INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Measurement of photosynthesis

Open-path infra-red gas analyser Li-6400 principles and basic functions

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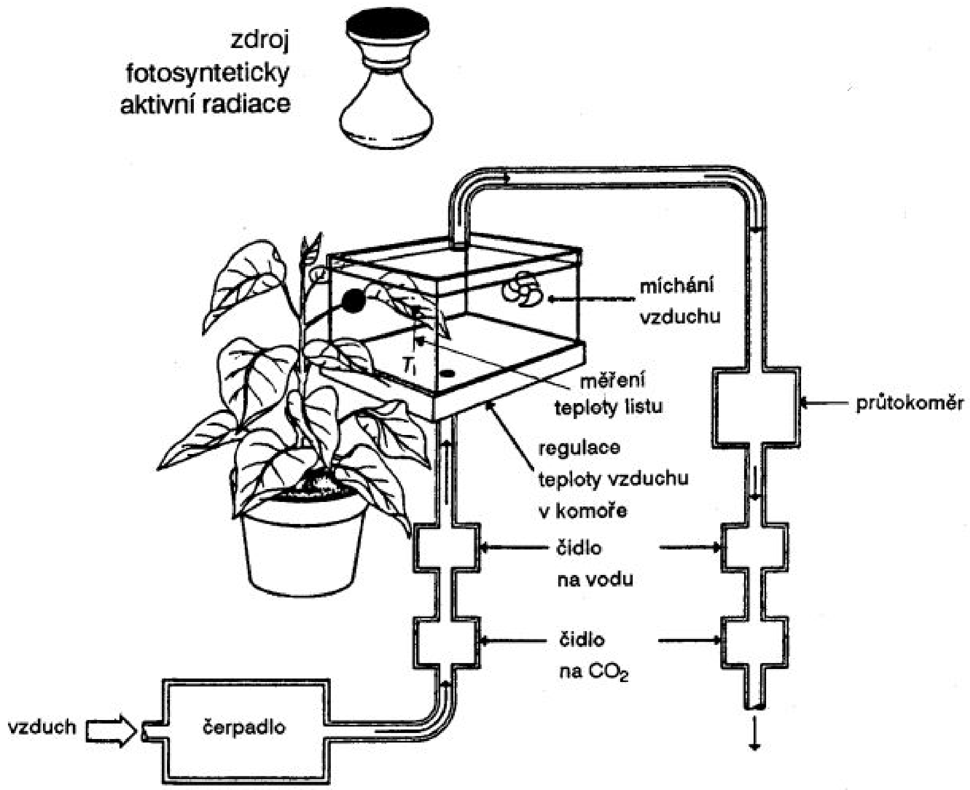
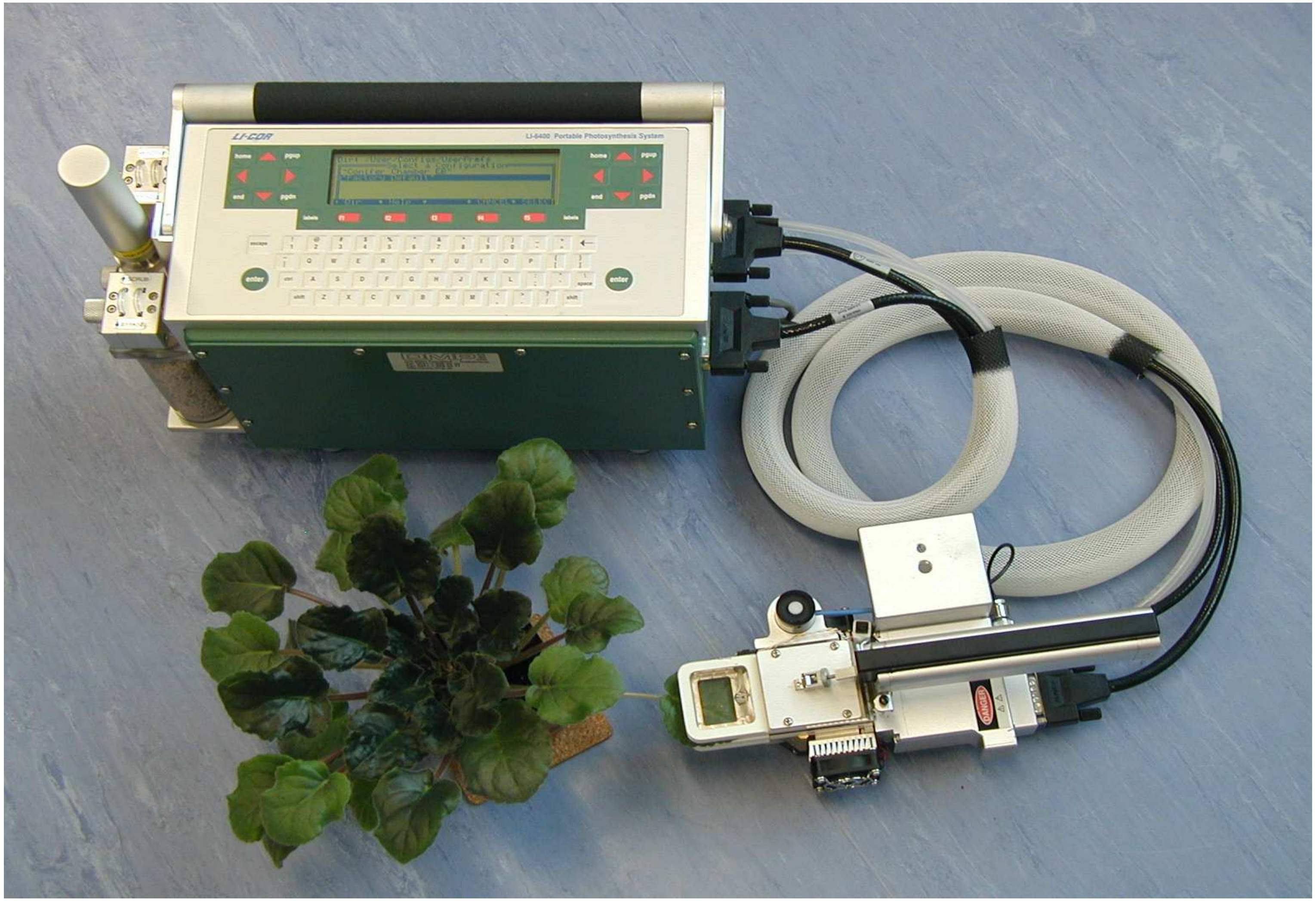
Tato akce se koná v rámci projektu:
Vybudování vědeckého týmu environmentální metabolomiky a ekofyziologie a jeho zapojení do mezinárodních sítí (ENVIMET; r.č. **CZ.1.07/2.3.00/20.0246**) realizovaného v rámci Operačního programu Vzdělávání pro konkurenceschopnost.

Gas-exchange technique



- accurate, continuous measurement of gas exchange (CO2, O2 and H2O) between plant tissues and the surrounding atmosphere
- changes in the concentration of H2O and CO2 are determined by an infrared gas analyser (**IRGA**)
- Lambert-Beer extinction law
  - $\mathbf{a_\lambda = 1\text{-}exp(\text{-}l.M.k_\lambda)}$
- **hierarchy**: leaves → ecosystem

