

Remote sensing of solar-excited plant fluorescence as a measure of photosynthetic rate

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Abstract

Leaf level net photosynthetic rates (P_N) of laurel oak (*Quercus hemispherica*) juveniles grown under contrasting nutrient and CO₂ regimes were negatively correlated with red to far-red ratios, R/FR (690/760 nm), steady-state, solar-excited fluorescence ratios ($r^2 = 0.66$, $n = 12$) measured across 12 plant canopies. Laurel oak juveniles that had been subjected to nitrogen stress over a period of a year demonstrated higher R/FR than their counterparts that had been provided with sufficient nitrogen. Plants that had been grown at elevated CO₂ concentrations, EC [700 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$] also exhibited significantly higher R/FR when subjected to normal ambient carbon dioxide concentrations than their counterparts grown under ambient concentrations, AC [380 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$]. All fluorescence measurements were obtained by observing a multi-plant canopy using a unique solar-blind passive sensor. This sensor, which utilizes Fraunhofer-line discrimination techniques, detects radiation at the cores of the lines comprising the atmospheric oxygen A- and B-bands, centered at 762 and 688 nm, respectively. These results support the use of solar-excited steady-state plant fluorescence as a potential tool for remote measurement of canopy radiation use efficiency.

Additional key words: chlorophyll; CO₂ concentration; laurel oak; nitrogen; *Quercus*; photon use efficiency; R/FR ratio.

Introduction

The ability of the terrestrial biosphere to sequester carbon dioxide (CO₂), which is being emitted into the atmosphere due to consumption of fossil fuels, is a matter of great debate. It is critical to know the direction and the quantities of the carbon exchange between the earth and the atmosphere because this knowledge has direct impact on energy use policy. The mechanisms by which the terrestrial biosphere sequesters carbon are diverse and interconnected. Net CO₂ flux values, typically determined using eddy covariance towers, do not provide information as to the relative sizes of the sources and sinks of CO₂. For example, within a forest canopy, the trees and under-canopy plants take up CO₂ during daylight and release some during the night, generally with a net uptake. However, the total CO₂ fluxes can be dominated by terms which include uptake and release by the soil and degra-

dation of the organic underbush including bark, twigs, and fallen leaves (especially during and after senescence) (Dixon *et al.* 1994, Goulden *et al.* 1996). The ability of researchers to differentiate among source and sink terms will better allow them to extrapolate net biosphere behavior in the future and to predict what will happen in response to particular remediative measures (Bazzaz 1997, 1998). Moreover, although a grid of eddy-covariance towers is being maintained in North American, European, and increasingly in tropical forest systems through various carbon flux networks, it is proving difficult to scale up from point measurements of net ecosystem exchange (NEE). Remote sensing techniques that can provide measurements of total canopy photosynthetic rate (and thus photon use efficiency, PUE) have considerable potential for the monitoring of forest function at the land-

Received 6 August 2001, accepted 14 March 2002.

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Acknowledgments: The authors acknowledge financial support from the U.S. Department of Energy and National Aeronautics and Space Administration under the Small Business Innovation Research program as well as internal funding from *Aerodyne Research, Inc.*

scape and regional scale. Such techniques would be very important for productivity models that rely on estimates of PUE (Landsberg and Waring 1997, Coops 1999).

We are pursuing an approach to P_N measurement that differs from the conventional approach of using leaf-level cuvette measurements—the measurement of *in situ* steady-state solar-excited plant fluorescence (Carter *et al.* 1990, 1996, Theisen *et al.* 1994). Steady-state solar excited plant fluorescence has been suggested as a suitable approach for remote sensing at the leaf, whole plant, or multi-plant canopy level. In order to test the hypothesis that steady-state solar-induced fluorescence is correlated with P_N , juveniles of laurel oak, *Quercus hemispherica*, were grown in a controlled environment greenhouse at

Materials and methods

Plant growth: All plants were grown in greenhouse facilities at Harvard University. This research greenhouse is equipped with closed loop heating and cooling so that both temperature and the CO₂ environment can be precisely controlled in six independent zones. CO₂ is controlled through an automatic monitoring and injection system. This system utilizes a single *LiCor-6251* infrared gas analyzer (IRGA, *LiCor*, Lincoln, NE, USA).

