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Investigations of Photon Emission from Wheat during Ozone Exposure by Means of a Portable Single Photon Counter

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Summary

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Photon emission is inherently associated with fundamental biological processes such as cell division, photosynthesis, stress or death of organisms. For demonstrating the potential use of photon emission for monitoring stress effects on plants, we designed and performed a series of experiments about the physiological effects of ozone.

The photon emission of wheat (*Triticum aestivum* cv. Perlo) was recorded by means of a portable single photon counter. The results showed that photon emission of green plants acts as an indicator of ozone stress response and that a portable photon counter can be used successfully for detailed and sensitive in situ investigations of photon emission from stressed plants. This analytical method may complement other techniques used in plant physiology, especially those for studying effects on photosynthesis.

Introduction

Almost all living systems emit light of very low intensity, the so-called biophoton emission. This can be measured by means of the well established method of single photon counting. Especially in green plants, two kinds of photon emission can be distinguished: 1) Photoluminescence (PL) is re-emitted after

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previous light excitation of plants due to recombination reactions of separated and stabilized charge pairs in the electron transport chain of the photosynthetic unit (AMESZ & VAN GORKOM 1978, HIDEG & al. 1989). 2) A spontaneous ultraweak photon emission (UPE) is emerging after the decay of photoluminescence (after some tens of minutes) and arises from several biochemical reactions within living tissues (ABELES 1986, BOVERIS & al. 1983, SCOTT & al. 1989).

Singlet oxygen and excited triplet carbonyls are discussed as ubiquitous sources of photon emission (ABELES 1986). In green plants it is assumed that chlorophyll, acting as an efficient acceptor of energy from endogenous excited species (BOHNE & al. 1986), is the dominant source of the UPE (HIDEG & al. 1990, ROSCHGER & al. 1992).

Photon emission is intimately connected with fundamental biological processes such as cell devision, photosynthesis, stress or death of organisms. In particular, photon emission of perturbated systems is considered as a tool to estimate deviations of the homeostasis in living systems (SLAWINSKI & al. 1992, KOCHEL 1992).

Materials and Methods

Photon counter. In the present study the photon emission of green plants was recorded by means of a portable single photon counter based on a red-sensitive photomultiplier for photon counting.

The Hamamatsu photomultiplier R4457P has a S-20 photocathode (4 x 13 mm) and a spectral response of 185-830 nm λ_{max}= 370 nm). The anode luminous sensitivity is 1360 $A \cdot lm^{-1}$ and the current amplification amounts $4 \cdot 10^6$ at 1000 V. The dark count rate (14 cps at +25° C and 820 V) was recorded after each sample measurement. The photomultiplier output signal is amplified and shaped by means of an Amptek A111 PAD amplifier/discriminator and fed to a multi-channel scaler (MCS-II) from Tennelec/Nucleus plugged in a laptop. All components of the detector unit are enclosed in a cylinder of 6 cm diameter and 23 cm length. The detector unit was fitted in a light-tight measuring chamber (40x30x30 cm).

The excitation of photoluminescence (duration: 5 s after 10 min dark adaptation of each sample) was performed by two techniques: 1) red light induced photoluminescence excited with Siemens LED, λ_{max} = 635±15nm, intensity: $3 \cdot 10^{-4}$ W/cm^2. 2) far-red light induced photoluminescence excited with incandescent light (Osram Concentra lamp, 30 W combined with an interference filter, λ_{max} = 756±52nm, intensity: $5 \cdot 10^{-4}$ W/cm^2). The latter far-red excitation generally induces a transient increase of the photoluminescence i.e. a relative maximum of the decay curve.

Plants and Stressor. Winter wheat *Triticum aestivum L.* (cv. Perlo) was pot-cultivated in greenhouse compartments adapted as fumigation-chambers at the Research Center Seibersdorf, Austria. For further cultivation details see (SOJA & SOJA 1995). The uppermost, fully developed leaf was detached and placed in water. Photoluminescence was measured from an area of 0.5 x 2 cm in the middle part of the leaf. The wheat plants were ozone fumigated with 80 $nl \cdot l^{-1}$ for 8 h a day (from 9:00 a.m. to 5:00 p.m.) from tillering till grain-filling.

