19° Task Force Meeting of the UNECE ICP Vegetation, 30st January – 2nd February, 2006, Caernarfon, Wales, UK

Ecophysiological measurements and remote sensing techniques: an approach for the detection of early ozone injury

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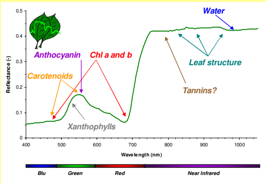
OBJECTIVE

- The research activities are addressed to find optical signals of the oxidative stress linked to ozone exposure. The intention of connecting 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

- 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 remote analysis of the reflected light can be used to infer the "physiological" status of a plant.

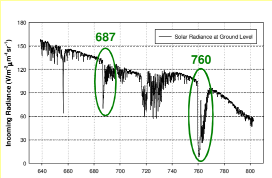


Leaf spectral reflectance over the visible and near infrared range. Leaf variables that determine the "shape" of the curve are reported.

ADVANCES

- Besides traditional remote sensing techniques (vegetation optical indexes) we extracted a recently developed index connected to the xanthophylls cycle, the *Photochemical Reflectance Index*, PRI=$(R_{531}-R_{570})/(R_{531}+R_{570})$.

- Capitalizing on our recent results, we employed an advanced method for the detection of the steady state chlorophyll fluorescence. This method relies on the use of a very high spectral resolution spectroradiometric system (0.06nm sampling step). Such spectral resolution enables us to explore the leaf radiance field within two narrow "dark" bands at 687 and 760nm, 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".



Ground level incident solar radiance. The "wells" – dark lines positioned at 687 and 760nm are outlined.

MATERIALS & METHODS

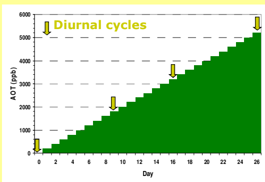
PLANT MATERIAL & TREATMENT

- Rooted cuttings of a poplar clone (*Populus deltoides* x *maximowiczii* Eridano), known for O_3-sensitivity, were grown for 2 months in plastic pots containing a steam sterilized soil:peat:perlite (1:1:1 volume) mix in a greenhouse and watered every day. Uniform plants were transferred to fumigation chambers after the appearance and complete expansion of the 10th leaf.
- Plants were exposed to chronic O_3 fumigation (80 ppb O_3, 5 h, d^{-1}) for 26 consecutive 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.

MEASUREMENTS

- Measurements were carried out on three plants per thesis, on the 5th leaf of each plants.
- 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 PPFD (direct & diffuse)	Rotronic 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 Passive Fluorescence	HH FS Spectrometer, ASD 2 Spectrometers, OceanOptics



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

- Dark adapted and illuminated (PPFD>1000 µmol m^{-2} s^{-1}) samples were collected for subsequent chemical extraction of xanthophylls and phenols (*data not shown*).

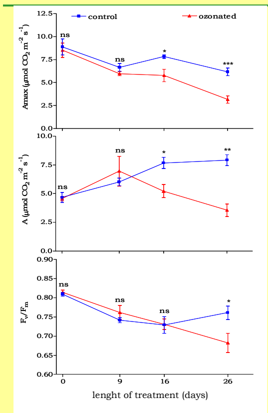
FIELD SETUP

- 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 measured on two separate plants.

RESULTS

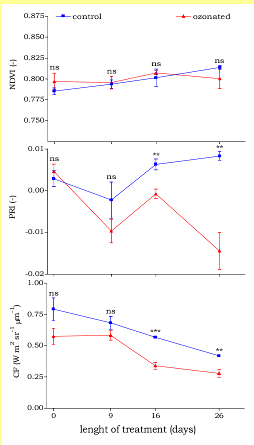
In the graphics below, measurements of control and ozonated plants were taken 0, 9, 16 and 26 days after the start of fumigation. Values represent means ± S.E. Comparison between means was performed according to Student's *t*-test (* : $P\leq0.05$; ** : $P\leq0.01$; *** : $P\leq0.001$).

PHYSIOLOGY & VISUAL ASSESSMENT



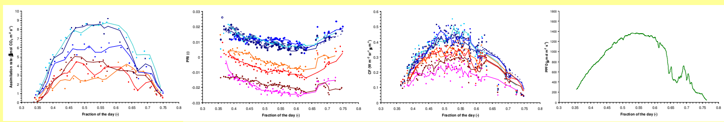
- Both **photosynthetic activity at saturation light level (Amax) and at maximum solar irradiance (A)** under natural conditions (e.g. approximately at solar noon) were significantly reduced by O_3 treatment from day 16 on.
- **Photochemical efficiency of PSII** (Fv/Fm) from active fluorescence was able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

PROXIMAL SENSING



- **Traditional RS techniques** were not able to detect any difference between control and treated leaves. On the left it is showed the time course of the most popular index sensing vegetation "vigor": the *Normalized Difference Vegetation Index* (NDVI) measured during diurnal cycles at maximum solar irradiance. Other traditional indexes tested provided similar results (not shown).
- **PRI index**, connected to the xanthophylls depoxidation state, is significantly different, and lower (greater depoxidation), for treated plants with respect to control, in the last two diurnal cycles.
- Similarly, **steady state passive chlorophyll fluorescence** (CF) is significantly different in the last two cycles. CF 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, CF and incident PPFD, are reported. Blue and red dots refer to control and ozonated plants, respectively. Time is expressed as fraction of the day (e.g. 0.5 is noon).



- 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, CF also separates these three groups. Incoming PPFD is reported on the right for reference.

CONCLUSIONS AND PERSPECTIVES

- This study shows that ozone injury may be sensed remotely by the proposed advanced RS techniques. The employed spectral index (PRI, related to xanthophylls cycle) and the steady state passive fluorescence under natural solar conditions were able to discriminate ozone 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. In this framework we developed an additional spectral index operating at 760 nm which was able to detect treated plants (*not shown*). This index, if proven connected to phenolic substances accumulation as we suspect, may help in identifying oxidative stress.

ACKNOWLEDGEMENTS

E. Pellegrini and A. Francini are acknowledged for their help at the San Piero a Grado fumigation facility. We also thank F. Fava for his support during the field campaign. Research was supported by the ERSAF-Lombardia and by the MIUR-PRIN Projects, Rome.