Juveniles were started from seeds collected in northern central Florida. The soil was a mixture of sand, peat, and vermiculite (40 : 40 : 20). Seedlings were grown under two nutrient and two CO₂ regimes in a full factorial design. During the first year of growth, all plants were given 18 g of slow release fertilizer (*Osmocote*, 20 : 20 : 20 NPK). Plants in the high nutrient treatment were given supplemental fertilizer monthly (Peter's solution NPK 20 : 20 : 20) at a rate of approximately 4 g m⁻² (40 kg ha⁻¹). Low nutrient plants were not given any additional nutrients. The CO₂ treatments were ambient, AC [$\sim 380 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$] and elevated, EC [$\sim 700 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$] and were replicated in three blocks. The temperature was maintained at 25/22 °C (day/night). Plants were watered regularly.

Measurement conditions: Fluorescence, P_N , and leaf Chl measurements were made during the second year of growth in mid-October. Plants were between 70 and 150 cm tall. All measurements were made outside under an open sky on clear days at AC. Ambient temperatures ranged from 10–20 °C. Since the plants grown under EC were measured under AC, they were effectively being stressed during the measurements. Measurements of all treatments from one block were measured on the same day. Several plants of each replicate were arranged to form a canopy that filled the field of view of the passive fluorescence sensor. In order to lessen radiation transport effects, the field of view was arranged to not be optically opaque – *i.e.*, the vegetation index was less than one.

Harvard University. Laurel oak is a fast growing and very common oak species that occupies a range of soil types in the southeastern United States. It is a dominant canopy tree in many mesic forests in this region. Laurel oak is typically evergreen, flushes multiple times throughout the year and maintains a full canopy year-round under warm conditions. In order to create a range of photosynthetic capacities, plants were grown at two different nutrient levels and at two different CO₂ concentrations. Multi-plant canopy measurements of steady-state solar excited plant fluorescence were obtained in conjunction with leaf level CO₂ uptake and chlorophyll (Chl) concentration measurements.

Typical integration times for the fluorescence measurements were on the order of 600 s; measurements on each replicate were taken twice and averaged.

Remote sensing of Chl fluorescence: The recently invented solar blind fluorescence sensor operates as a Fraunhofer line discriminator, detecting radiation at the cores of the lines comprising the atmospheric oxygen A- and B-bands, centered at 762 and 688 nm, respectively (Kebabian 1996). These bands also correspond to wavelengths in the far-red and red Chl fluorescence bands. The sensor avoids the use of an interferometer or monochromator. As radiation collected from the fluorescing plants is passed through a cell containing oxygen at low pressure, the oxygen absorbs some of the incident energy and subsequently re-emits photons which are detected by a photomultiplier tube (*i.e.*, a fluorescence-induced fluorescence approach). This induced fluorescence signal is directly proportional to the absolute intensity of the plant fluorescence in the narrow oxygen absorption bands. Furthermore, since the oxygen in the cell absorbs radiation at exactly the wavelengths that are strongly absorbed by oxygen in the atmosphere, the response to residual incident sunlight is minimal. The sensor is described in detail in Kebabian *et al.* (1999).

All plant fluorescence values are reported as the ratio of the fluorescence at 690 nm (red, R) to that at 760 nm (far red, FR). The efficacy of this fluorescence ratio (R/FR) as a measure of plant stress is attributed to two factors. First, the ambient Chl reabsorbs the R emission at 690 nm (unlike the FR emission at 760 nm). Thus its observed intensity is sensitive to the Chl concentration. Second, the FR emission is apparently less responsive than the R emission to the state of the electron transport mechanisms at normal physiological temperatures (Lichtenthaler and Rinderle 1988, Agati *et al.* 1995, Govindjee 1995, Buschmann *et al.* 2000).

