AIP Review of Scientific Instruments

Photoacoustics as a tool for the diagnosis of radicular stress: Measurements in eucalyptus seedlings

P. R. Barja, A. M. Mansanares, E. C. da Silva, and P. L. C. A. Alves

Citation: Review of Scientific Instruments **74**, 709 (2003); doi: 10.1063/1.1518567
View online: http://dx.doi.org/10.1063/1.1518567
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/74/1?ver=pdfcov
Published by the AIP Publishing

Photoacoustics as a tool for the diagnosis of radicular stress: Measurements in eucalyptus seedlings

P. R. Barja
Universidade do Vale do Paraíba-UNIVAP, Research and Development Institute-IP&D, Av. Shishima Hifumi 2911, 12244-000 São José dos Campos, SP, Brazil

A. M. Mansanares[a] and E. C. da Silva
Instituto de Física Gleb Wataghin, Universidade Estadual de Campinas, Unicamp, Caixa Postal 6165, 13083-970 Campinas, São Paulo, Brazil

P. L. C. A. Alves
Departamento de Biologia Aplicada, FCAVJ-Unesp, 14870-000 Jaboticabal, SP, Brazil

(Presented on 26 June 2002)

In reforesting companies (cellulose industry), eucalyptus is usually cultivated in small plastic containers (50 mL). As seedlings remain for about 120 days in these containers—until transplantation—their roots become space restricted, with consequent limitations in water and nutrient absorption. These restrictions may lead to plant stress, decreasing productivity. In this work, we used the photoacoustic technique to evaluate the photosynthetic activity of *Eucalyptus grandis*, *E. urophylla* and *E. urograndis* seedlings subjected to this limited space availability, seeking a correlation with morphological parameters and fluorescence measurements in these seedlings. Photoacoustic, fluorescence, and morphological analysis were conducted every 15 days, from 45 to 120 days after sowing. Fluorescence and photosynthetic rate were evaluated *in vivo* and *in situ*, the latter one using the open photoacoustic technique. Data show that root dry matter diminished markedly at 90 and 120 days after sowing; this behavior showed a high correlation with the gas exchange component of the photoacoustic signal, as well as with the fluorescence ratio Fv/Fm. These results indicate that the soil volume of the container becomes insufficient for the roots after 90 days, probably leading to a nutritional deficiency in plants, which explains the decrease observed in the photosynthetic rate of seedlings.
[DOI: 10.1063/1.1518567]

I. INTRODUCTION

The utility of the photoacoustic technique in studying leaf photosynthesis is corroborated by the reviews published in this area.[1–4] The photoacoustic technique is based on the absorption of modulated light by the sample with the successive release of modulated heat and (in the case of vegetal leaves) gas evolution.

The first photoacoustic measurements of leaf photosynthesis were performed with the leaf usually being cut and enclosed in the cell.[5] This has changed with the development of the open photoacoustic cell (OPC)[6–8] composed by an electret microphone that uses its own chamber as the acoustic cell, with the sample acting as one of the walls. Since the sample itself closes the chamber, it is not necessary to cut a leaf disk, or to detach the leaf from the plant for measurements, so the OPC allows *in vivo* and *in situ* monitoring of the photosynthetic activity in plants, avoiding dehydration of the sample. Part of the leaf remains exposed to the outside, capturing external CO_2, which minimizes changes in the photoacoustic chamber atmosphere. Several studies on photosynthesis were carried out with the OPC technique, such as the evidence of heterosis in maize hybrids through photosynthesis induction measurements[9] and the energy storage determination.[10]

The two most cultivated species of eucalyptus in Brazil are the fast growing *Eucalyptus grandis* and the *E. urophylla* that grows lower, but is more tolerant of water-limited conditions.[11,12] The crossing of these two species generates the hybrid *E. urograndis*, which presumably grows faster and is more resistant to dry regions.[13] For these reasons, the *E. urograndis* hybrid has been the most planted by reforesting companies in Brazil in recent years.

In this work, *in vivo* and *in situ* photosynthesis induction measurements were performed in leaves of *Eucalyptus grandis*, *Eucalyptus urophylla*, and *Eucalyptus urograndis* plants for different ages of the seedlings. This was done to determine if the photosynthetic behavior of these plants was influenced by a root stress due to the limited volume of the receptacle utilized for each sample.