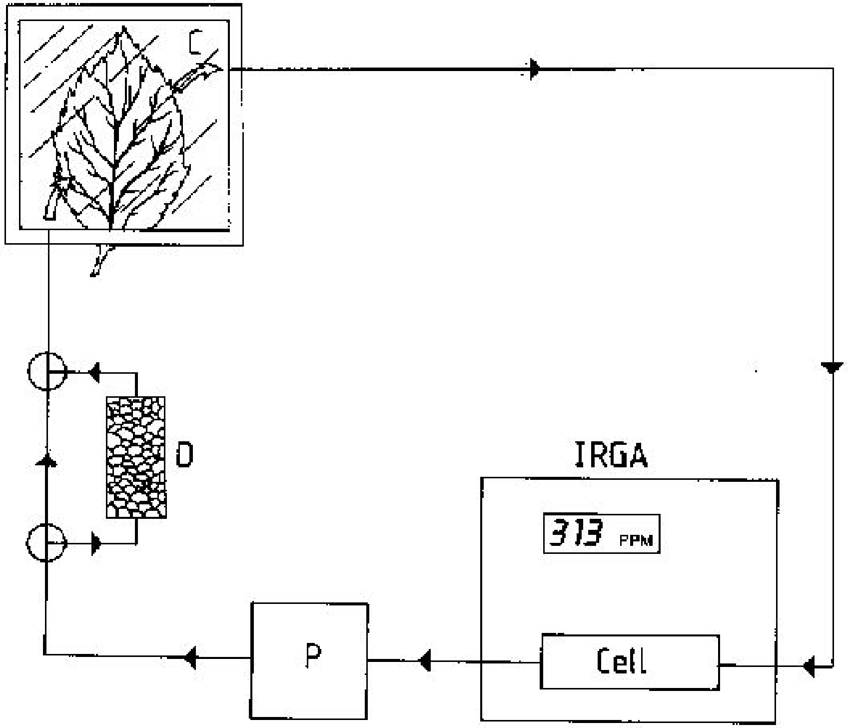
Gas-exchange system LI-6400

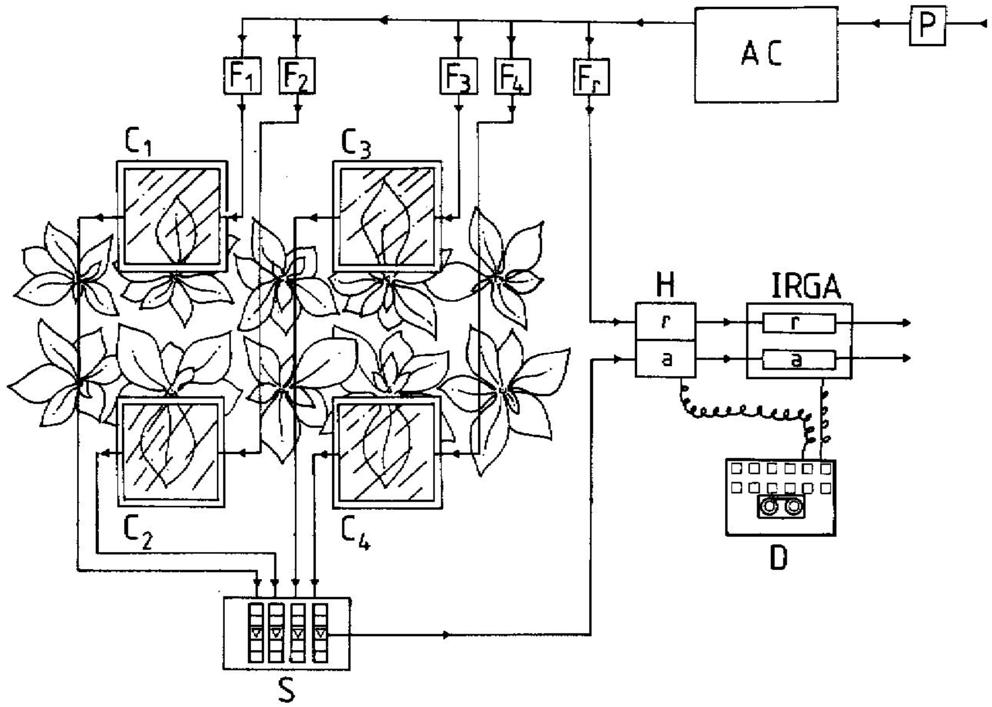


Basic types of devices

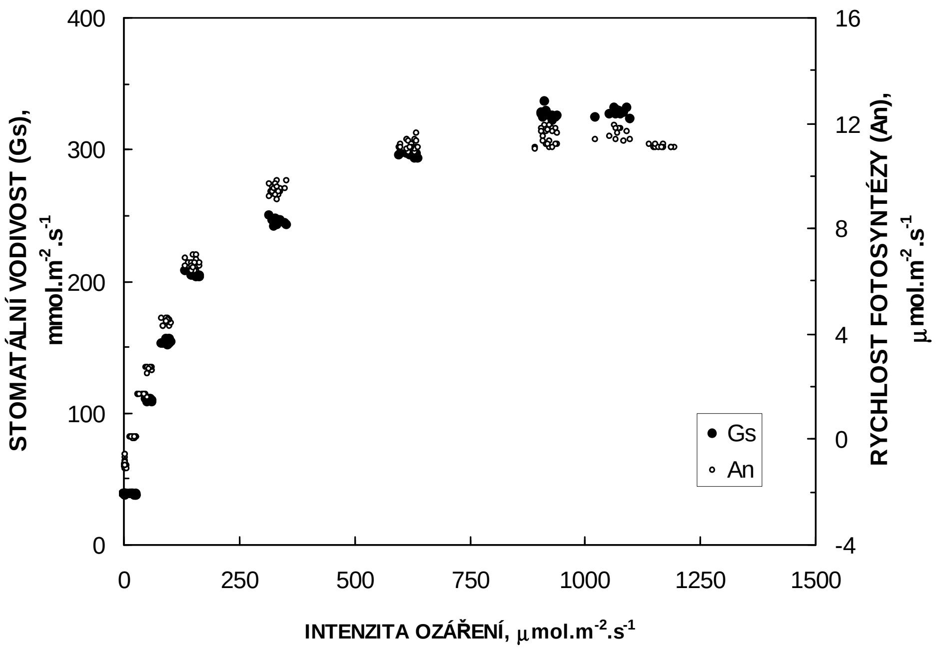
Closed system x **Open-path system**

(Licor, LI-6200) *(Licor, LI-6400; Ciras-1, PP Systems)*





Basic photosynthetic characteristics



$A = P - R_L - R_D$

• **Light Response Curve**

A_N-I (FAR, PPFD)

CO_2 = constant

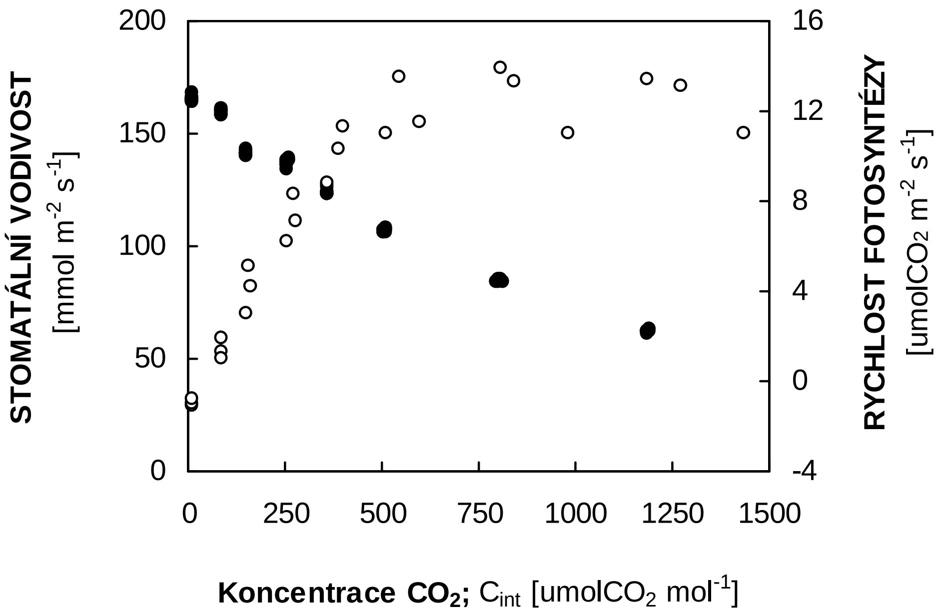
variable light intensity

• **CO_2 Response Curve**

A_N-Ci

irradiance = constant = saturated

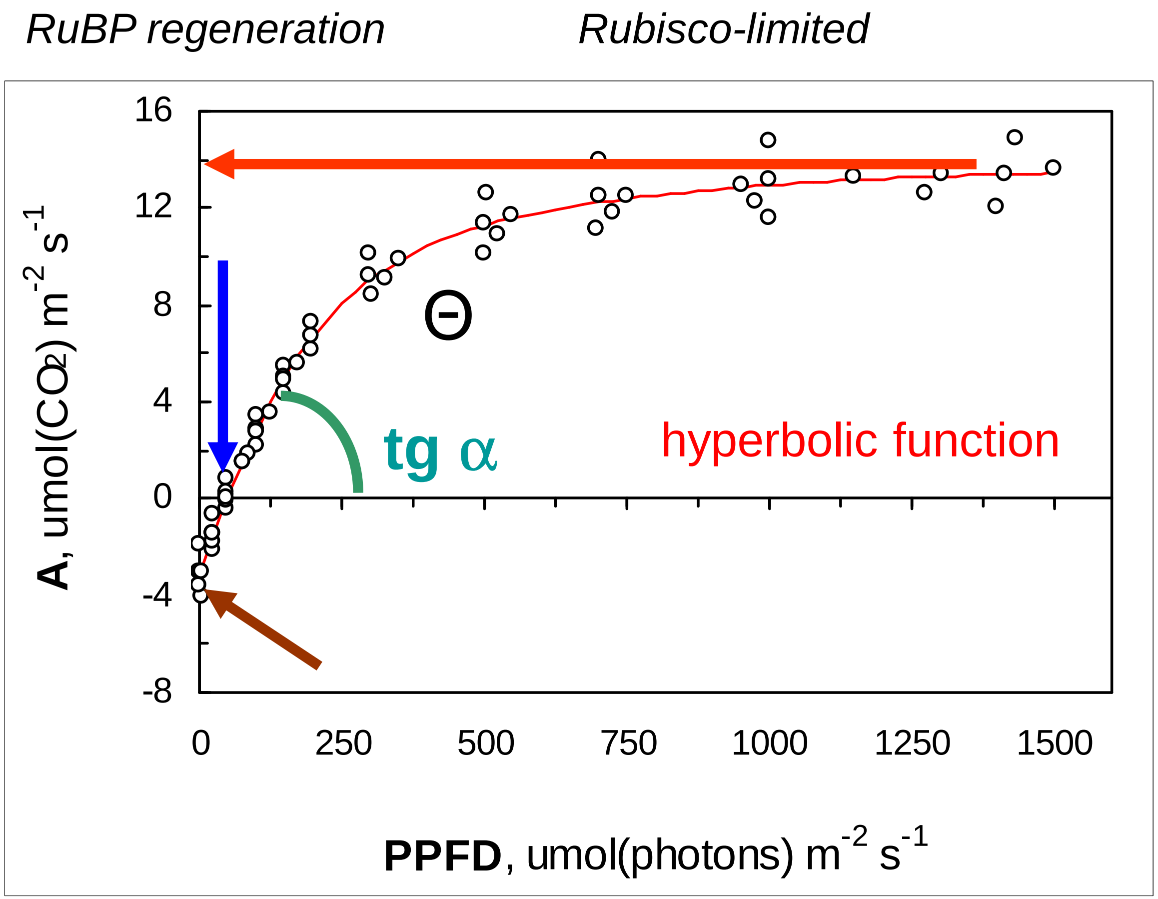
variable CO_2 concentration



3 examples

- differences in sun- and shade-acclimated leaves/plants
- effect of dynamic light environment
  - photosynthetic induction curves
- effect of sky conditions
  - C assimilation during sunny and cloudy days

Light response curve



R_D – dark (mitochondrial) respiration
$\mu mol(CO_2)\ m^{-2}\ s^{-1}$

Γ_I – compensation irradiance
$\mu mol(photon)\ m^{-2}\ s^{-1}$

AQE (tg α) – apparent quantum efficiency
$mol(CO_2)\ mol^{-1}(photons)$

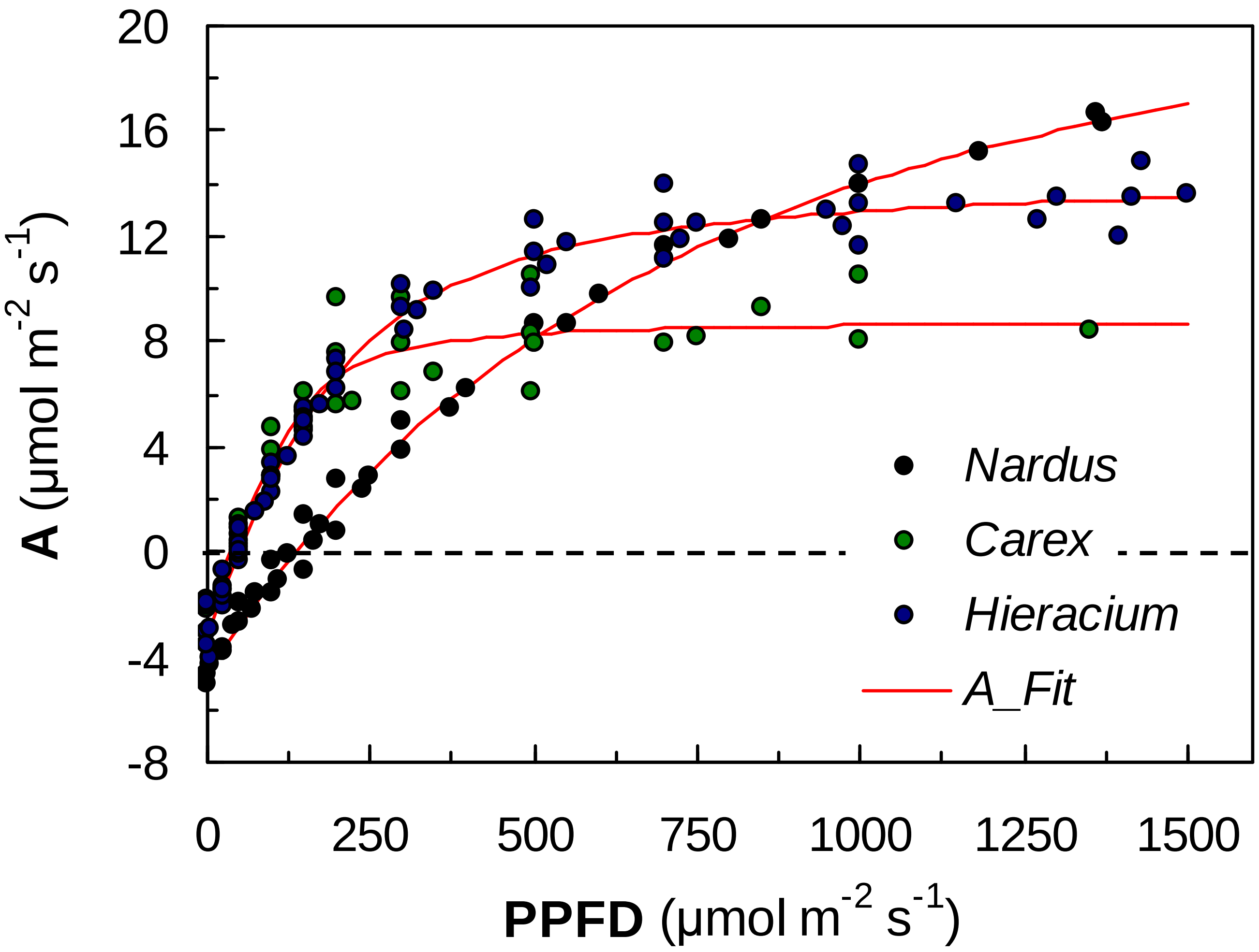
Θ – curvature (0 – 1)
dimensionless

A_{max} – light-saturated assimilation rate
$\mu mol(CO_2)\ m^{-2}\ s^{-1}$

$$A = \frac{AQE \cdot I + A_{max} - \sqrt{(AQE \cdot I + A_{max})^2 - 4 \cdot AQE \cdot I \cdot \Theta \cdot A_{max}}}{2 \cdot \Theta} - R_D$$

