A new fluorimeter built at Orsay allowed us to measure at a distance of up to 6 m both the steady-state and the maximum chlorophyll fluorescence. This instrument has been applied continuously during 17 days of water stress development to follow the chlorophyll fluorescence parameters of a potted grapevine. Gas-exchange rates for H₂O and CO₂ and chlorophyll fluorescence parameters of the same leaf were recorded concurrently. It was shown that: (1) Under well-watered conditions, before noon, a correlation was found between net photosynthetic rate and the rate of electron transport calculated from fluorescence measurements. After several hours of high light exposure, CO₂ assimilation (A) started to decrease more than the rate of electron transport (ETR). Under drought conditions, the above-mentioned correspondence was lost: when A almost vanished due to high stomatal closure, the ETR was still about 50% of the control value. It is suggested that under these conditions, the ratio of photorespiration to CO₂ assimilation increased. (2) Light response of the quantum yield of ETR became increasingly different between morning and afternoon as water stress progressed, thus serving as a good indicator of plant water status. (3) A simple fluorescence parameter, Fs, accurately reflected the plant physiological state. Over the range of light intensities used in this study, this parameter changed in parallel with irradiance in well-watered plants. With increasing water stress, Fs changed in opposite direction to irradiance changes. The response of Fs to rapid changes in irradiance was fast (within seconds). The potential of this parameter for remote sensing of water stress is discussed. © Elsevier Science Inc., 2000

INTRODUCTION

The interest of chlorophyll fluorescence as a useful signal reflecting plant photochemistry has been widely reviewed (Bolhär-Nordenkampf et al., 1989; Krause and Weis, 1991; Schreiber et al., 1994; Lichtenthaler, 1996). This is a nondestructive and noninvasive signal, easy to use for many purposes in laboratory and fieldwork. For these reasons efforts have been made to relate chlorophyll fluorescence parameters, mainly the electron transport rate from PS II (ETR), with actual rates of CO₂ assimilation (Edwards and Baker, 1993; Genty et al., 1989; Schindler and Lichtenthaler, 1996; Valentini et al., 1995; Weis and Berry, 1987). The results have shown good agreement between CO₂ assimilation and ETR in C₄ plants, but not as good agreement in C₃ plants, due to the contribution of other processes to electron use.

Photorespiration and the Mehler reaction are the main processes related to the imbalance between CO₂ assimilation and ETR. The first consists of the oxygen-
ation of ribulose-1,5-bisphosphate by Rubisco, which, according to the enzyme properties, is likely to increase when CO₂ availability in the chloroplast is reduced, as occurs under water stress due to stomatal closure. The photorespiratory pathway itself consumes only about half of the NADPH synthesized by the chloroplastic electron transport chain in respect to the consumption by CO₂ assimilation. However, this cycle, which evolves one molecule of CO₂ per each two molecules of O₂, is always coupled to CO₂ assimilation through the recycling of the evolved CO₂. Both processes together represent a combined cycle, the C₃-C₅ cycle that according to a steady-state biochemical model recently presented (Takeba and Kozaki, 1998) is able to maintain about 75% of maximum ETR in a situation in which no net CO₂ assimilation is observed (that is, only by internally recycling the CO₂ evolved by the photorespiratory pathway). The Mehler reaction consists of a direct reduction of O₂ by the electron transport chain at the ferredoxin level (Asada, 1999). Both processes increase under water stress as a consequence of reduced CO₂ availability in the chloroplast, which increases the ratio O₂/CO₂, and both have been suggested many times as important electron consumers under water stress (Cornic and Briantais, 1991; Flexas et al., 1999a; Flexas et al., 1999b; Flexas et al., 1999c; Osmond et al., 1997; Wingler et al., 1999), and their importance in water-stressed grapevines has been recently demonstrated (Flexas et al., 1999c).