II. MATERIALS AND METHODS

A. Photoacoustic setup

The experimental setup is already depicted elsewhere.[10] There are two light sources: a xenon arc lamp (Oriel, mod.6128, 1000 W) and a halogen lamp (Ushio/ELC, 250 W). A chopper (PAR, mod. 192) and a monochromator

[a]Electronic mail: manoel@ifi.unicamp.br

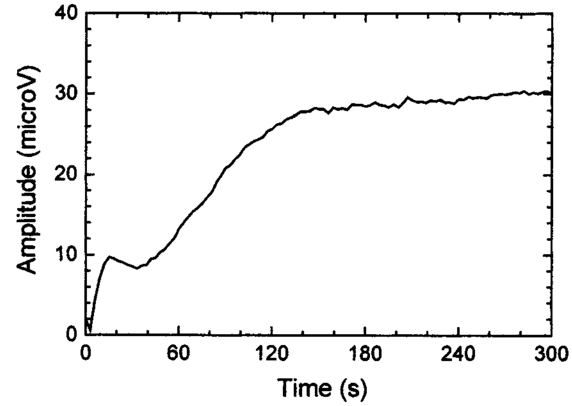

FIG. 1. Typical photosynthesis induction curve (amplitude of the photobaric component as a function of time) obtained for *E. urograndis* leaf (45 days old seedling) previously dark adapted for 24 h. Modulated light attributes: $\lambda = 680$ nm, modulation frequency of 17 Hz, and intensity of 10 W/m^2.

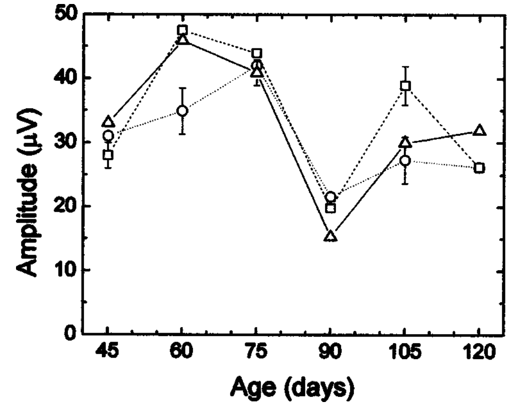

FIG. 2. Amplitude of the steady-state photobaric signal as a function of the age of the eucalyptus plants (previously dark adapted for 24 h). Each point is the mean value of about four measurements. Solid line/triangles: *E. urograndis*; dashed line/squares: *E. urophylla*, and dotted line/circles: *E. grandis* leaves.

(Oriel, mod. 77250) are used in front of the xenon lamp in order to obtain modulated light of a determined wavelength (680 nm). Measurements were carried out at 17 Hz. Optical filters limit the light of the halogen lamp to the visible part of the spectrum. Intensity of the modulated red light, used for photosynthesis induction, was 10 W/m^2, while that of continuous white light, used for photosynthesis saturation, was about 350 W/m^2. A double-branched optical cable is used to guide each light beam up to the photoacoustic cell. The chopper and the photoacoustic cell microphone are connected to a lock-in amplifier (PAR-EG&G, mod. 5210) that measures the amplitude and phase of the microphone signal. The lock in is connected through a general purpose interface bus to a microcomputer for data acquisition. The typical time constant used is 1 s, which gives the time response of the setup.

The OPC is already described in literature.[7,14] It is composed by an electret microphone, with the photoacoustic (PA) microphone chamber being closed by the leaf itself. The sensitivity is of about 10 mV/Pa.

B. Plant materials

All eucalyptus plants utilized in this work were cultivated in small pots (50 ml) under 50% shade conditions and were irrigated and fertilized daily. Measurements were performed in eucalyptus leaves from seedlings between 45 and 120 days old, in fifteen days intervals. Fully expanded leaves of the second pair were selected for measurements. Seedlings were dark adapted at ambient temperature for about 24 h in the laboratory before measurements. After this dark period, plants were moved to the experimental setup, and a selected part of an undetached leaf was fixed to the OPC and exposed to the modulated light. Average values were taken over about four measurements for each sample and condition.

III. RESULTS AND DISCUSSION

Figure 1 shows a typical curve of the photobaric signal amplitude in photosynthesis induction measurements in an *E. urograndis* leaf. At time $t = 0$, modulated light was switched on and the PA signal (amplitude and phase) was recorded as a function of time. For all species of eucalyptus studied, photobaric component presents a fast increase in the signal amplitude followed by a decrease to the initial level in the first minute of illumination. After this, amplitude increases up to a steady state, reached after about 4 min.

Figure 2 shows the amplitude of the steady-state photobaric signal as a function of the age of the seedlings for the three species. These data indicate that all species studied reach maximal photosynthesis rate between 60 and 75 days old. Measurements in older samples reveal a sudden decrease in the photosynthetic rate for 90 days old seedlings.

The reduction of the photosynthetic activity observed for 90 days old seedlings could be correlated with the stress due to the insufficiency of space in the pot. Figure 3 shows the dried root weight as a function of the age of the seedlings for *E. urophylla*. As one can see, there is a minimum around 90 days, thus correlating to the photoacoustic measurements of the oxygen evolution. Similar results were obtained for the other species. In fact, the deficit of nutrients and lack of space in the pot may inhibit both mineral absorption and growing of the root system, reducing nutrient acquisition. As a result, activation/synthesis of enzymes involved in photo-

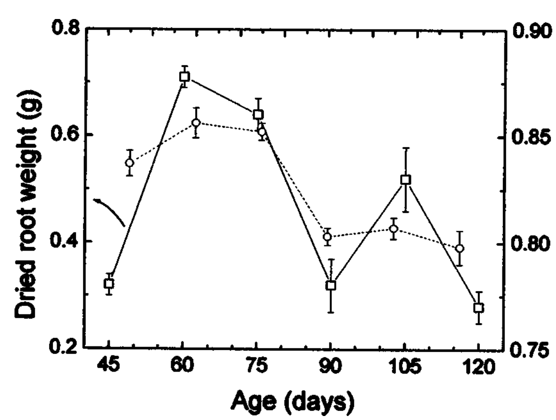

FIG. 3. Dried root weight and the ratio between the variable fluorescence Fv and maximum fluorescence Fm as a function of the age of the seedling for *E. urophylla*.

synthetic processes may be damaged, with immediate effects in photosynthesis.