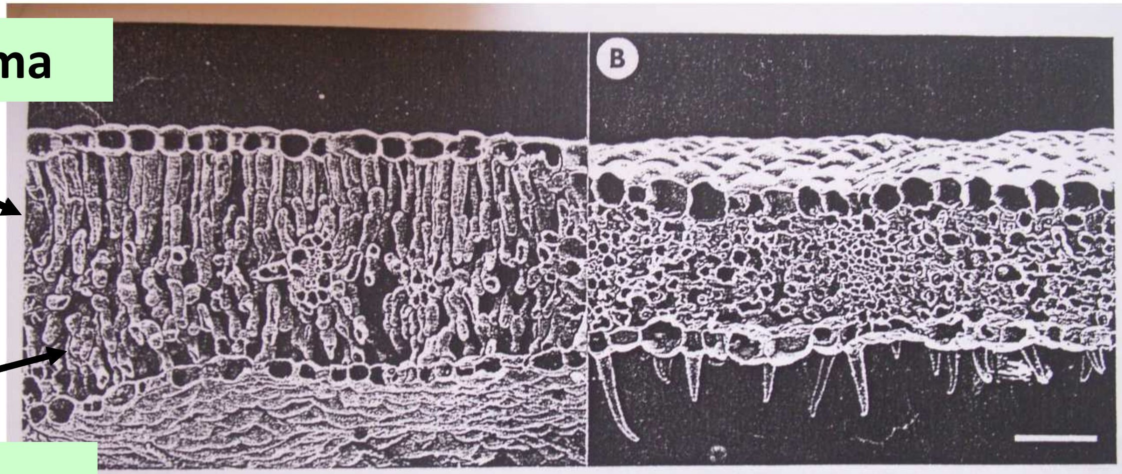
Prioul JL, Chartier P (1977) Annals of Botany, 41, 789–900.

Examples of A-PPFD curves



Herbaceous species (CzechGlobe)

Sun- x shade-acclimated leaves



Leaf anatomy of *Thermopsis montana* and *Smilacina stellata*. Leaves of (a) *T. montana* that develop in the sun typically have well-developed columnar palisade, whereas only spongy tissue is present within (b) *S. stellata* (scale bar = 100 µm).

Vogelmann T.C. and G. Martin: PCE (1993) 16, 65-72

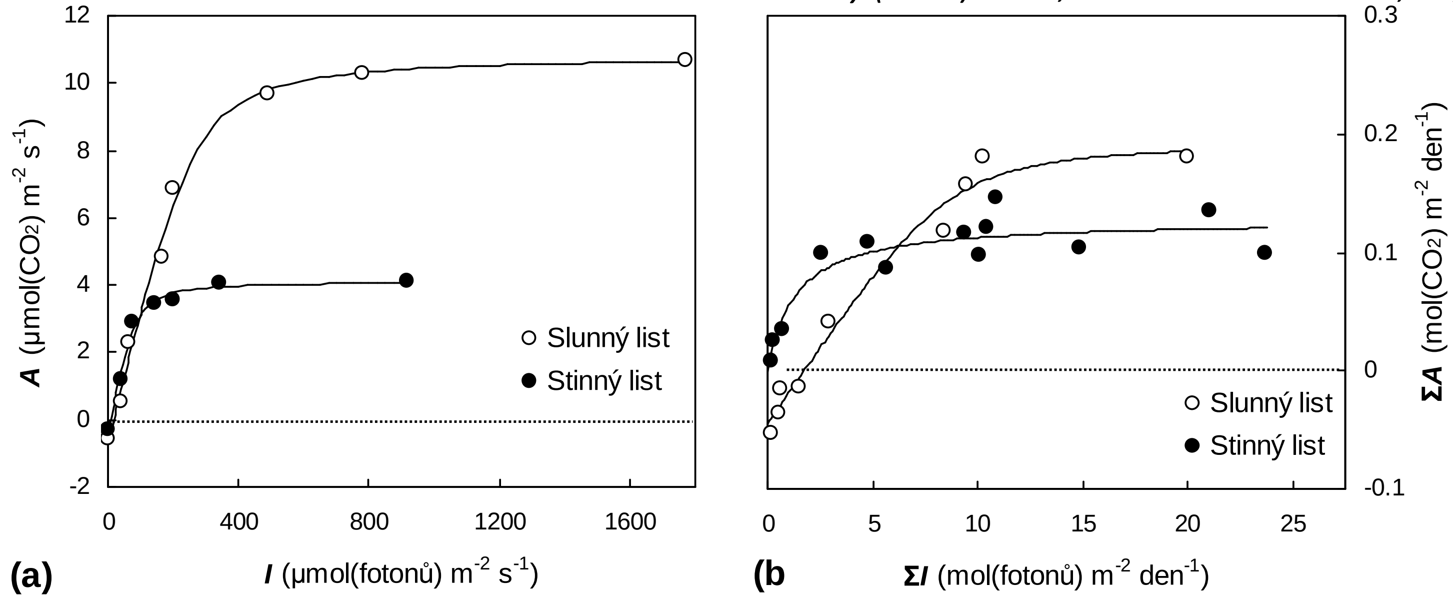
Shade-acclimated leaves:

- thinner leaves but larger area (higher specific leaf area – SLA; $cm^2\ g^{-1}$)
- lower number of stomata per unit leaf area (stomata are bigger)
- bigger chloroplasts with irregularly oriented granna
- higher chlorophyll and carotenoids content per unit mass ($mg\ g_{DW}^{-1}$)
- lower conductance to CO2 in the mesophyll
- lower Nitrogen content – lower Rubisco content

investments into the protein structures connected with the efficient photochemical reactions (primary phase of photosynthesis)

Functional differences

Sims et Pearcy (1994) Plant, Cell and Environment, 17, 881–887.



Shade-acclimated leaves/plants:

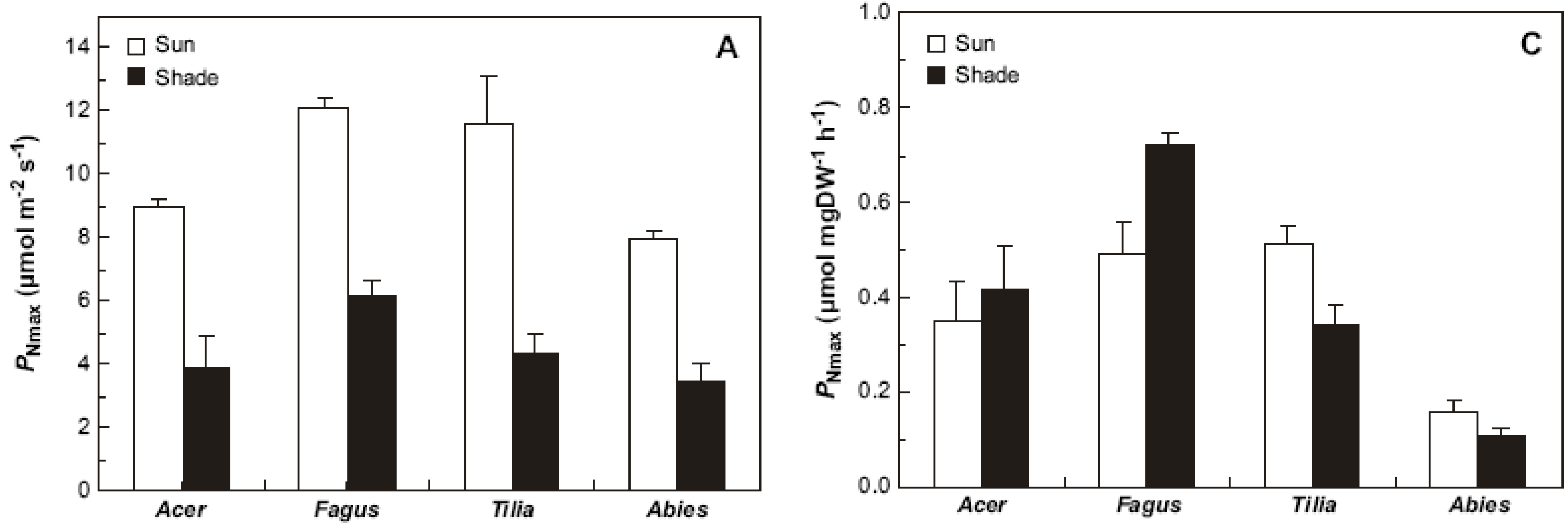
- lower mitochondrial resp. (R_D)
- lower compensation irrad. (Γ_I)
- higher quantum effic. (AQE)
- lower light-saturated rate of CO_2 assimilation (A_{max})

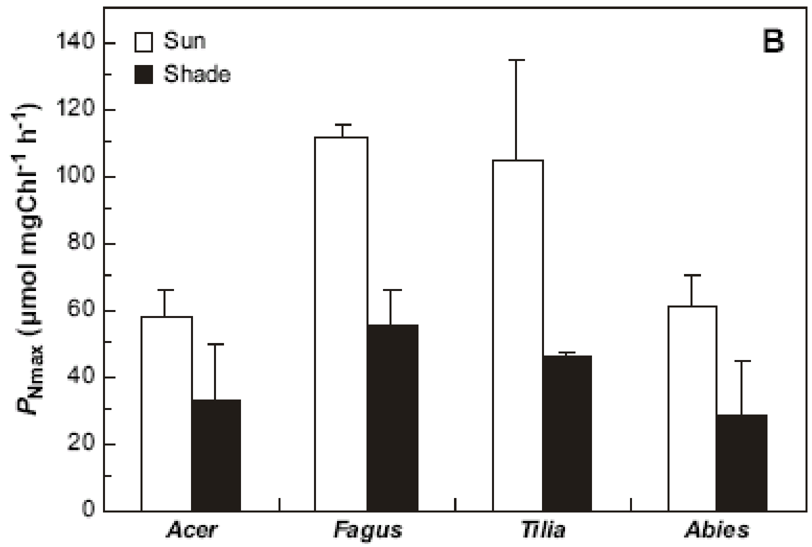
Shade-acclimated leaves/plants:

- higher sum of assimilated CO_2 (ΣA) at lower amount of daily irradiance (ΣI)
- more effective in dynamic light environment
  - faster induction, slower deactivation of photosynthesis

Assimilation of a canopy depends on its structure (sun/shade leaves ratio)

CO_2 Assimilation Rate – Units



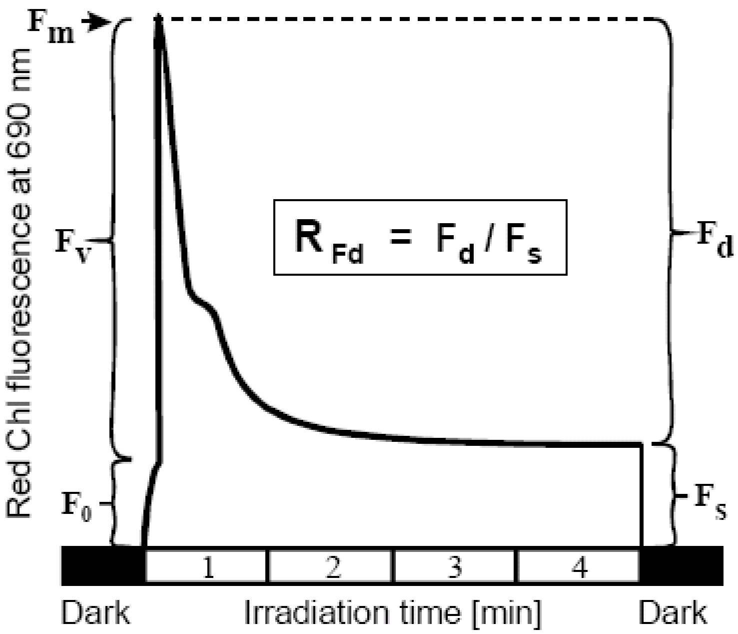


Kubiske ME, Pregitzer KS Funct. Ecol. 11 (1997) 24-32:

- shade leaves of shade-intolerant species respond to shade primarily by altering SLA,
- whereas shade-tolerant species respond largely via biochemical acclimation of the photosynthetic apparatus.

