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Solar-induced fluorescence and photochemical reflectance index for detecting plants physiological status

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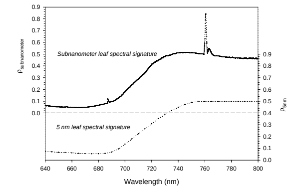
OBJECTIVE

- The main goal of the research was to investigate the potential of non-invasive remote sensing techniques for monitoring plant stress and photosynthetic status by focusing on the detection of steady-state fluorescence and the xanthophyll de-epoxidation state at leaf level, in the context of a chronic fumigation experiment.

BACKGROUND: PRI & Fs

- Besides traditional remote sensing techniques (vegetation optical indexes) we used the Photochemical Reflectance Index (PRI) that is correlated to the xanthophyll heat dissipation and may be employed to discriminate non-photochemical quenching. PRI is defined as $(R_{531}-R_{570})/(R_{531}+R_{570})$.

- We estimated steady-state fluorescence (Fs) by exploiting a variation of the FLD principle applied in the 687 and 760 nm atmospheric oxygen absorption bands (Meroni and Colombo, 2006). An index of fluorescence efficiency, normalized fluorescence (NFs), was calculated by rationing the estimated fluorescence with the radiation incident in a nearby restricted spectral range not including the Fraunhofer line, i.e. in the continuum.



Comparison of a subnanometer leaf spectral signature derived from the HR2000 devices with a FWHM of 0.13 nm and a reference green leaf reflectance recorded by a traditional spectrometer with a FWHM of 3.5 nm.

MATERIALS & METHODS

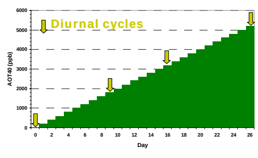
PLANT MATERIAL & TREATMENT

- Rooted cuttings of a poplar clone (*Populus deltoides* x *maximowiczii* Eridano) known for its O_3-sensitivity, were grown in a greenhouse for 2 months in pots containing a steam sterilized soil:peat:perlite (1:1:1 volume) mix and watered regularly. Uniform plants were transferred to fumigation chambers after the complete expansion of the 10th leaf.
- Plants were exposed to chronic O_3 fumigation (80 ppb O_3, 5 h d^{-1}) for 26 days in a controlled environment fumigation facility. Control plants were exposed to charcoal-filtered air only in two chambers identical to those mentioned above. O_3 exposure was expressed in terms of AOT40 (de Leeuw and Zantvoort, 1997, *Environ. Pollut.*, 96, 89-98).

MEASUREMENTS

- Radiometry and gas exchanges measurements were carried out on three plants per thesis on the 5th and 4th leaf of each plant, respectively.
- Visual assessment of ozone injuries and F_v/F_m (photochemical efficiency of PSII) were recorded daily. Diurnal cycles of optical properties, chlorophyll *a* fluorescence (active) and gas exchanges were collected under natural solar illumination on days 0, 9, 16 and 26 after the start of fumigation.

Measurement	Device
Meteo	
Air temperature & humidity	Rotronic
PPFD (direct & diffuse)	BF3, Delta-T
Physiology	
Net Photosynthesis & Stomatal conductance	CIRAS, PP-System
Pigment concentration	HPLC, Dyonex
Fluorescence (active)	2 PAM2000 Fluorimeter, Waltz
Remote Sensing	
Optical properties & PRI	HH FS Spectrometer, ASD
Passive Fluorescence	2 Spectrometers, OceanOptics



Ozone AOT40 over the course of the experiment and days of measurement.

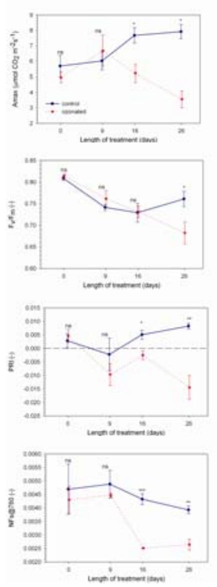
FIELD SET-UP

- Six leaves from separate plants were aligned in a custom-designed sample holder that permits the spectrometers to view either the leaf sample or the white reference panel and allows the gas analyzer to operate on the same plant at the same time. Active fluorescence was also measured on two different plants.

