Photosynthetic induction in *Eucalyptus urograndis* seedlings and cuttings measured by an open photoacoustic cell


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Abstract

Photosynthetic induction in leaves of four-month-old *Eucalyptus urograndis* seedlings and of cuttings obtained from adult trees that were previously dark-adapted was studied by the *in vivo* and *in situ* Open Photoacoustic Cell Technique. Results for the gas exchange component of the photoacoustic (PA) signal were interpreted considering that the gas uptake component would have a phase angle nearly opposite to that of the oxygen evolution component. By subtracting the thermal component from the total PA signal, we studied the competition between gas uptake and oxygen evolution during the photosynthetic induction. Seedlings presented a net oxygen evolution prior to cuttings, but cuttings reached a higher steady-state photosynthetic activity. The chlorophyll (Chl) \(a/b\) ratio and the Chl fluorescence induction characteristic \(F_{v}/F_{m}\) were significantly higher for cuttings, while there was no difference between samples in stomata density and leaf thickness. Thus the differences in PA signals of seedlings and cuttings are associated to differences between the photosystem 2 antenna systems of these samples.

Additional key words: chlorophyll fluorescence; clone differences, gas uptake, leaf thickness; oxygen evolution, photobaric signal; photothermal signal; stomata density.

Introduction

Working with leaf discs and isolated chloroplasts, Inoue *et al.* (1979) simultaneously measured the induction kinetics of the photoacoustic (PA) signal and of the chlorophyll (Chl) fluorescence, showing that the PA transient was associated with photosynthesis. Nowadays, the usefulness of the PA technique in the study of leaf photosynthesis is already established (Fork and Herbert 1993, Malkin and Canaani 1994, Malkin and Puchenkov 1997, Buschmann 1999). The principle of the technique relies on photon absorption and consequent heat and oxygen release. The amplitude (magnitude) and phase (delay with respect to the photon absorption) of the PA signal carry information about optical and thermal parameters of the sample.

The absorbed radiant energy is partially converted into photochemical energy and heat, being partially re-emitted as Chl fluorescence in the photosynthetic process. The PA-signal phase carries information about the delay between photon excitation and pressure variation, which depends on the coefficients of heat and gas diffusion. The signal phase also depends on the time constants of various steps in the electron transfer chain (delayed generation of heat). Poulet *et al.* (1983) determined the oxygen diffusion coefficient and estimated the limiting time constant on the donor side of photosystem 2 (PS2). Korpil and Osander (1992) discussed a more complete model of mass diffusion.

Most PA measurements of photosynthesis have been performed with the leaf being cut and enclosed in the cell (Bults *et al.*, 1982, Canaani *et al.*, 1982, Havaux *et al.*, 1987, Malkin 1987, Dau and Hansen 1989, Charland *et al.* 1992). In 1987, an Open Photoacoustic Cell (OPC) was conceived (Perondi and Miranda 1987, Marquezini *et al.*, 1991, Pereira *et al.* 1994). A commercial electret...
phone that uses its own chamber as the acoustic cell forms this compact device, with the sample acting as one of the microphone walls. As the leaf itself closes the PA chamber (with the abaxial surface turned to the inside of the chamber), it is not necessary to cut a leaf disc, neither to detach the leaf from the plant for measurements. Thus the OPC allows in vivo and in situ monitoring of photosynthetic activity in plants, avoiding dehydration of the sample. Part of the leaf remains exposed to the outside, capturing external CO₂, which minimises changes in the PA chamber atmosphere. The OPC technique has been used in studies of, e.g., the effect of dehydration in soybean leaves (Pereira et al. 1992), the evidence of heterosis in maize hybrids (da Silva et al. 1995), the energy storage determination (Barja and Mansanares 1998), and the effects of irradiance and temperature in Eucalyptus leaves (Barja et al. 2001).