Lichtenthaler et al. Plant Physiology et Biochemistry 45: 577-588, 2007.

Fluorescence Decrease Ratio



F_0 = initial fluorescence

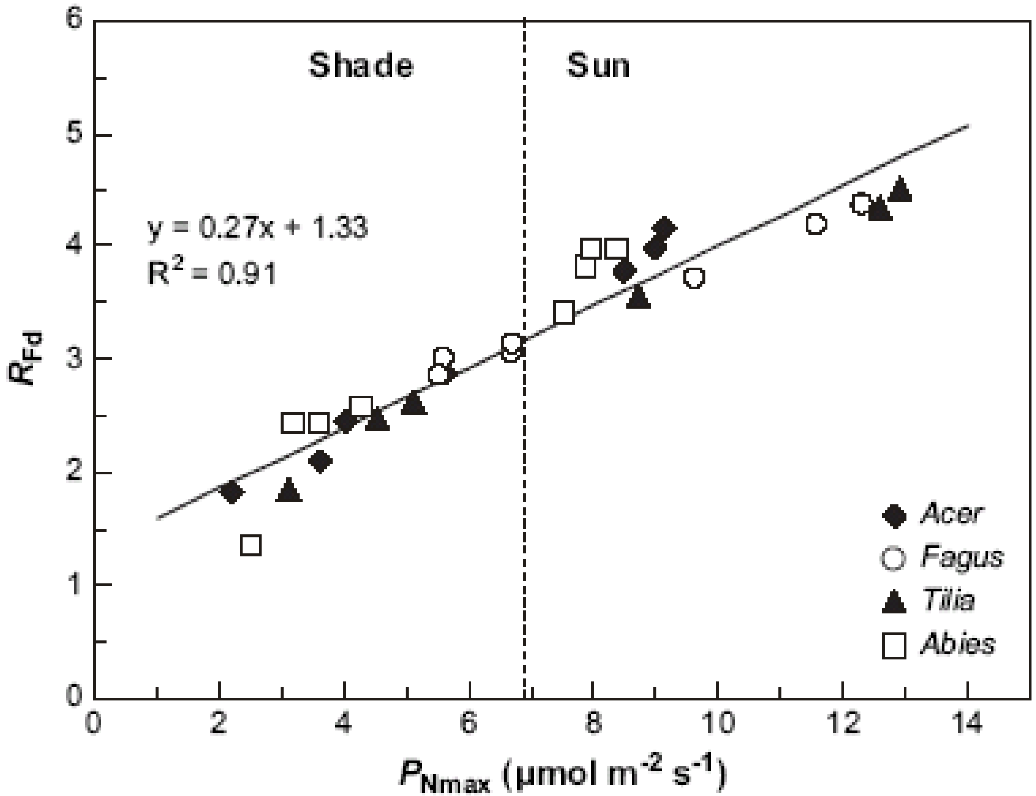
F_v = variable fluorescence

F_m = maximum fluorescence

F_d = fluorescence decrease

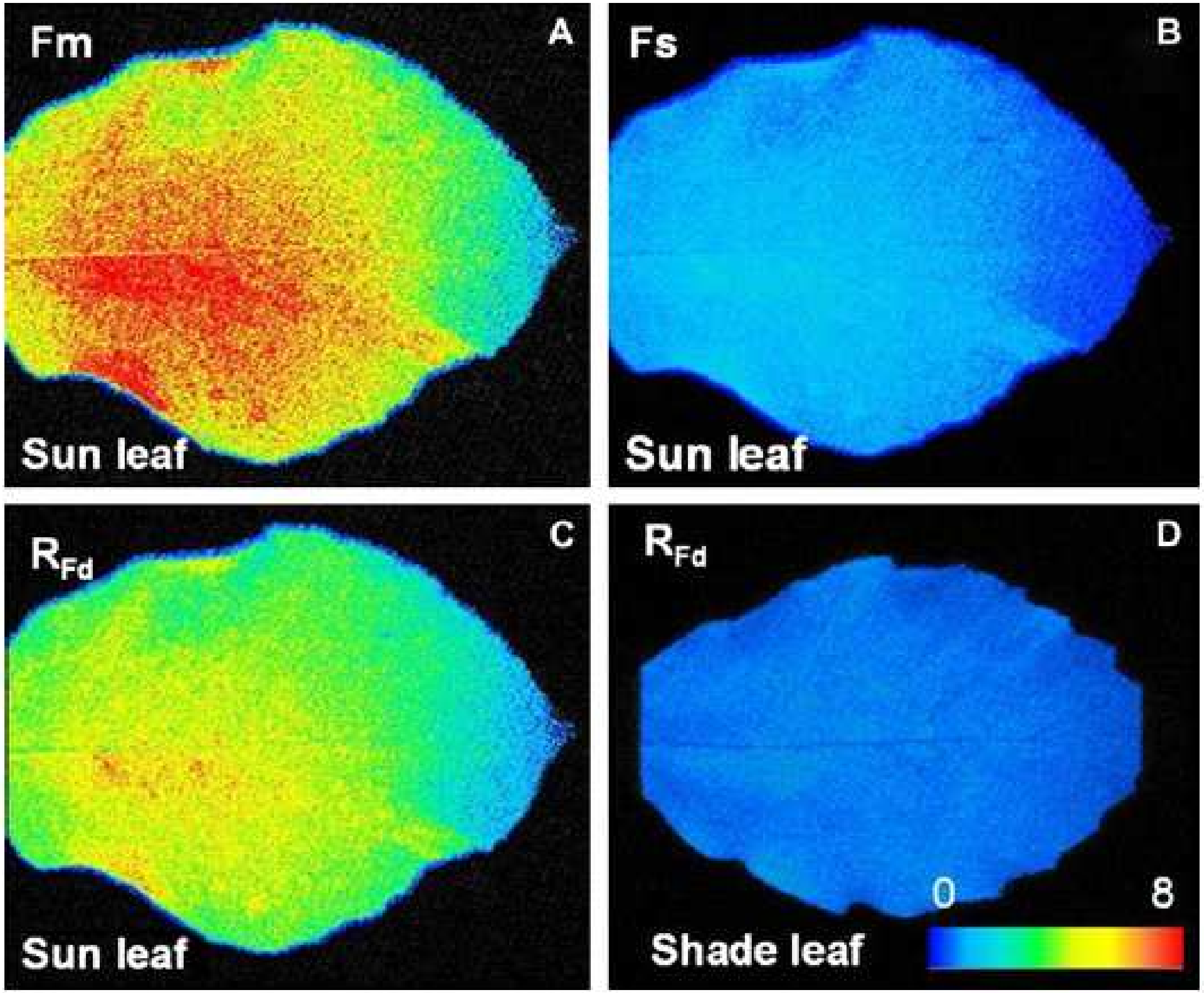
F_s = steady state fluorescence

R_{Fd} = fluorescence decrease ratio



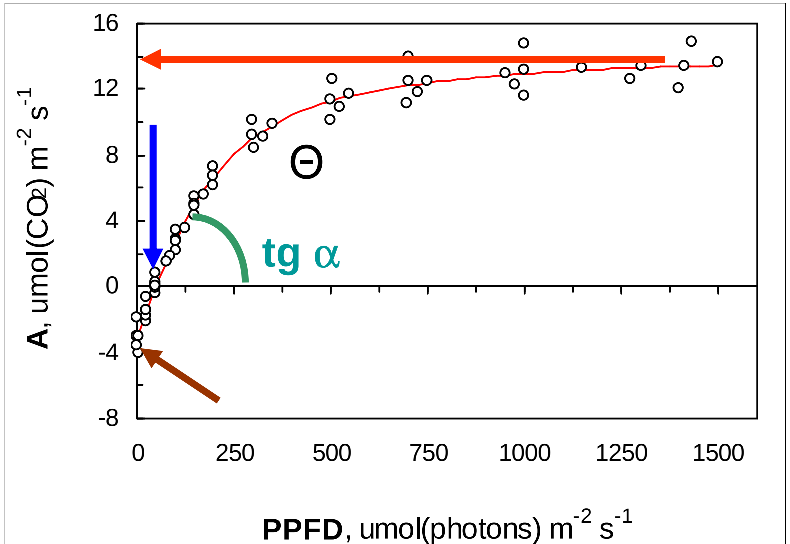
Lichtenthaler et al. Plant Physiology et Biochemistry 45: 577-588, 2007.

R_{Fd} Spatial Distribution

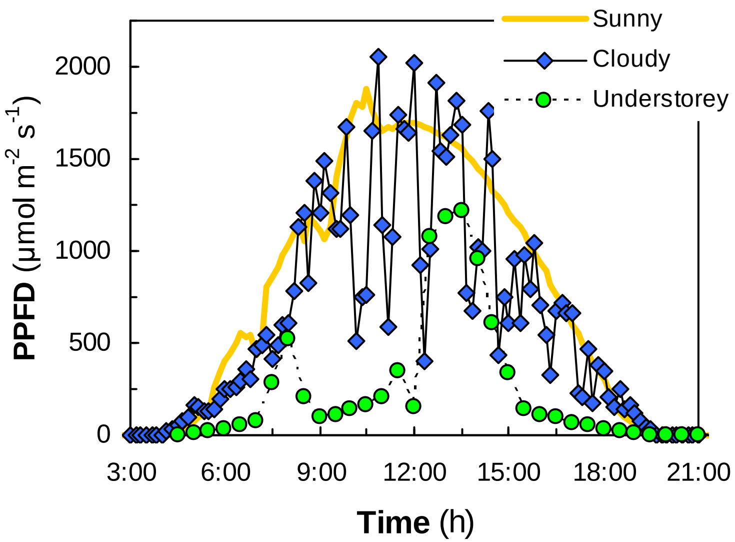


Lichtenthaler et al. Plant Physiology et Biochemistry 45: 577-588, 2007.