Results and Discussion

The change of photon emission in wheat leaves caused by the stress influence of ozone is demonstrated by three methods:

1) Red excitation of photoluminescence (PL): The measurements show (Fig. 1) that the suppression of the mean value of initial photoluminescence over all samples of three measuring days is not significant ($p > 0.15$). However, suppression clearly was found in the second half of each measuring day after 4-5 h fumigation. The mean values of the last 4 measurements on each day over all 3 days were significantly ($p < 0.025$) depressed in the ozone treated samples (Fig. 1b). This gives evidence for a reversible decrease of photoluminescence caused by ozone on a daily basis. Similarly, GRIMM & FUHRER 1992 describe a reversible impairment of chlorophyll fluorescence parameters during ozone fumigations of wheat. Based on our results, we conclude that after an ozone dose AOT40 (accumulated over threshold of 40 $nl \cdot l^{-1}$) of 160-200 ppb-h metabolic effects of ozone can be observed by means of red induced photoluminescence. During night the effects disappear, suggesting that the underlying processes are rather caused by metabolic changes than by damages of structural components.

Since a close relation between the PL and processes of photosynthesis is assumed (AMESZ & VAN GORKOM 1978), the suppression of PL of stressed samples indicates a possible disturbance of the photosynthetic unit. Influence of ozone on the photosynthetic apparatus is known from other investigations (e.g. JÄGER & al. 1986).

2) Far-red excitation of photoluminescence: The relative maximum of the PL-decay curve after far-red illumination ($\lambda_{ex} > 700$ nm) is suppressed after ozone fumigation of wheat leaves. This is evident from Fig. 2 which shows the results of two days measurements ($p < 0.01$ and $p < 0.005$) by means of the cumulated photon count rate in the time range when the relative maximum usually occurs. The dependence of the relative maximum on various exogeneous parameters (e.g. herbicides, different pH values, temperature etc.) reflects an intimate correlation with a well-functioning thylakoid membrane and an intact chloroplast (SCHMIDT & SENGER 1987 a,b). Therefor, this kind of measurement may also provide information about stress influence. For definite statements more detailed investigations would be necessary, which were beyond the scope of the present study.

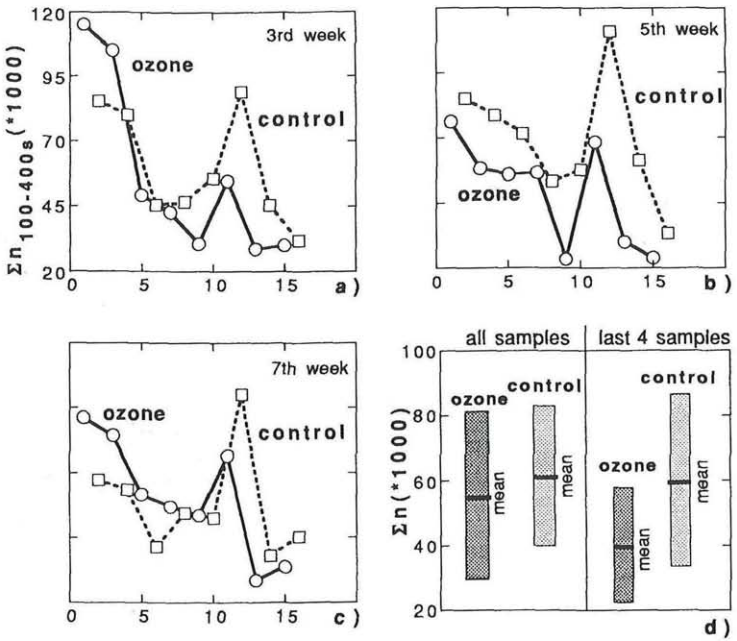

Fig. 1. Red induced photoluminescence for wheat after ozone treatment (80 $nl \cdot l^{-1}$ for 8 $h \cdot d^{-1}$). **a)** Example for a measurement on one day at the indicated week of ozone fumigation. Data points represent an accumulated photon count rate (Σn) of photoluminescence 100-400s after illumination. **b)** mean value (± standard deviation) of initial photoluminescence over all samples of 3 different measuring days and over the last 4 samples of 3 measuring days respectively (investigated in the afternoon after 4-5 hrs ozone fumigation).