Leaf-level P_N and Chl measurements: P_N measurements [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$] were made on eight to ten individual leaves, chosen randomly from each canopy, using a *Li-Cor 6400* portable photosynthesis system. Measurements were made under natural radiation at the same time as the fluorescence measurements. Chl was also measured on 8-10 randomly chosen leaves from each canopy using a *SPAD-502* meter (*Spectrum Technologies*, Plainfield, IL, USA) which provides relative Chl content in an area of

Results

Chl measurements for all the replicates as well as the mean values are presented in Fig. 1. As expected, plants grown under high nutrients show far higher Chl contents than those given none. However, for the same nutrient levels, there was no difference in relative Chl contents for plants grown under different CO_2 concentrations, a result

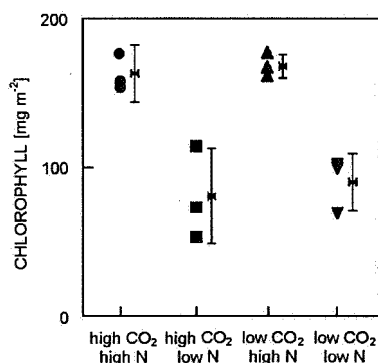


Fig. 1. Average chlorophyll concentration measured using a *SPAD* meter for laurel oaks grown under high and low nitrogen treatments and high and low ambient carbon dioxide concentrations. Individual points and means are shown. (Error bars show 1 standard deviation of the mean.)

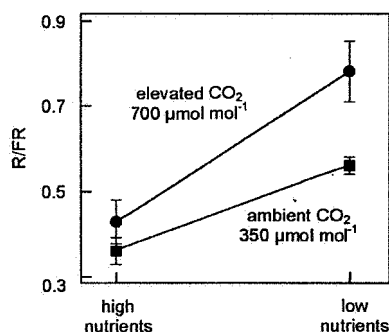


Fig. 2. Measured fluorescence ratios (690/760 nm) of laurel oaks grown with high and low nitrogen treatments under high and low carbon dioxide concentrations. Measurements were made using multi-plant canopies. ANOVA indicates that the observed differences for both carbon dioxide and nitrogen treatment effects are statistically valid (CO_2 effect, $p < 0.003$; nutrient effect, $p < 0.001$).

the leaf using an optical absorption technique (Markwell *et al.* 1995). Readings were converted to Chl concentrations using the following formula (following the procedures found in Cavender-Bares *et al.* 2000):

$$y = 5.53x - 62.90,$$

where y = Chl content [mg m^{-2}] and x = SPAD absorbance [relative].

that is consistent with other studies (Curtis and Wang 1998).

In contrast, the Chl fluorescence values (Fig. 2) indicate that the R/FR ratio was able to discern both long term stress induced by low nutrient levels and short-term stress imposed by exposing plants grown under EC to AC. The fluorescence measurements were obtained under AC within 15-120 min after the plants were removed from their controlled CO_2 environment. The data points represent the average of the values obtained for the three replicates and the error bars represent one standard deviation of the mean. ANOVA indicates that the plant fluorescence ratios could be separated by both N treatment and AC with high certainty ($p \leq 0.01$). In plants acclimated to EC, ribulose-1,5-bisphosphate carboxylase/oxygenase activity is reduced (Sage *et al.* 1989, Bowes 1991, Delgado *et al.* 1993, Sage 1994, Webber *et al.* 1994). When these plants are then subjected to lower CO_2 concentrations they show a reduced photosynthetic efficiency. These results are in accord with others obtained using bean plants that indicate that solar-excited fluorescence provides pre-visual stress detection (Kebabian *et al.* 1999).