Dynamic Light Environment



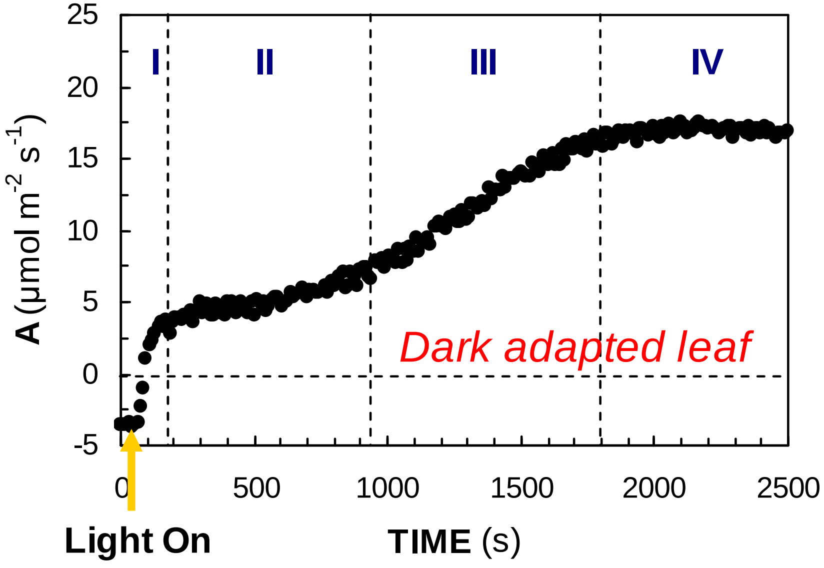
$$A = \frac{AQE \cdot I + A_{max} - \sqrt{(AQE \cdot I + A_{max})^2 - 4 \cdot AQE \cdot I \cdot \Theta \cdot A_{max}}}{2 \cdot \Theta} - R_D$$

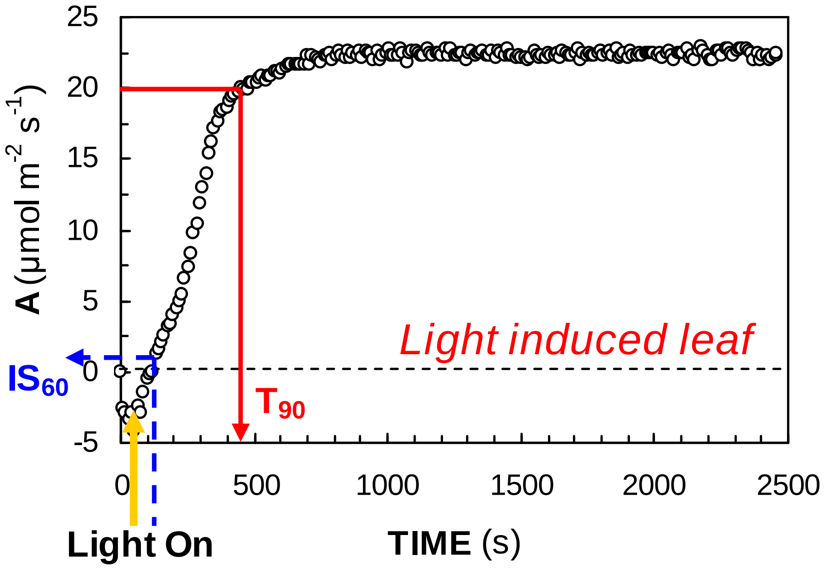


- <u>common approach</u>: carbon fixation based on the photosynthetic parameters estimated under steady-state conditions
- natural environment ≈ dynamic light fluctuation
  - (A) variable cloud cover,
  - (B) self-shading of leaves and trees → <u>**sun-flecks**</u>

- **effective utilization of fluctuating light requires fast photosynthetic induction**

Photosynthetic induction curve





- **photosynthetic induction curve (PIC)**
  - **dark adapted leaf**
  - **light pre-induced leaf**
- **phases of induction curve**

I. 1–2 min; rapid activation of enzymes associated with RuBP regeneration (fructose-1,6-bisphosphate, FBPase)

II. 10–15 min; biochemical limitation of photosynthesis; light regulation of Rubisco activity

III. tens of minutes; stomatal limitations, low intercellular CO2 concentration → slowing the rate of Rubisco activation (secondary limitation)

IV. fully light-induced leaf (A_{max}, G_{Smax})

- **basic parameters**

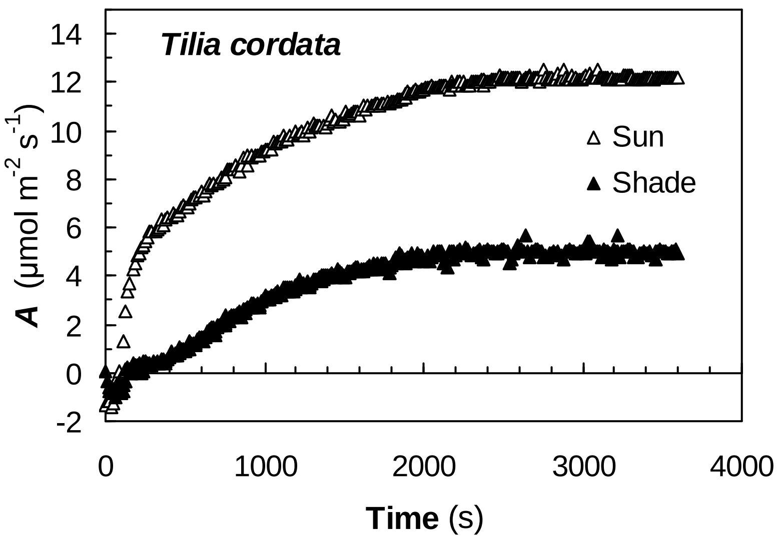
T_{90} – time required to reach 90% of A_{max}

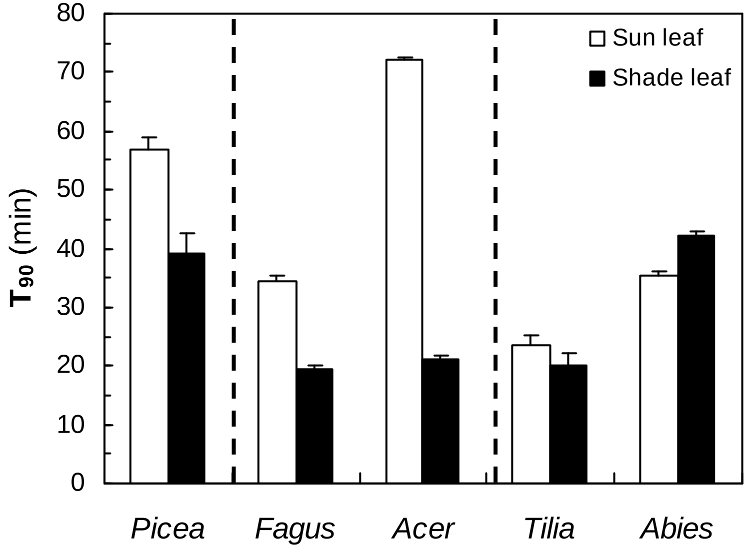
IS_{60} – induction state (%) after 60s

τ – time constant for Rubisco activation

PIC in sun and shade leaves

Urban et al. Tree Physiology, 27: 1207-1215, 2007.





- **Hypothesis:** shade acclimated leaves have faster photosynthetic induction
  - literature sources are inconsistent

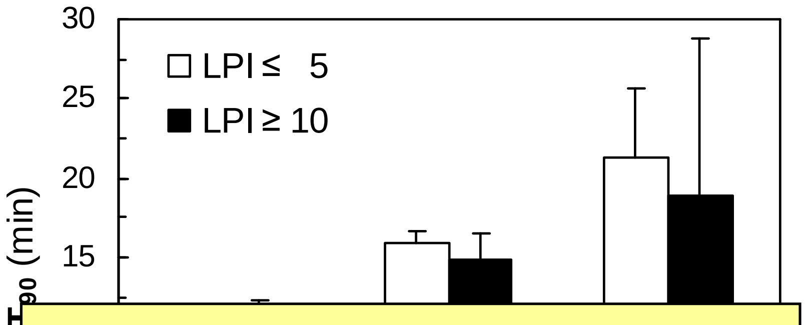
- 5 ecologically contrasting tree species
  - *Abies alba,Tilia cordata* - strongly shade-tolerant
  - *Fagus sylvatica, Acer pseudoplatanus* - intermediate in shade-tolerance
  - *Picea abies* - sun-demanding

- example of time course of CO2 assimilation during photosynthetic induction (linden)

- **shade intolerant tree species**
  - **T_{90} sun leaves >> T_{90} shade leaves**
- **strongly shade-tolerant tree species**
  - **T_{90} sun leaves ≤ T_{90} shade leaves**

PIC and leaf ontogeny

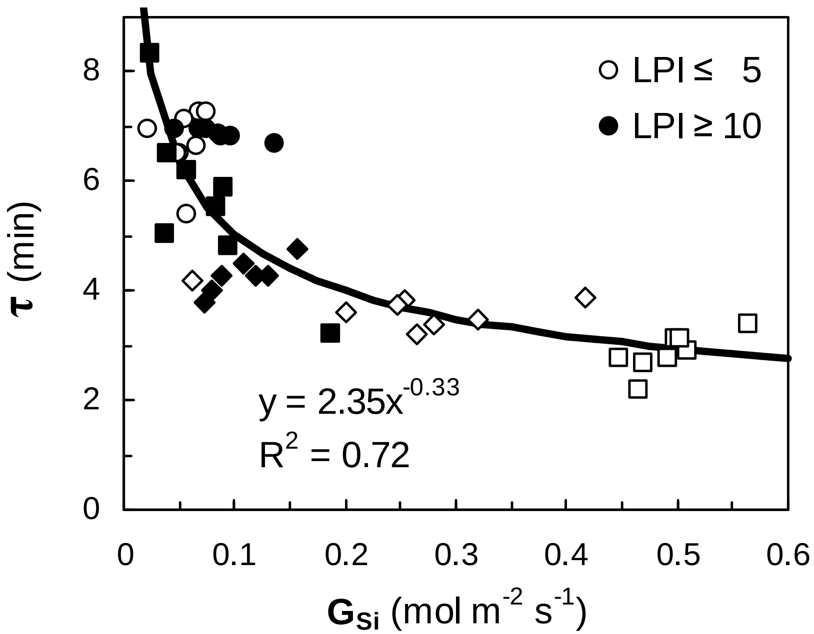
Urban et al. Tree Physiology, 28: 1189-1197, 2008.



- **Hypothesis:** activation of photosynthesis proceeds longer in older leaves
  - increase of resistances to CO_2

- results document an essential role of stomata in photosynthetic induction
  - transient drought periods

- leaf age was described with the Leaf Plastochron Index (LPI)
  - LPI≤5: young expanding leaves
  - LPI≥10: mature to senescing leaves
- 3 poplar clones (*Populus*)
  - *Populus alba*, *P. nigra*, *P. x euramericana*



- non-significant differences in IS_{60} and T_{90}
  - clones with fast initial induction required longer times for the full activation
- **max. stomatal limitation** during induction
  - young leaves 4–9%
  - mature leaves 16–30%
- time constant for **Rubisco activation** (τ)
  - tight correlation with initial G_S (G_{SI})

What is the role of CO2?

