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Leaf level detection of steady state fluorescence and PRI for early ozone injury assessments

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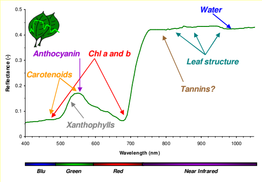
OBJECTIVE

- The research activities are addressed to find optical signals of the oxidative stress linked to ozone exposure of plants. The connection of ozone damage to remote sensing (RS) is motivated by the interest in developing a rapid and non-intrusive way of evaluation of plant physiological status (ground level RS) and by the appealing possibility of monitoring large areas (airborne and satellite RS).

BACKGROUND: PRI & Fs

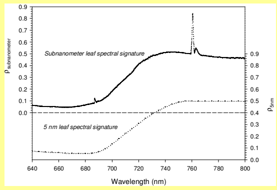
- The spectral reflectance of a leaf (ratio of reflected to incident radiation) is governed by leaf structure and biochemical components (e.g. photosynthetic pigments).
- Therefore, the spectral characteristics of radiation reflected by leaves can provide an understanding of their constituents and may be used to infer responses to growth conditions and adaptations to the environment.



Leaf spectral reflectance over the visible and near infrared range. Leaf variables that determine the "shape" of the curve are reported.

- Besides traditional remote sensing techniques (vegetation optical indexes) we used the Photochemical Reflectance Index (PRI) that is connected to the xanthophylls cycle and may be employed to discriminate non-photochemical quenching $PRI=(R_{531}-R_{570})/(R_{531}+R_{570})$.

- We employed an advanced method for the detection of the steady state chlorophyll fluorescence (Fs). This method relies on the use of a very high spectral resolution spectroradiometric system (0.06 nm sampling step). Such spectral resolution enables us to explore the leaf radiance field within two narrow "dark" bands at 687 and 760 nm, respectively, where the solar irradiance is strongly reduced due to molecular oxygen absorption by terrestrial atmosphere. The chlorophyll fluorescence is detected by exploiting the infilling of this "wells".



Comparison of a subnanometer leaf spectral signature derived from the HR2000 devices and a reference green leaf reflectance recorded by a traditional spectrometer with spectral resolution of 5 nm.

MATERIALS & METHODS

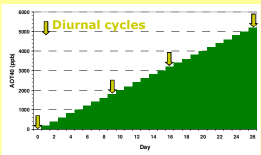
PLANT MATERIAL & TREATMENT

- Rooted cuttings of a poplar clone (*Populus deltoides* x *maximowiczii* Eridano), known for its O_3-sensitivity, were grown for 2 months in pots containing a steam sterilized soil:peat:perlite (1:1:1 volume) mix in a greenhouse 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 maintained under the same experimental conditions as O_3-treated plants, but exposed to charcoal-filtered air. O_3 exposure is expressed in terms of AOT40, (de Leeuw and Zantvoort, 1997, *Environ. Pollut.*, 96, 89).

MEASUREMENTS

- Measurements were carried out on three plants per thesis, on the 5th leaf of each plant.
- Visual assessment of ozone injuries and Amax (assimilation under saturating light condition) were recorded daily. Four diurnal cycles of optical properties, chlorophyll *a* fluorescence (active) and gas exchanges were collected outdoor under natural solar illumination.

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.

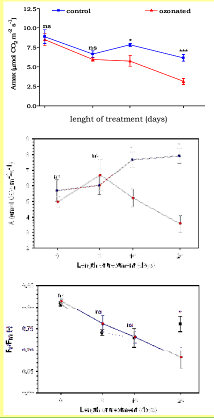
FIELD SET-UP

- Leaves from 6 plants were placed in a custom-designed sample holder that permits the spectrometers to view either the leaf sample or the white reference and allows the gas analyzer to operate on the same plant at the same time. Active fluorescence was also measured on two separate plants.

RESULTS

Measurements of control and ozonated plants were taken 0, 9, 16 and 26 days after the start of fumigation; results 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).

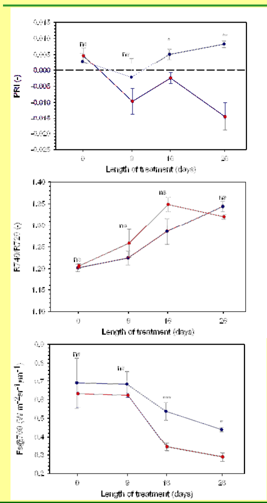
PHYSIOLOGY & VISUAL ASSESSMENT



- Both **photosynthetic activity at saturation light level (Amax) and at maximum solar irradiance (A)** under natural conditions (e.g. approximately at solar noon) were significantly reduced by O_3 treatment from day 16 on.
- **Photochemical efficiency of PSII** (Fv/Fm) from active fluorescence was able to detect a difference from day 26.
- **Visible symptoms** appeared at day 26 on 30% of treated leaves.

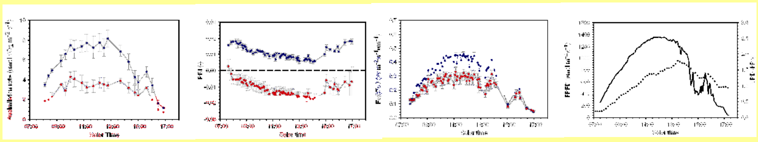
Time course of physiological measurements. Blue and red lines refer to control and ozonated plants, respectively

PROXIMAL SENSING



- **Traditional RS techniques** were not able to detect difference between control and treated leaves. On the left it is shown the time course of the narrow band reflectance ratio (740/720) measured during diurnal cycles at maximum solar irradiance. Other traditional indexes tested provided similar results (data not shown).
- **PRI index**, connected to the xanthophylls depoxidation state, is significantly different, and it was found lower (greater depoxidation) for treated plants with respect to control, in the last two diurnal cycles.
- Similarly, **steady state passive chlorophyll fluorescence** (Fs@760) is significantly different in the last two cycles. Fs is smaller for treated leaves, as found in other studies, when plants experienced a prolonged stressor. The general decreasing trend is due to the decreasing trend of solar irradiance.

- As an example, the last diurnal cycle measurements regarding Assimilation, PRI, Fs@760 and incident PPFD, are reported. Blue and red dots refer to control and ozonated plants, respectively.



- Assimilation time course shows higher rate for control plants for the whole day. PRI qualitatively separates three groups that correspond to control samples (higher curves), treated samples with and without visible symptoms (lower and middle curves, respectively). With more scatter in the data, Fs also separates these three groups. Incoming PPFD is reported on the right for reference.

CONCLUSIONS AND PERSPECTIVES

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 treated plants before active fluorescence and visual assessment and contemporary with gas exchange. This capability, together with being RS not intrusive and not at contact, makes RS appealing for the detection of ozone injury. Nevertheless, we are aware that the operational application of this technique in natural conditions is not at hand since both xanthophylls depoxidation and fluorescence reduction are rather generic indicators of ongoing stress. Thus, further study is needed to develop other spectral indicators more specific of oxidative stress.

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.

ACKNOWLEDGEMENTS

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