Despite these difficulties with C₃ plants, chlorophyll fluorescence has been shown to be an interesting tool for plant stress detection (Cecchi et al., 1994; Cerovic et al., 1996; Gintner et al., 1994; Moya et al., 1992; Moya et al., 1995). The potential of fluorosensing water stress has been reported recently for several plants including grapevines using parameters other than ETR, such as the chlorophyll fluorescence mean lifetime (Cerovic et al., 1996; Schnuck et al., 1992) or nonphotochemical quenching of chlorophyll fluorescence (Flexas et al., 1998; Flexas et al., 1999a; Flexas et al., 1999b; Schultz, 1997). Moreover, it has been noticed that water stress induces marked effects on the daily pattern of steady-state chlorophyll fluorescence (Fₛ) (Cerovic et al., 1996; Flexas et al., 1999a; Flexas et al., 1999b; Rosema et al., 1998).

The aims of the present work were:

1. To test the capacity of the new fluorimeter developed at the LURE (Orsay, France) by I. Moya for measuring at a distance of 0.5 m to 6 m both the steady state and the maximum chlorophyll fluorescence in vivo.

2. To test the utility of several chlorophyll fluorescence parameters for plant water stress detection, paying special attention to the time resolution and spatial correlation of Fₛ changes to light over a range of values of the plant water status.

Simultaneous measurements of chlorophyll fluorescence with the newly constructed frequency-induced pulse amplitude modulation fluorimeter (FIPAM) and gas-exchange rates with a CO₂/H₂O porometer (LI-6400, Li-Cor Inc., Lincoln, NE, USA) were performed continuously (night and day) during the 17 days of a drought cycle. Some experiments with artificial light were performed to complete the study.

MATERIAL AND METHODS

Plant Material

One-year-old plants of Vitis vinifera (L.) cultivar Cabernet Sauvignon were grown in a greenhouse at Orsay (vicinity of Paris, France), under natural light and temperature conditions in small pots (0.5 L) with horticultural substrate. Pots were covered with aluminum foil to avoid soil water evaporation, and periodically irrigated to maintain them at field capacity until the onset of measurements, which were performed during the summer of 1996. The last fully expanded leaf of the main shoot was used for measurements.

Environmental Conditions

Measurements were performed under natural greenhouse conditions during the summer. Air and leaf temperature (inside and outside the Li-6400 chamber) were continuously recorded using thermocouples coupled both to the FIPAM (measurements every 30 s) and to the Li-6400 (measurements every 5 minutes). Photosynthetic Photon Flux Density (PPFD) was also recorded on the leaf surface with a quantum meter coupled to the Li-6400.

Environmental heterogeneity was present during the experiment, with sunny and cloudy days, as well as sunny and cloudy intervals within the same day. The iron greenhouse structure also caused a temporally unavoidable light interception that shaded the leaf for short periods during the diurnal time courses, which caused discontinuities in the profile of light interception by the leaf. These sudden discontinuities served to aid observation of the rapid response of photosynthesis to changes in the light environment. About 1,200 μmol photon m⁻² s⁻¹ PPFD were recorded at midday sunny peaks during sunny days. Air temperature inside the greenhouse varied between 15°C and 20°C during the night and dawn, to peaks of 30°C to 35°C at midday (data not shown).

Plant Water Status

Water stress was induced by withholding watering. Daily water loss was followed by successive pot weighing during the experiment. Leaf discs were taken periodically from leaves similar to those used for photosynthetic measurements. The samples were taken always in the early morning to avoid differences in water content due to water loss during the day. The leaf water deficit (LWD) was estimated from disc fresh weight and the weight of
the same discs after 24 h in distilled water at 4°C (full turgor), as follows: LWD=(turgid weight−fresh weight)/turgid weight.

**Chlorophyll Fluorescence Measurements**

A new fluorimeter was designed and built at LURE (Orsay, France) by I. Moya, with the aim of continuously recording fluorescence parameters (Fs and Fm) during several days, at a distance from the leaf sufficient to avoid any interference with the natural illumination of the leaf. This distance was 0.6 m in our particular experiment.