Leakey et al. (2005)

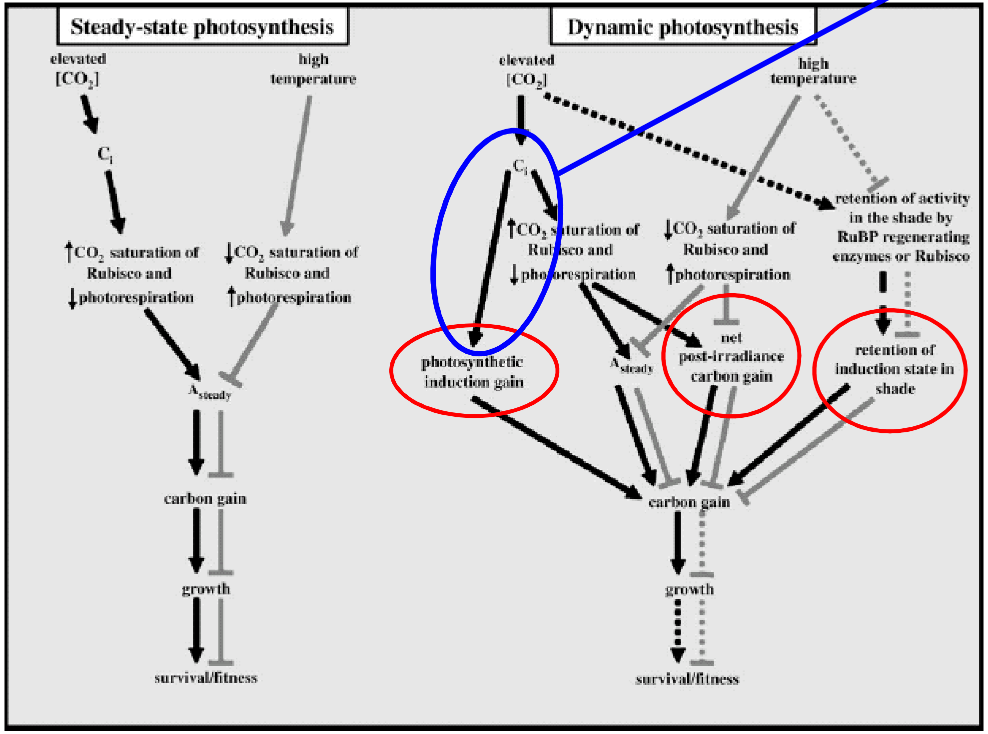


***Figure**: A simplified diagram comparing the mechanisms by which elevated [CO_2] and high temperature impact photosynthesis under steady-state or dynamic irradiance regimes. Arrows indicate positive effects, barred lines indicate negative effects.*

- **Does elevated [CO_2] lead to faster photosynthetic induction?**

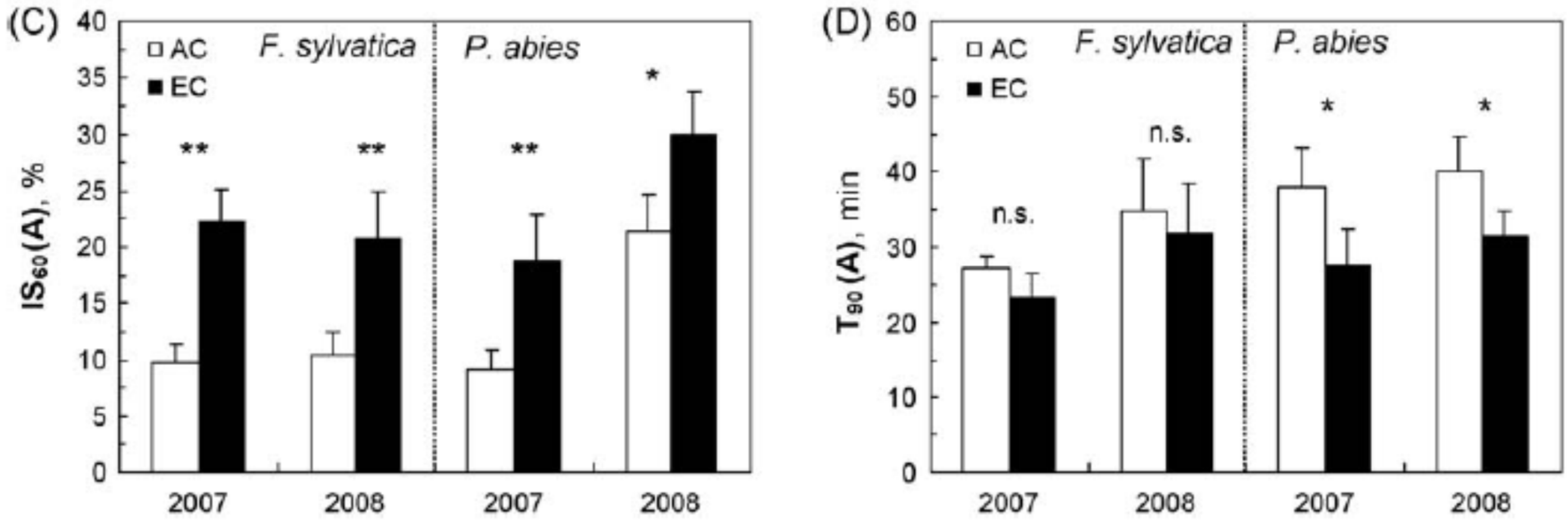
1) → decrease of Rubisco amount
2) → decrease of stomatal conductance

- the reduced maximum activity of Rubisco and stomatal conductance should be reached faster in elevated [CO_2] plants than ambient ones
  - even if actual induction rates are not altered by elevated [CO_2],

- **two distinctive tree species**
  - broad-leafed *Fagus sylvatica*
  - coniferous *Picea abies*
- **variants**
  - AC – 385 μmol(CO_2) mol^{-1}
  - EC – 700 μmol(CO_2) mol^{-1}

Effect of elevated CO_2 concentration

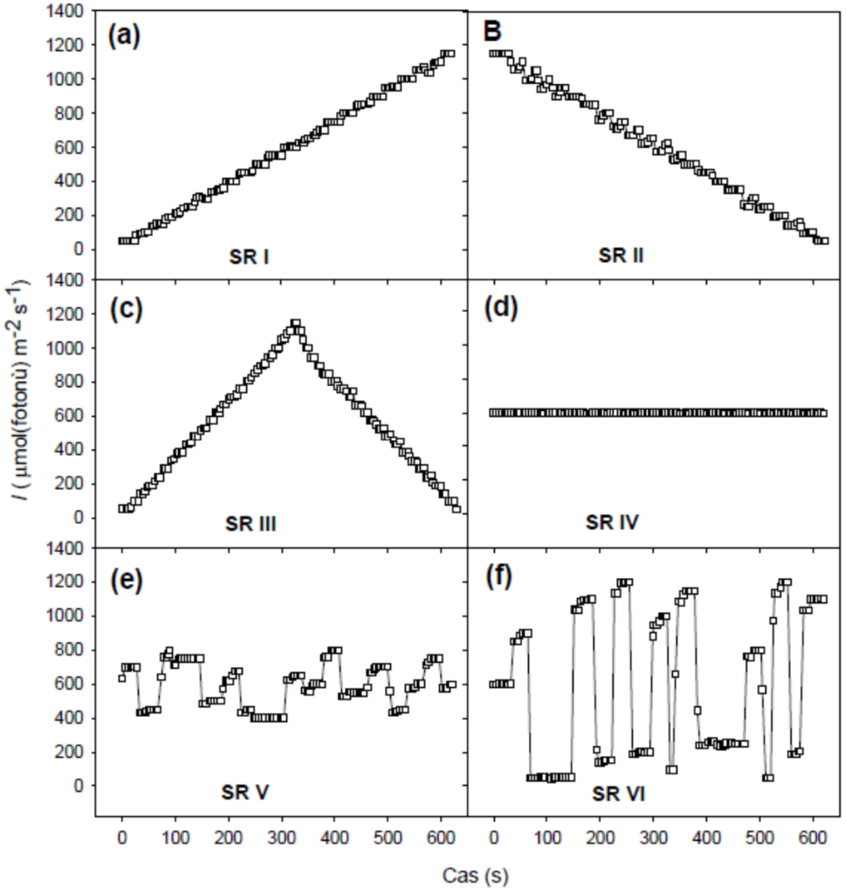
Košvancová et al. Plant Science 177: 123-130, 2009.

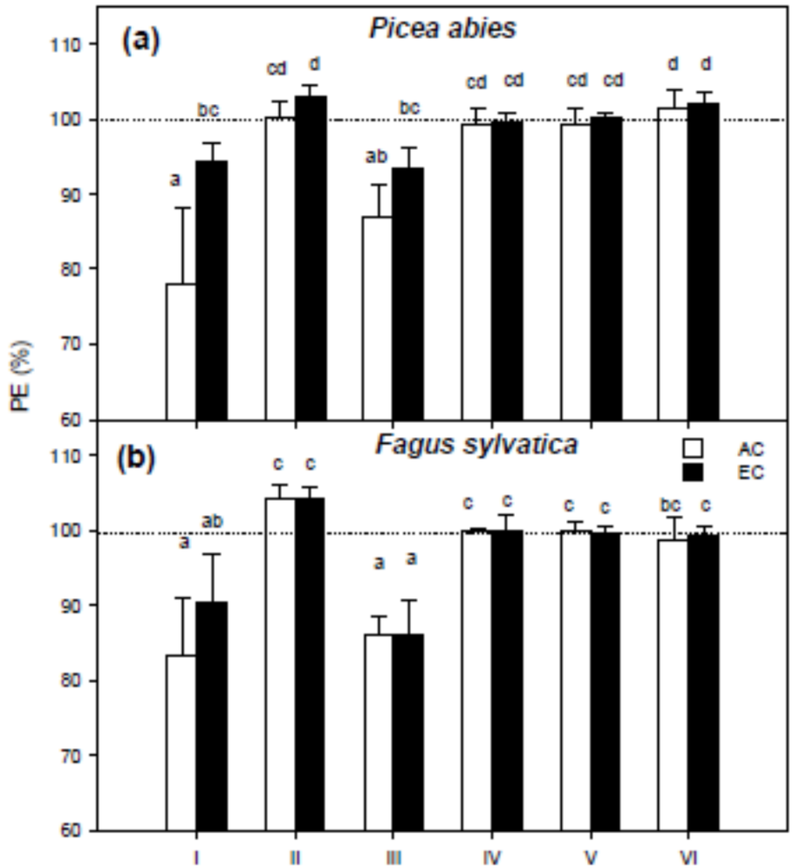


- EC stimulated the initial phase of photosynthetic induction (IS60)
  - F. sylvatica and P. abies
- stimulation of the secondary phase of photosynthetic induction T90(A)
  - reflecting mainly stomatal opening
  - only in P. abies
  - F. sylvatica: reduced rate of stomatal opening by EC

Effect of elevated CO_2 concentration

Holišová et al. J.Env.Quality 41: 1931-1938, 2012.