Figure 3 also shows the ratio between the variable fluorescence Fv and the maximum fluorescence Fm plotted against the age of the seedlings. The measurement of the fluorescence of the chlorophyll is made in function of time, after the start of incidence of intense white light on the sample (previously in the dark). In the beginning, there was a low level of fluorescence F_0, since the electron acceptors in the Photosystem II were available. As time goes by, these acceptors become unavailable because of the large amount of electrons produced as a result of light absorption. The photochemical path is therefore saturated, and fluorescence of chlorophyll increases, reaching finally a maximum value Fm. The difference (Fm-F_0) is named variable fluorescence Fv. Hence, the ratio Fv/Fm is a measure of the availability of electron acceptors in the Photosystem II in the absence of light. Figure 3 presents a reduction in the ratio Fv/Fm after 90 days for *E. urophylla*. This result also indicates that the photosynthetic system is being affected by the restriction of the volume of pot.

Comparison between species in measurements for 90 days old seedlings shows that *E. grandis* seems to be the most resistant to this stress, while *E. urograndis* is the most affected. However, subsequent measurements show that, one month after the decay of photosynthesis activity attributed to radicular stress, *E. urograndis* totally recuperates the same photosynthetic capacity verified in 45 days old seedlings. Concluding, one can say that all eucalyptus species studied presented a partial restoration of their photosynthetic capacities one month after the manifestation of the radicular stress. This recuperation may never be complete because of the limited supply of nutrients in the small pot used for each sample.

IV. CONCLUSIONS

The study presented here shows the application of the OPC in investigating how the photosynthetic behavior of eucalyptus leaves is affected by the availability of soil and nutrients for the seedlings. This kind of study can only be performed through *in vivo* and *in situ* measurements, since suspensions of chloroplasts can not reproduce the behavior of living samples and the response of detached leaves can be distorted by dehydration.

Data presented here show that parameters as the soil volume available to each sample have a strong influence in the development of the eucalyptus plants. Results indicate that the photosynthetic behavior of seedlings has been affected by a radicular stress, with the soil volume of the pot becoming insufficient for the roots, causing a nutritional deficiency in plants. Investigating this stress through the photoacoustic technique, the present work can be helpful for an optimized planning of eucalyptus reforestation.

ACKNOWLEDGMENTS

The authors wish to thank the Brazilian agencies CAPES, CNPq, and FAPESP, for financial support, and Votorantim Celulose e Papel Ltda., for supplying the samples.

[1] D. C. Fork and S. K. Herbert, Photochem. Photobiol. **57**, 207 (1993).
[2] S. Malkin and O. Canaani, Annu. Rev. Plant Physiol. Plant Mol. Biol. **45**, 493 (1994).
[3] S. Malkin and O. Puchenkov, in Progress in Photothermal and Photoacoustic Science, Life and Earth Sciences, Vol. 3, edited by A. Mandelis and P. Hess (SPIE, Bellingham, WA, 1997), pp. 17–56.
[4] S. K. Herbert, T. Han, and T. C. Vogelmann, Photosynth. Res. **66**, 13 (2000).
[5] S. Malkin, Planta **171**, 65 (1987).
[6] L. F. Perondi and L. C. M. Miranda, J. Appl. Phys. **62**, 2955 (1987).
[7] M. V. Marquezini, N. Cella, A. M. Mansanares, H. Vargas, and L. C. M. Miranda, Meas. Sci. Technol. **2**, 396 (1991).
[8] A. C. Pereira, G. de O. Neto, H. Vargas, N. Cella, and L. C. M. Miranda, Rev. Sci. Instrum. **65**, 1512 (1994).
[9] W. J. Silva, L. M. Prioli, L. C. M. Miranda, N. Cella, H. Vargas, and A. M. Mansanares, Plant Sci. **104**, 177 (1995).
[10] P. R. Barja and A. M. Mansanares, Instrum. Sci. Technol. **26**, 209 (1998).
[11] S. T. Blake, Austrobaileya **1**, 7 (1977).
[12] L. D. Pryor, E. R. Williams, and B. V. Gunn, Aust. Syst. Bot. **8**, 7 (1995).
[13] M. T. Inoue and S. Oda, Proceedings of the International Symposium on Forest Tree Physiology, Nancy, 1998, p. 69.
[14] P. R. Barja, A. M. Mansanares, and E. C. da Silva, Acoust. Phys. **47**, 16 (2001).