The FIPAM fluorimeter is based on fluorescence excitation by a laser diode (635 nm, 10 mW, SDL Inc.). The beam is modulated at different frequencies with constant amplitude and duration (2 μs) and focused on the leaf by a microscope objective, from distances adjustable in the range of 0.5 m to 6 m, depending on the laser source. The resulting spot has a rectangular shape of 0.5 mm by 4 mm at a distance of 1 m. The new concept of saturating the fluorescence yield by increasing the frequency of modulation makes a bridge between the PAM technique, widely applied among plant physiologists (Schreiber, 1983) and other LIDAR systems capable of detecting chlorophyll fluorescence at distances greater than 10 m, but restricted to measuring the stationary fluorescence level (Fs). We have already used the FIPAM fluorimeter at distances of about 6 m using a 100-mW laser diode (Philips CQL 822/D, Eindhoven, The Netherlands) instead of the 10-mW one used in the present work. The market availability of high-power laser diodes and other solid-state, high frequency modulation lasers, which can be used for chlorophyll excitation, is growing very fast. There is no doubt that remote sensing measurements with the FIPAM method over distances higher than 10 m will be feasible in the near future.

With our system, the basal fluorescence value when all photosystems are closed—that is, at complete darkness (Fo), as well as at the steady-state chlorophyll fluorescence emission under a given irradiance (Fs)—are measured with a frequency of only 1 Hz, which corresponds to an average intensity of 0.05 μmol photons m⁻² s⁻¹. At this frequency no actinic effect is observed even in complete darkness. Maximum fluorescence when all centers are closed, both in darkness (Fm) or under a given irradiance (Fm'), are induced by increasing the frequency to 100 kHz. Under these conditions, the average intensity ranges between 2,000 and 10,000 μmol m⁻² s⁻¹, depending on focusing. We ensure that actual maximum chlorophyll fluorescence has been reached by recording the complete induction kinetics, with a time resolution of 10 ms (not shown). The leaf fluorescence is collected by a 15-cm Frenel lens and focused on a PIN photodiode (Hamamatsu S3590) after passing through a high-pass filter (Schott RG665). The signal is processed by specially designed electronics locked to the frequency of excitation pulses, which make the pulsed response insensitive to continuous illumination even under conditions that saturate fluorescence. Two signals are obtained in parallel: a fluorescence signal (Fs) and a continuous signal (Rcont), which is proportional to the ambient light reflected by the leaf that passes through the detection filter. It has been observed that Rcont is proportional to the PPFD intensity. Under our experimental conditions, changes in the incident light due to solar position induced only minor decorrelation between Rcont and PPFD. Therefore, after calibration, Rcont can be used to follow changes in PPFD. The fact that Rcont and Fs originate from exactly the same leaf area enables a precise correlation between these two signals.

The instrument is controlled by a computer with a specially designed program, which allows continuous measurement over several days. Every second the Fs and Rcont signals are measured together with the air, leaf, detector, and laser temperatures. Corrections were applied to make the experiments insensitive to temperature changes of the instrument. The mean of 30 measurements is calculated every 30 s. The zero of the fluorescence signal is measured for each cycle by triggering the measurement in the absence of the excitation pulse. This value is automatically subtracted from the fluorescence signal. Thus, the Fs value is free of any electronic drift. This is of particular importance since the experiment lasted for several days.

In this experiment the frequency of Fs measurements was initially set to one measurement each 10 minutes. The same procedure was used to measure Fv/Fm and ΔF/Fm'. We refer to Fv/Fm when measurements are taken by night (i.e., all photochemical quenching relaxed) and to ΔF/Fm' (Genty et al., 1989) when measurements were taken in the presence of actinic light (i.e., after dawn). Since ΔF/Fm' represents the quantum yield of PSII photochemistry, the electron transport rate from PSII was calculated by multiplying ΔF/Fm' by incident PPFD. The result is expressed in relative units because it considers neither the leaf absorbance nor the factor of PSI-PSII excitation distribution. Most workers accept this parameter as a good estimate of the linear electron transport from PSII (Bilger et al., 1995; Cornic and Briantais, 1991; Flexas et al., 1999a; Flexas et al., 1999b; Flexas et al., 1999c; Genty et al., 1989; Krall and Edwards, 1992), although it has been recently suggested that this does not hold under CO₂-limited photosynthesis and high irradiance (Rosema et al., 1998).

**Gas-Exchange Parameters**

The photosynthetic performance (both fluorescence and gas exchange) of a single leaf was followed during the 17 days of the experiment to avoid any effect due to plant or leaf variability. The Li-6400 chamber was placed in a
The frequency of saturation pulses was decreased to one pulse each 20 minutes and laser focus was slightly changed. Again, plants were grown under greenhouse light and temperature conditions and irrigated periodically to maintain the soil at field capacity, and water stress was induced by withholding watering. Leaf water deficit was estimated as described above.