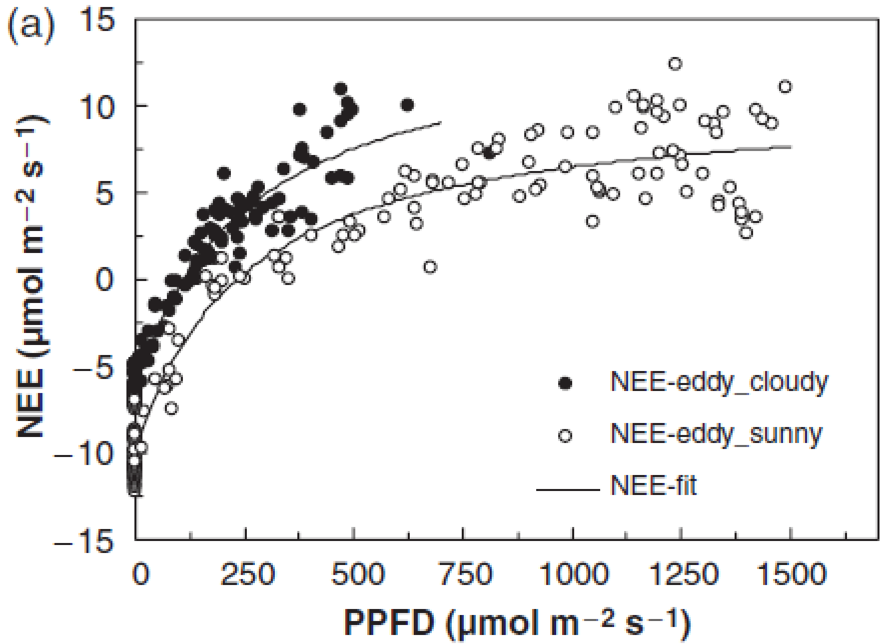




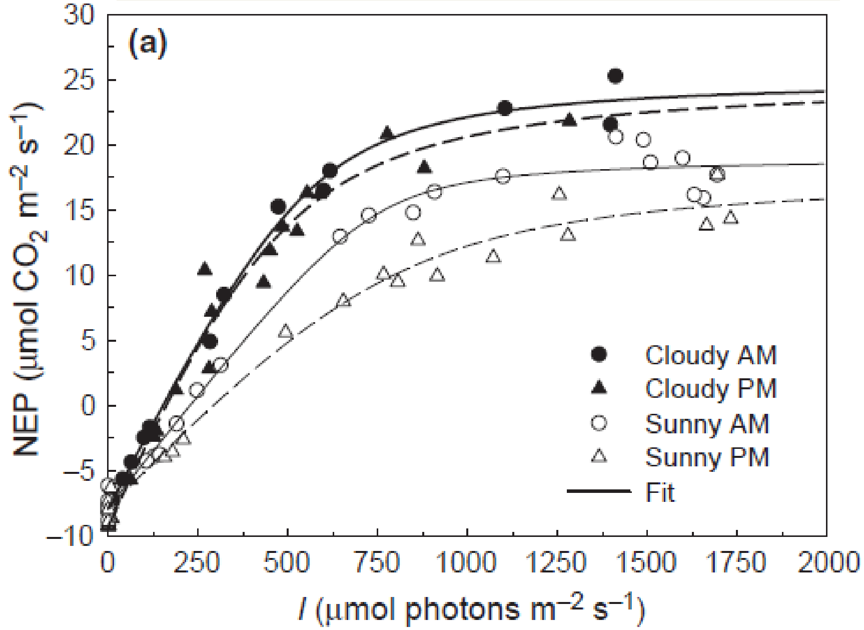
- different time courses of incident irradiance, whereas same duration (600 s) and total amount of radiation (35.88 mmol photons m^{-2})
- stimulation of photosynthetic efficiency by EC only when leaves were pre-exposed to low light intensity and photosynthetic induction was required
- EC had only a minor effect on the rate of induction loss and postillumination CO_2 fixation

Sky conditions: cloudy x sunny days

Urban O. et al.: GCB 13: 157-168, 2007.

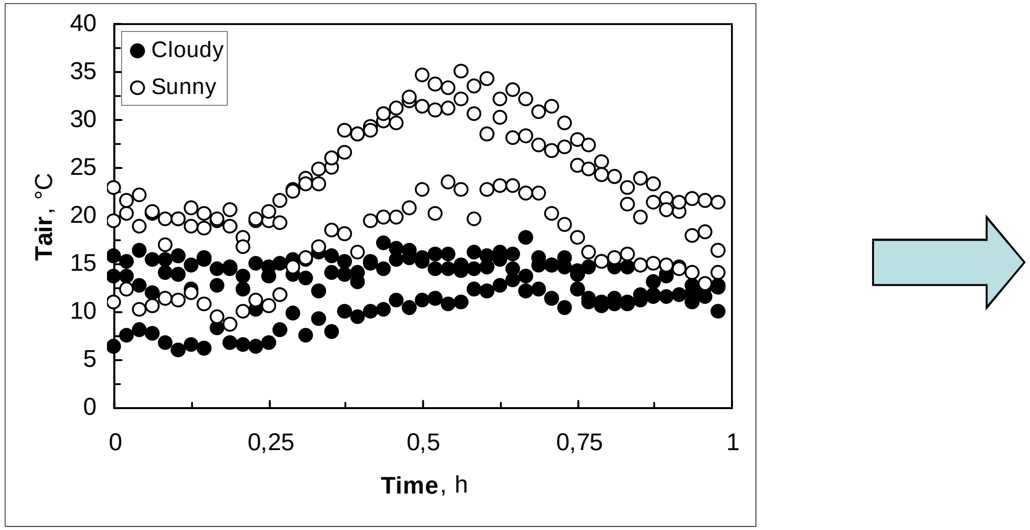


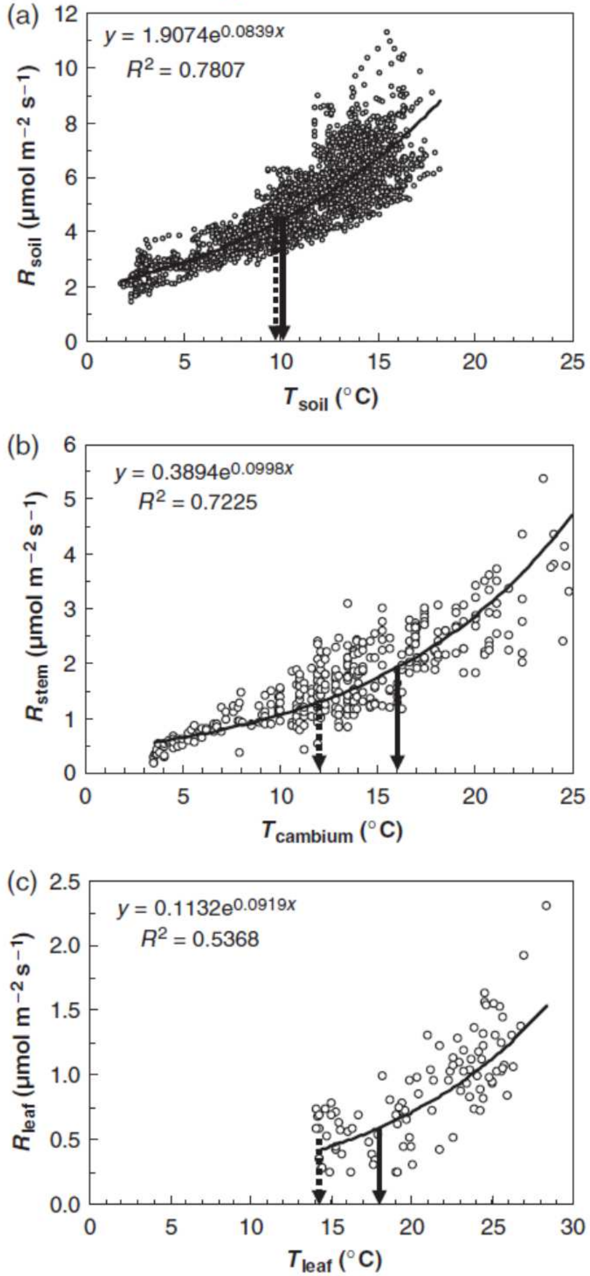
Urban O. et al.: Func.Ecol. 26: 46-55, 2012



- eddy-covariance measurements
- significantly higher NEE during cloudy sky at corresponding PPFDs
  - diffuse index: > 0.7 (cloudy sky), < 0.3 (clear sky)
  - AQE higher by 20%, Γ_I lower by 50% $\Rightarrow$ energy of solar radiation is used more efficiently in CO_2 assimilation
- **dimming effect** $\Rightarrow$ important global change
- hysteresis response of CO2 assimilation during sunny days

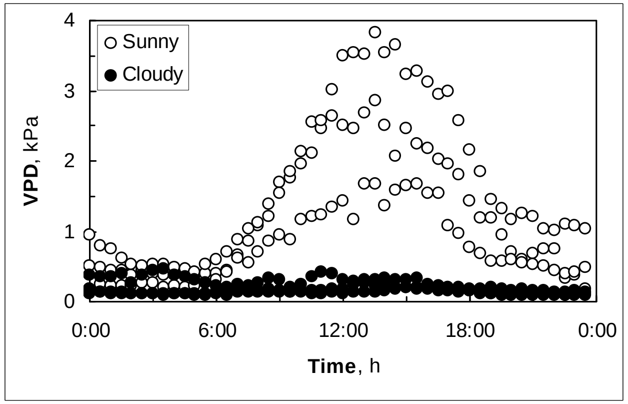
Why? I. low temperature – low respiration



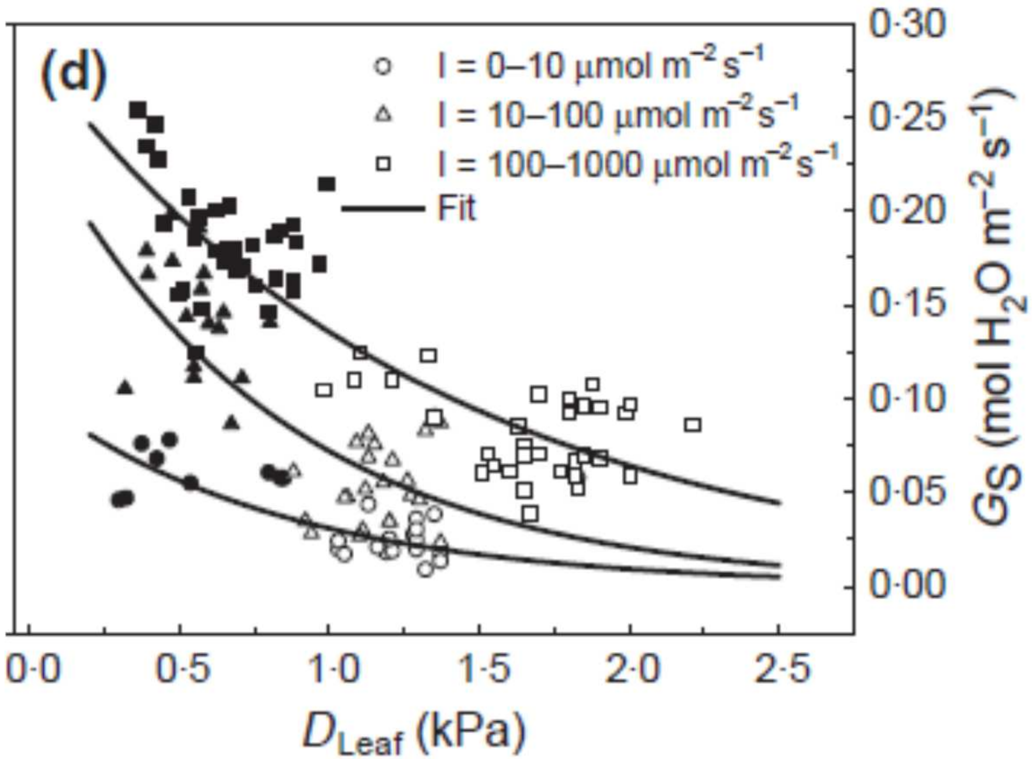


- respiration of all ecosystem components increases with temperature
  - chamber measurements
  - SAMTOC/SAMTOL
- **exponential relationship**
- **the main source of CO_2 – soil – has relatively constant temperature**
  - **dense forests**