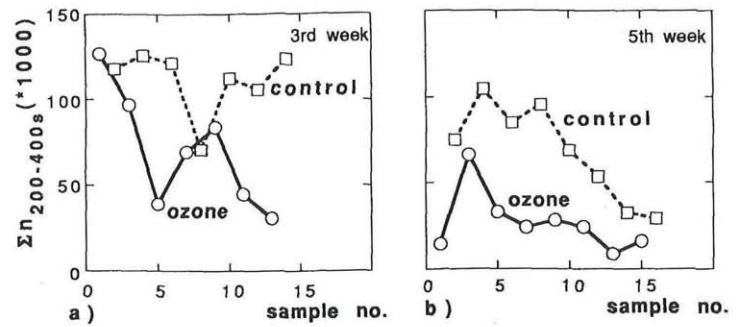

Fig. 2. Far-red induced photoluminescence of wheat leaves after ozone treatment measured on two different days **a)** and **b)** at the indicated week of fumigation (80 $nl \cdot l^{-1}$ for 8 $h \cdot d^{-1}$). Data points represent an accumulated photon count rate (Σn) of photoluminescence 200-400s after illumination.

3) Ultraweak photon emission: Generally, the ultraweak photon emission increases during deoxygenation (nitrogen or argon treatment) and decreases in a following reoxygenation (air treatment) (ROSCHGER & al. 1992). In ozone treated wheat leaves, this behaviour has changed as is shown in one example in Fig. 3a) and for more samples by means of the accumulated photon count rate of the anaerobic phase normalized to the mean value in the previous aerobic phase in Fig. 3b).

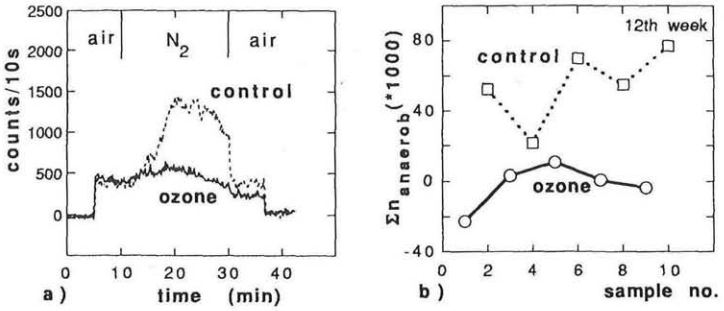

Fig. 3. Anaerobic phase-induced kinetics of ultraweak photon emission in wheat leaves after ozone fumigation (80 $nl \cdot l^{-1}$ for 8 $h \cdot d^{-1}$). **a)** Example of the type of kinetics for one sample of ozone group and control group, respectively (start of measurement after 15 min dark adaptation). **b)** Accumulated photon count rate (Σn) of the anaerobic phase minus a mean initial count rate in the aerobic phase for several samples.

The increase of UPE in the anaerobic phase of untreated samples points to triplet excited species which are efficiently quenched by oxygen in the post-anaerobic phase (ROSCHGER & al. 1992). The response of plants on the alterating conditions of oxygenation and deoxygenation is lost in the ozone treated wheat. A similar change of the anaerobic kinetics of UPE after ozone exposure at different duration and concentration was also demonstrated in duckweed (KATZINGER & al. 1994).

In conclusion, this investigation shows that three kinds of photon emission of plants (the red induced photoluminescence, the far-red induced photoluminescence and the ultraweak photon emission) demonstrate significant changes in physiological behavior of wheat after ozone treatment. These effects were found by means of a portable photon counter which now offers new techniques for more detailed in situ investigations of these phenomena in cooperation with various disciplines in bioscience e.g. plant physiology.

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