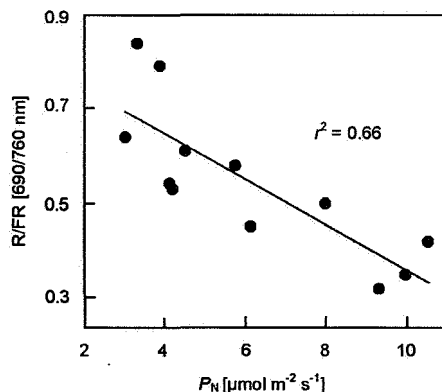


Fig. 3. Measured canopy fluorescence ratios of the laurel oak replicates plotted *versus* the measured net photosynthetic rate (P_N) at the leaf level. Assimilation rate values are the average of several leaf readings per plant. The r^2 value was obtained from a linear fit to the data.

Fig. 3 presents CO_2 assimilation values obtained from the replicates in close time proximity to the fluorescence

measurements. P_N among the replicates varied by almost a factor of four under full sun conditions. The highest P_N values were exhibited by trees grown at high N and low CO₂ concentrations. Correspondingly, the most stressed trees were those grown at low N and high CO₂. The values indicate that N stress resulted in a reduction of P_N by *ca.* 50 % for the plants grown in both AC and EC. Given that the leaf Chl content was reduced by only 35 %, N stress likely affected photosynthetic efficiency beyond the decreased photon harvesting capacity resulting from low leaf Chl content. The effect of the short-term CO₂ stress on P_N is also substantial. The trees grown at high N and EC exhibited a *ca.* 40 % reduction in P_N compared to their counterparts grown at AC. The trees grown at low N

Discussion

Juvenile laurel oak trees that had been subjected to N stress over a period of a year demonstrated higher R/FR fluorescence ratios than their counterparts that had been provided with sufficient nitrogen. ANOVA indicates that the measured differences were statistically significant. Plants that had been grown at EC and then subjected to AC also exhibited statistically significant higher R/FR ratios than their counterparts grown at AC. These plants were subjected to short-term stress caused by exposing the plants to concentrations of CO₂ lower than those to which they were acclimated. Near simultaneous P_N of all the plants indicated that the R/FR fluorescence ratios showed a strong negative linear correlation to P_N ($r^2 = 0.66$, $n = 12$), providing support for the hypothesis that solar excited steady state plant fluorescence is fundamentally related to physiological function. This technique shows promise for remote detection of photon use efficiency, an important parameter used in large-scale productivity models (Field *et al.* 1998, Cramer *et al.* 1999, Gamon and Qiu 1999).

The technique described here relies totally on solar irradiation and provides steady-state fluorescence levels of the plant unperturbed by the measurement process. The response is instantaneous and offers the opportunity to measure a quantity empirically correlated with P_N . Other studies have also indicated that steady state plant fluorescence, whether solar-induced (Carter *et al.* 1990, 1996, Theisen *et al.* 1994) or induced by artificial irradiation (Flexas *et al.* 2000, 2002), is linked to carbon assimilation and physiological function.

The differences in P_N and concomitantly in R/FR are caused by two different factors in these studies. The first factor is simply differences in leaf Chl content, a result of long term stress caused by N deprivation. The behavior of the fluorescence ratio in this case is easily understood in terms of the optical properties of the leaf Chl. Fluorescence emission at 690 nm is strongly reabsorbed by the leaf Chl; however, at 760 nm the leaf is essentially non-

absorbing and all the fluorescence escapes the leaf. As Chl content increases, the 690 nm fluorescence will thus not increase as quickly as the emission at 760 nm. Thus, all other things being constant, the fluorescence ratio should decrease as Chl content increases, in accord with the results shown here.

Overall, we found a significant negative correlation between R/FR (690/760 nm) and averaged leaf-level photosynthesis rates ($r^2 = 0.66$, $n = 12$) assuming a simple linear fit to the data. These results show that the R/FR steady state fluorescence ratio tracked changes in P_N resulting from both short- and long-term stress. This indicates that passive, steady-state plant fluorescence measurements are capable of providing quantitative information about P_N by the plant canopy during sunlight hours.