For measurements, plants were dark-adapted for 2 hours in a dark room. The temperature was maintained constant at 25°C throughout the experiment. The artificial diurnal cycle on a single leaf was provided by a 250-W slide projector whose light intensity was varied using a rotating dimmer coupled to a stepping motor. The motor was controlled by a program run on a Hewlett Packard 9816 computer. The light beam was filtered through a 2-cm layer of a copper sulphate solution (1 M) to minimize spectral changes when varying light intensity. A different plant was used for each diurnal cycle, during which gas-exchange and chlorophyll fluorescence measurements were performed as described above.

RESULTS

Leaf Water Status

Figure 1 shows the decrease in soil water content (estimated as pot weight loss) during the water stress development. Water loss was due only to plant transpiration, as the pots were covered with aluminum foil to prevent evaporation from the soil surface. The progressive reduction of the slope of weight decrease revealed that leaves adjusted their transpiration rate gradually in response to

<table>
<thead>
<tr>
<th>Day</th>
<th>Sunlight</th>
<th>LWD (%) (±1.5%)</th>
<th>A/g (±15%)</th>
<th>ETR/A (±15%)</th>
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<tr>
<td>5/08/96</td>
<td>S</td>
<td>5.1</td>
<td>not m.</td>
<td>not m.</td>
</tr>
<tr>
<td>8/08/96</td>
<td>S</td>
<td>5.8</td>
<td>103.3 (130)</td>
<td>not m.</td>
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<tr>
<td>9/08/96</td>
<td>S</td>
<td>7.6</td>
<td>101.5 (147.1)</td>
<td>13.5 (33.4)</td>
</tr>
<tr>
<td>10/08/96</td>
<td>C</td>
<td>not m.</td>
<td>116.7 (136.4)</td>
<td>12 (13.1)</td>
</tr>
<tr>
<td>11/08/96</td>
<td>S</td>
<td>not m.</td>
<td>123.8 (99.6)</td>
<td>15 (20.8)</td>
</tr>
<tr>
<td>12/08/96</td>
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<td>not m.</td>
<td>116.6 (184)</td>
<td>16.4 (20.4)</td>
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<tr>
<td>13/08/96</td>
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<td>155.8 (143.5)</td>
<td>18.7 (26.1)</td>
</tr>
<tr>
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<td>22.1 (37.9)</td>
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<tr>
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<td>38.7 (104.8)</td>
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<tr>
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<td>5/07/97</td>
<td>S</td>
<td>10.7</td>
<td>1095.9 (n.s.)</td>
<td>221.7 (n.s.)</td>
</tr>
</tbody>
</table>

*Leaf water deficit (LWD, mean for three replicates), water use efficiency, and the ratio of electron transport to CO₂ assimilation at 200 µmol photon m⁻² s⁻¹ (A/g in µmol CO₂ µmol H₂O⁻¹; ETR/A in µmol electrons µmol CO₂⁻¹, assuming a leaf absorptance of 0.84 and equal distribution of energy between the two photosystems). Values in brackets represent afternoon data. Sunlight (S), sunny days, up to 800 µmol photon m⁻² s⁻¹ or more; cloudy days (C), less than 500 µmol photon m⁻² s⁻¹. not m. = not measured; n.s. = nonsignificant because of the low and scattered values of both A and g (see Fig. 8b).
soil water availability. Recorded diurnal time courses of leaf transpiration and stomatal conductance confirmed this adjustment (data not shown). As can be seen (Table 1), the studied range of LWD (from 5 to 10%) was far from the 30% known to cause strong reductions in photosynthetic capacity (Cornic, 1994). The 1997 LWD values were within the range of the 1996 experiment (Table 1).