RESULTS

TIME SERIES OF MIDDAY MEASUREMENTS

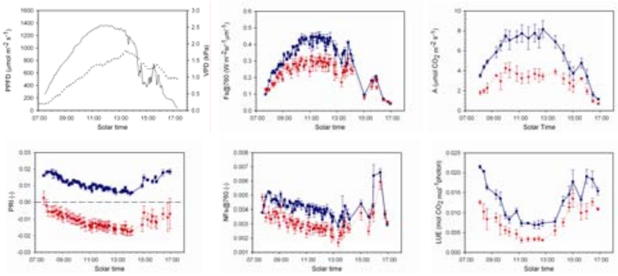
Time series of the investigated parameters measured at maximum solar irradiance (e.g. at midday) are reported in the graphics below. Values represent means ± S.E. Comparison between means was performed according to Student's *t*-test (* : P≤0.05; ** : P≤0.01; *** : P≤0.001).



- **Photosynthetic activity under maximum solar irradiance (Amax)** (e.g. around midday) was significantly reduced by O_3 treatment from day 16 on.
- **Photochemical efficiency of PSII** (F_v/F_m) from active fluorescence was able to detect a difference from day 26.
- **Visible symptoms** appeared at day 26 on 30% of treated leaves.
- **Traditional RS techniques** were not able to detect differences between control and treated leaves (data not shown).
- **PRI index** of treated samples was significantly lower (greater de-epoxidation of xanthophyll-cycle pigments) on day 16 and the difference in PRI value became larger on day 26.
- Similarly, **normalized fluorescence** (NFs@760) was significantly different in the last two cycles. NFs was smaller for treated leaves, as found in other studies, when plants experienced a prolonged stressor.

DAILY COURSES

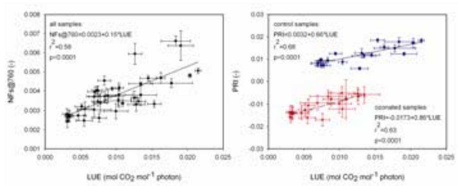
- As an example, the last diurnal cycle measurements regarding incident PPFD, PRI, Fs@760, NFs@760, A and light-use efficiency (LUE = A/incoming PPFD) are reported. Blue and red dots refer to control and ozonated plants, respectively.



- On day 26 the different physiological status between healthy and fumigated samples was clearly detected in terms of A and LUE, passive fluorescence and PRI.

COMPARISON OF LUE AND PASSIVE MEASUREMENTS

- The relationships between LUE and remotely-sensed energy dissipation pathways are shown in figure.



- A unique significant linear regression was found between LUE and NFs for both theses.
- Two regression lines with the same slopes and different intercepts described the relationships between LUE and PRI for controls and treated samples.

- NFs appears to be directly linked to photosynthesis while PRI may be sensitive to both photosynthesis and the photo-protection level that is activated to withstand the given irradiance level.

CONCLUSIONS

This study shows that O_3 injury may be remotely sensed by using advanced RS techniques. The spectral index PRI and the steady-state passive fluorescence under natural solar conditions (Fs) were able to discriminate control and treated plants before active fluorescence and visual assessment and contemporary with gas exchange measurements. Furthermore LUE showed a significant linear correlation with NFs for both theses, thus indicating a link between photochemistry and passive fluorescence, regardless of the stress experienced. On the contrary, a single relationship with PRI was not found. In fact, treated samples showed greater exploitation of the xanthophyll de-epoxidation cycle with respect to controls to attain analogous assimilation levels. These observations provide support for the use of NFs and PRI in monitoring plant physiological status: a reduction in fluorescence may be regarded as an indicator of plant stress if it is accompanied by a higher level of NPQ (i.e. lower PRI).

REFERENCES

Meroni M. and Colombo R., (2006). Leaf level detection of solar induced chlorophyll fluorescence by means of a subnanometer resolution spectroradiometer, Remote Sensing of Environment. 103-4: 438-448.

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