The two most cultivated species of Eucalyptus in Brazil are the fast growing E. grandis W. Hill ex Maiden and the E. urophylla S.T. Blake, that grows less, but is more tolerant to water-limited conditions (Blake 1977, Pryor et al. 1995). The crossing of these two species generates the hybrid E. urograndis, which presumably grows faster and is more resistant to dry regions (Blake et al. 1988, Inoue and Oda 1988). For these reasons, the E. urograndis hybrid is nowadays the most planted by reforesting companies in Brazil. Due to the unequivocal economic importance of the Eucalyptus culture, it is important to study this hybrid, not completely characterised until now.

In this work, we measured the in vivo and in situ photosynthetic induction in leaves of E. urograndis seedlings and cuttings (the so-called clones), using the PA technique. Our goal was to characterise the photosynthetic behaviour of the E. urograndis C041 cuttings, since cuttings are gradually replacing seedlings in reforesting practice due to faster growing in the field and better adaptability to surrounding conditions.

Materials and methods

PA setup: The experimental scheme utilised has two light sources: a Xenon arc lamp (Oriel, model 6128, 1000 W) and a tungsten lamp (Ushio/ELC, 250 W). To obtain modulated radiation of a given wavelength (680±10 nm), we put a chopper (PAR, model 192) and a monochromator (Oriel, model 77250) in front of the Xenon lamp. Measurements were carried out at 17 Hz. Optical filters limited radiation of the tungsten lamp to the visible part of the spectrum. Irradiance by the modulated red radiation used for photosynthetic induction was 10 W m⁻², while that of the continuous “white light” used for photosynthesis saturation was about 350 W m⁻². A double-branched optical cable guided each radiation beam up to the acoustic cell. The chopper and the PA cell microphone were connected to a lock-in amplifier (PAR-EGandG, model 5210), that selectivity amplifies the PA signal taking into account the modulation frequency. The lock-in was connected through a GPIB to a microcomputer for data acquisition. Typical time-constant used was 1 s, which gives the time response of the set-up.

OPC is already characterised in the literature (Perondi and Miranda 1987, Marquezini et al. 1991, Pereira et al. 1992, 1994, Barja and Mansanares 1998). The sensitivity of the electret microphone was about 10 mV Pa⁻¹.

Plants: Both E. urograndis seedlings and C041 cuttings (obtained of adult trees) were cultivated in small pots (50 cm³) under 50 % shade. They were irrigated and fertilised daily. The use of such recipients in the present work follows their current use by the reforesting company that provided the samples. Four-months-old seedlings and cuttings were transferred to the laboratory where they were dark-adapted for at least 10 h at ambient temperature. After this dark period, the plants were moved to the experimental set-up and a selected part of a non-detached leaf was fixed to the OPC and exposed to the radiation coming from the optical cable. Fully expanded leaves of the second pair were selected for measurements. Average values were taken over about 10 measurements for each kind of sample.

Photosynthetic induction: When plants are dark-adapted for a certain time, their photosynthetic reaction centres deactivate. After dark-adaptation, radiation incidence gives rise to the photosynthetic induction, i.e., the restart of photosynthetic processes (Prinsley and Leegeood 1986, Malkin 1987, Gardeström 1993, Sassenrath-Cole and Pearly 1994). Therefore, the initial level of PA signal corresponds solely to temperature oscillation (thermal component). As reaction centres readapt to irradiance, photosynthesis starts and the production of oxygen takes place, giving rise to the so-called photobaric signal (gas component) and eventually reaching the steady state. Simultaneous incidence of modulated (10 W m⁻², Xenon lamp) and continuous (250 W m⁻², tungsten lamp) radiation gives the maximal thermal component, since the non-modulated radiation saturates the gas exchange component. One must subtract the thermal component from the total PA signal to analyse the photosynthetic activity of the plants studied.

Actually, when only modulated radiation is present, the thermal component is lower than that observed under simultaneous incidence of both modulated and continuous radiation, due to energy storage. However, considering typical energy storage for eucalyptus plants (see Barja and Mansanares 1998) would implicate a correction of about 3 % of the total signal, which does not affect the analysis being made in the present case. For this reason,