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In cases where the P_N and thus fluorescence ratios change as a function of short term stress, however, the explanation must derive from how the photochemistry is affected by the underlying physiological processes. Previous studies using this sensor tentatively suggest that the fluorescence at 690 nm is far more sensitive to short term stress than that at 760 nm (Kebabian *et al.* 1999). Chl fluorescence at 690 nm is largely emitted from photosystem (PS) 2 with minor contributions from emission from PS1. At this wavelength, fluorescence intensity depends on photochemical and non-photochemical de-excitation of the energy absorbed by the light-harvesting complexes (Schreiber *et al.* 1998). Under normal conditions, electron transport and CO₂ fixation determine the balance between these two processes. This balance protects the photochemical apparatus from over-reduction from excess photons (Krause and Weis 1991, Melis 1999). Even if P_N is reduced, safe dissipation of excess energy may continue through electron transport processes including photorespiration (Heber *et al.* 1995), the Mehler-ascorbate pathway (Asada 1999) and cyclic electron flow around PS2 (Canaani and Havaux 1990) or PS1 (Katona *et al.* 1992). Many stress factors, including water stress and excess photons, also affect non-photochemical processes. These non-photochemical quenching processes represent non-radiative pathways of de-excitation of incoming radiant energy, mainly through heat dissipation induced by high trans-thylakoid ΔpH and de-epoxidation of the xanthophyll cycle (Demmig-Adams and Adams 1992, Björkman and Demmig-Adams 1994, Horton *et al.* 1996, Gilmore 1997, Niyogi 1999), as well as through spillover from PS2 to PS1 (State I-State II transitions) re-

sulting from the aggregation and redistribution of light-harvesting Chl-proteins between the two photosystems (Horton *et al.* 1996, Gilmore 1997).

The mechanisms that affect the emission at 760 nm are far less well understood. Historically, it has been generally assumed that PS1 contributes little fluorescence intensity at any wavelength at room temperature. If 760 nm emission emanated primarily from PS2, mechanisms similar to those described above would presumably apply. However, both Agati (1998) and Pfündel (1998) point out that PS1 provides a substantial contribution to the fluorescence at 735 nm; Pfündel estimates that, in some cases, its contribution could be more than 50%. Presumably, at 760 nm, PS1 fluorescence could easily be dominant. In support of this thesis, recent results from isolated intact PS1 indicates that the fluorescence associated with excitation of PS1 (from maize) extends out to wavelengths longer than 800 nm (Croce *et al.* 2000). No variable fluorescence (*i.e.*, induction kinetics) is observed from PS1 under routine conditions (Lavergne and Trissl 1995). Thus, its intensity is fairly constant under all but extreme conditions. Consequently, if the fluorescence at 760 nm principally emanates from PS1, we expect that the main differences in R/FR (690/760 nm) largely result from differential fluorescence quenching at 690 nm. In this limit, the simple negative correlation of P_N with fluorescence ratios based on the short-term stress (CO_2 con-

centrations) in the present experiment may only hold if non-photochemical quenching is minimal or constant. A recent study found that the relationship between steady-state fluorescence at 690 nm and carbon assimilation can vary significantly with water stress due to an increase in non-photochemical quenching (Flexas *et al.* 2002).

The present study indicates that this solar-blind, passive sensor may be suitable for use as a monitor of CO_2 uptake by plant canopies under ambient conditions if the caveats presented above and corrections for canopy dependent radiation transport effects are considered (Liang *et al.* 1997, Pearson 1997, Pearson and Settle 1997, Walthall 1997). Additional research is necessary to determine the physiological mechanisms underlying the relationship between steady state fluorescence ratios of the plant canopy and leaf-level P_N , as well as to determine conditions under which this relationship might not hold. Many large-scale ecosystem flux models incorporate a PUE parameter to estimate net primary productivity (see, for example, Field *et al.* 1998, Cramer *et al.* 1999, Gamon and Qiu 1999). PUE, a measure of canopy photosynthesis, is generally measured on the ground rather than remotely, assumed to be constant or calculated indirectly due to the lack of appropriate techniques. Given the results presented here, this sensor offers the potential for remote detection of PUE and improved estimates of P_N at the ecosystem level.

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