Effects of Water Stress on the Diurnal Time Course of Chlorophyll Fluorescence and Gas Exchange (Experiment with Natural Light)

Figures 2A and 2B show the diurnal pattern of chlorophyll fluorescence parameters under irrigated conditions during a sunny day. Through the night, a marked decrease of Fm and a slight increase in Fo (Fig. 2A) caused a substantial decrease in Fv/Fm (Fig. 2B). The origin of these phenomena, present in most of the recorded cycles (see Figs. 3 and 4), seems to be the repetition of saturating pulses in the same leaf area during the whole night. During the morning, the Fs pattern followed quite well that of PPFD, but this relationship was not completely maintained during the afternoon. The relationship between ΔF/Fm' and irradiance during the day (Fig. 2D) shows that for any given irradiance, values corresponding to the morning were similar to those of the afternoon. Only points corresponding to the dawn showed a different pattern. The diurnal time course of electron transport rate (Fig. 2C) followed the diurnal pattern of irradiance. The rate of CO₂ assimilation also followed the same pattern during the morning (Fig. 2C). However, from midday on, a progressive decrease in CO₂ assimilation was recorded and was not accompanied by concomi-

Figure 2. Diurnal time course of chlorophyll fluorescence and gas-exchange under irrigation conditions on a sunny day (9 August 1996). (A) Chlorophyll fluorescence. Dots represent values of Fm and Fm'. Continuous line represents values of Fo and Fs. The spikes of Fo during the night are due to incomplete reopening of closed centres during the 30 seconds after a saturating pulse. (B) Variable fluorescence, Fv/Fm and ΔF/Fm' (dots). The dotted line is the PPFD measured with the FIPAM. (C) Relative electron transport rate (ETR) estimated from chlorophyll fluorescence measurements (continuous thin line), rate of CO₂ assimilation (A) measured by gas exchange (continuous thick line) and PPFD measured with the internal quantum meter of the gas-exchange analyzer chamber (dotted line). (D) The relationship between ΔF/Fm' and PPFD, replotted from Fig. 2B. Solid triangles are morning data and empty circles are afternoon data.
tant decreases in electron transport rate, which caused an increase of the ratio ETR/A during the afternoon (see also Table 1).

The chlorophyll fluorescence diurnal pattern of irrigated plants during a cloudy day (Figs. 3A and 3B) showed the same trends and relationships described for a sunny day, but with changes not so marked along the day. Interestingly, on this day, which had maximum irradiances lower than 400 μmol photons m⁻² s⁻¹, the diurnal pattern of CO₂ assimilation followed quite well that of electron transport rate during the whole day, with no imbalance detected in the afternoon (Fig. 3C). The slope of the relationship between ΔF/Fm’ and irradiance was similar to that of the previous day (Fig. 3D).

In comparison with irrigated plants, drought stress induced a more pronounced response of chlorophyll fluorescence parameters to light intensity (dawn increase of Fs, diurnal time course of ΔF/Fm’: Figs. 4A and 4B). An interesting aspect was observed in the relationship between ΔF/Fm’ and light intensity by comparing Figs. 2D and 4D. Two different patterns, corresponding to morning and afternoon data, were clearly distinguished for the stressed plant, whereas only one pattern was present for the control plant. During the morning the quantum yield of PSII was similar to that of irrigated plants for any given irradiance, but clearly lower in the afternoon. Such afternoon quenching of ΔF/Fm’ did not reverse after several hours of darkness, so the maximum Fs, diurnal time course of ΔF/Fm’; Figs. 4A and 4B).

Figure 3. Diurnal time course of chlorophyll fluorescence and gas exchange under irrigation conditions on a cloudy day (10 August 1996). (A) Chlorophyll fluorescence. Dots represent values of Fm and Fm’. Continuous line represents values of Fo and Fs. The spikes of Fo during the night are due to incomplete reopening of closed centres during the 30 seconds after a saturating pulse. (B) Variable fluorescence. Fv/Fm and ΔF/Fm’ (dots). The dotted line is the PPFD measured with the FIPAM. (C) Relative electron transport rate (ETR) estimated from chlorophyll fluorescence measurements (continuous thin line), rate of CO₂ assimilation (A) measured by gas exchange (continuous thick line) and PPFD measured with the internal quantum meter of the gas-exchange analyzer chamber (dotted line). (D) The relationship between ΔF/Fm’ and PPFD, replotted from Fig. 3B. Solid triangles are morning data and empty circles are afternoon data.
Figure 4. Diurnal time course of chlorophyll fluorescence and gas exchange under drought conditions on a sunny day (17 August 1996). (A) Chlorophyll fluorescence. Dots represent values of Fm and Fm’. Continuous line represents values of Fo and Fs. The spikes of Fo during the night are due to incomplete reopening of closed centres during the 30 seconds after a saturating pulse. (B) Variable fluorescence, Fv/Fm and ΔF/Fm’ (dots). Dotted line is the PPFD measured with the FIPAM. (C) Relative electron transport rate (ETR) estimated from chlorophyll fluorescence measurements (continuous thin line), rate of CO₂ assimilation (A) measured by gas exchange (continuous thick line) and PPFD measured with the internal quantum meter of the gas-exchange analyzer chamber (dotted line). (D) The relationship between ΔF/Fm’ and PPFD, replotted from (B). Solid triangles are morning data and empty circles are afternoon data.