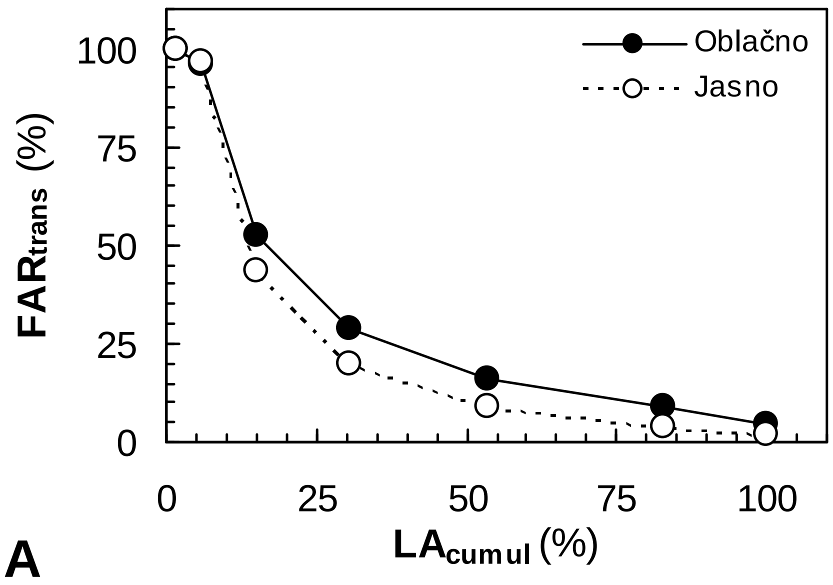
Why? II. lower vapour pressure deficit

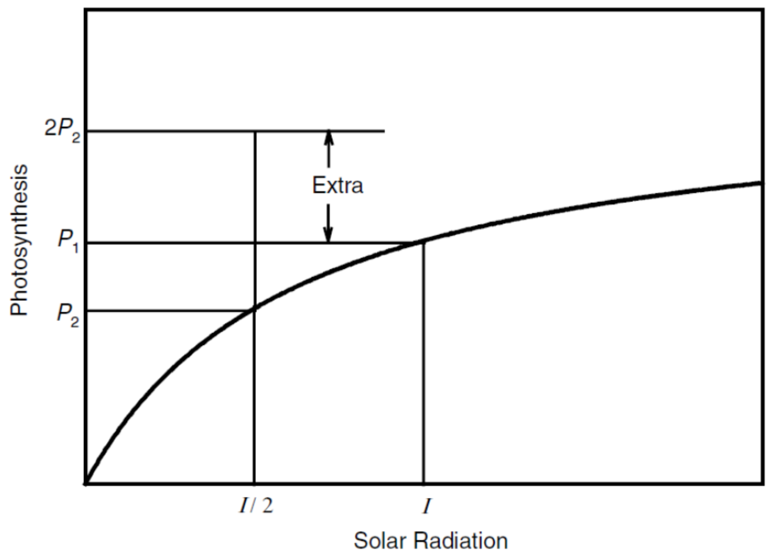


- high VPD (D) values -- closure of stomatal apertures and reduction of stomatal conductance for CO2 diffusion
- **reduction of intercellular CO2 concentration – reduction of CO2 assimilation rate**
- **main reason of NEE hysteresis curve**
  - **(midday) photosythetic depression**



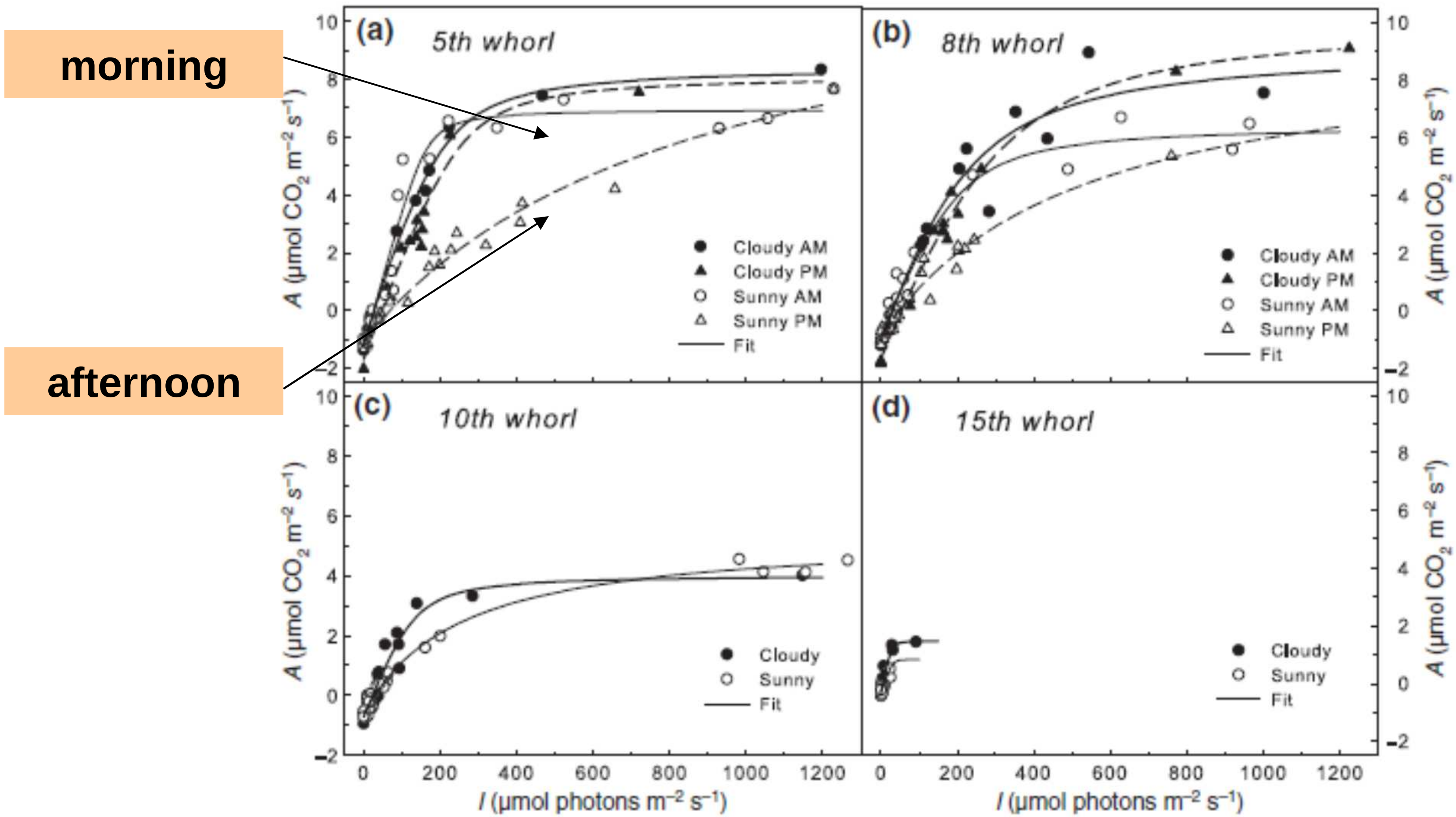
Why? III. effective penetration of diffuse light





- main reason for higher NEE
- diffuse radiation penetrates more efficiently into lower parts of a canopy
- lower extinction coefficients
  - ≈ 25% (ca 0.6 x 0.8)
- bigger part of leaf area is photosynthetically productive
  - 30 – 40 μmol m^{-2} s^{-1} (Γ_I)
- up to ≈ 70% of LA may be a source of CO_2 during sunny days
- uniform illumination of the canopy is better
  - consequence of the hyperbolic shape of LRC

Vertical distribution of photos.

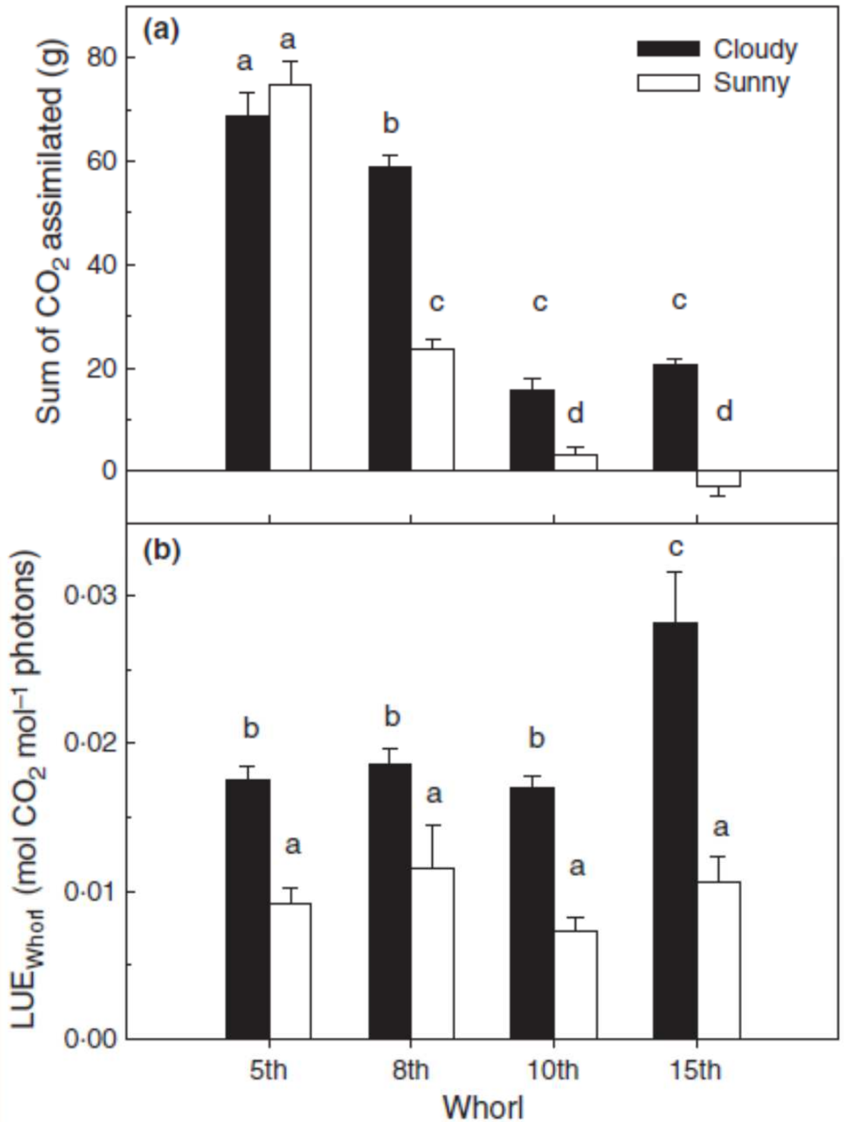


- light response of CO_2 assimilation (daily course measurements)
- hysteresis response of sun shoots from the upper canopy layers
  - negative (lower A values in the afternoon at the same irradiance)

Modelling of C assimilation

$$A = \frac{AQE \cdot I + A_{max} - \sqrt{(AQE \cdot I + A_{max})^2 - 4 \cdot AQE \cdot I \cdot \Theta \cdot A_{max}}}{2 \cdot \Theta} - R_D$$

- model based on LRC parameters
- detail measurement of radiation regime within canopy
- under cloudy skies
  - lower parts have significantly positive C balance (24 hours)
  - higher light use efficiency (LEU; irrad. above zero)



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- Lichtenthaler H.K., Ač A., Marek M.V., Kalina J., Urban O.: Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry* **45**: 577-588, 2007.
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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Thank you for your attention!

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