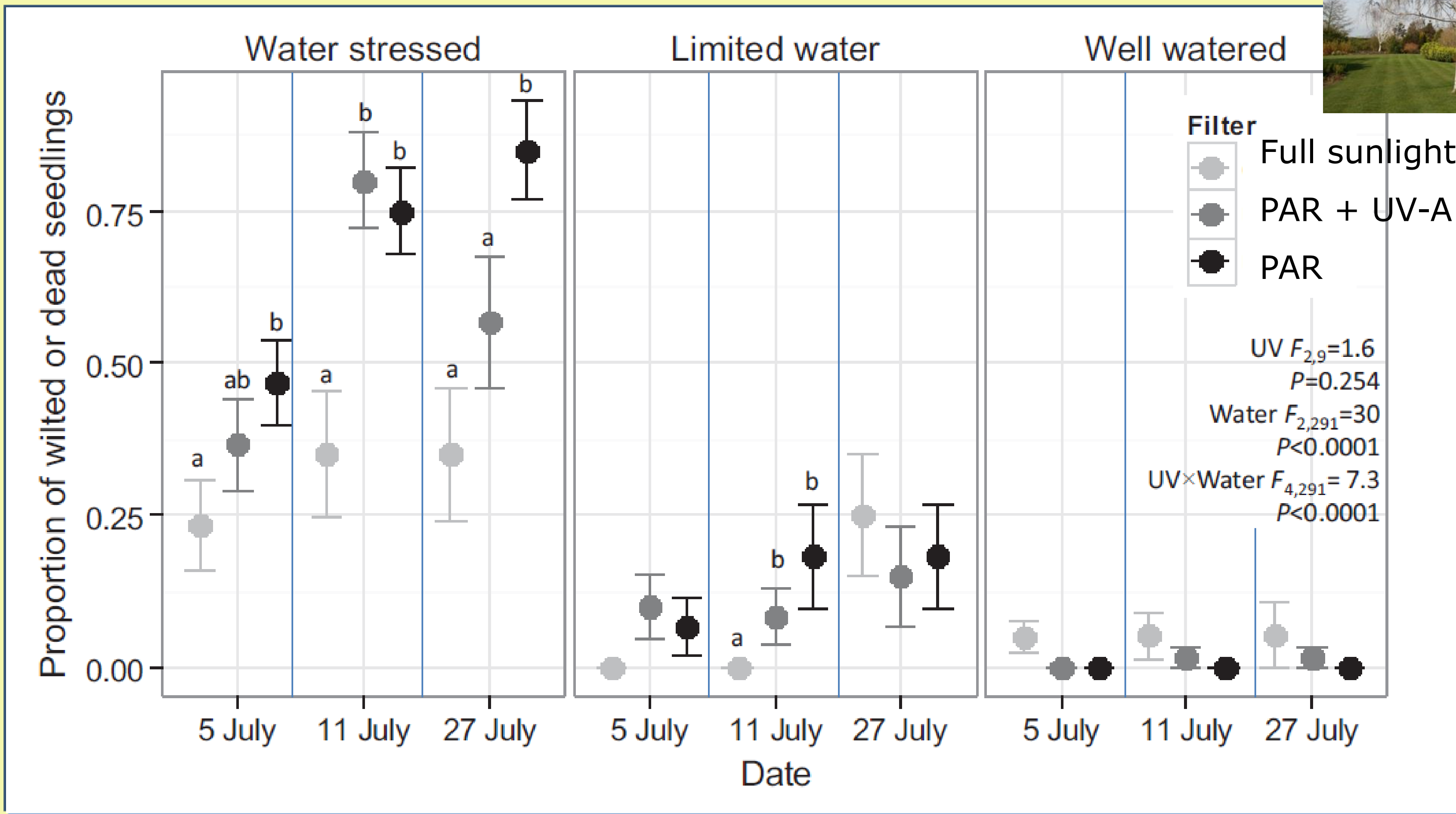
UV-B induced stockier plant architecture; a novel tool for horticulture!

Why have plants evolved to exploit UV-B as a source of information?

Did early plants "evolve" recognition of UV-B radiation to fine-tune UV-B protection?

Did early algal ancestors "evolve" recognition of UV-B radiation to switch on drought and/or heat stress defences?

Has UV a functional role in drought tolerance in birch?



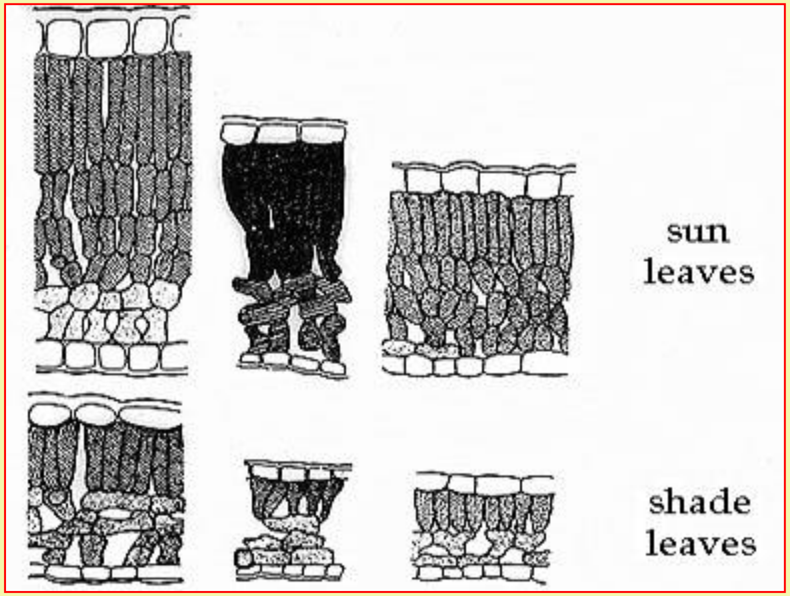
Robson et al., PCE (2014)

Are all plant UV-B responses about UV-B protection?

UV-B phenotype

- Smaller, thicker leaves
- Decreased water loss

Similar to sun/shade leaves



Are plants exploiting UV-B a proxy for e.g. high PAR, drought and/or heat?

Hypothesis:

UV-B perception has evolved into a general source of information about the environment

The end!