followed the diurnal pattern of irradiance, CO₂ assimilation was almost absent during most of the day, due to complete stomatal closure only 3 hours after dawn (plot not shown).

In water-stressed plants the diurnal time course of Fs showed an opposite pattern to that of well-watered conditions; that is, it showed an inverse correlation with PPFD. Figure 5 shows details (periods of 4 hours) of drought-associated change in the Fs response to PPFD for three different days after withholding water. It is clear that under irrigated conditions, there was a positive correlation between Fs and irradiance, in spite of the large variations in irradiance (Fig. 5A). However, only 5 days later, under a mild water stress, this pattern had changed. The positive correlation was maintained at low light intensities (below 250 μmol photons m⁻² s⁻¹), but at high light intensities there was an inverse correlation between the two parameters (Fig. 5B). Three days later, a negative correlation was found even at low light intensities (Fig. 5C).

Effects of Progressive Soil Drying along the Days on Stomatal Conductance, CO₂ Assimilation, and Electron Transport Rate

Progressive soil drying was accompanied by different degrees of reduction in A, g, and ETR (Figs. 6A and 6B). Average values of these parameters were obtained from measurements taken at 200 μmol photon m⁻² s⁻¹ for the full period of water stress development. This PPFD was chosen because it is present both on sunny and cloudy days. Stomatal closure was an early response to soil drying, accompanied by a concomitant decrease of CO₂ assimilation. However, CO₂ assimilation showed a slightly lower decrease, and therefore the intrinsic water use efficiency (A/g) progressively increased with water stress (Fig. 6 and Table 1). Such increases were high when taken
Experiment with Artificial Light

The "diurnal" cycles performed with artificial light were made to verify the above results under controlled conditions, avoiding frequent changes in incident light intensity over the leaf, as well as large variations of temperature. In addition, we could check that the Fv/Fm quenching endured by oversaturating pulses during the night did not affect the dependence of other fluorescence parameters on water stress. Figure 7A shows the diurnal time course of Fs and Fm for a well-irrigated plant. Both Fo and Fm values remained constant during the night, and thus Fv/Fm was stable (Fig. 7B). It is interesting to note that similar to the experiment performed with natural light, Fs increased suddenly at dawn, when light intensity was less than 10 μmol photon m⁻² s⁻¹, but to a much lower extent than in the previous experiment. Also, this effect was reversed in a shorter time. The quenching of Fm observed at dawn was much lower than in the previous experiment, and its duration was much shorter. The diurnal time course of Fs followed that of PPFD (Fig. 7A). Again, under water stress conditions, this pattern was inverted (not shown).

Figure 8A shows the diurnal time course of CO₂ assimilation and electron transport rate for irrigated plants. A good coincidence was observed between both parameters during the morning. At higher light intensities (midday), electron transport continued to rise until the midday light peak, while CO₂ assimilation reached its maximum value at about 500 μmol photon m⁻² s⁻¹. The decorrelation is larger during the afternoon, as noticed in the experiment with natural light.

When the plant reached a water deficit similar to that of the experiment with natural light, CO₂ assimilation was almost completely absent (Fig. 8B), in accordance with an almost complete stomatal closure (not shown). Electron transport rate, however, was still maintained at about 50% to 60% of control values (Fig. 8B). The plot of ΔF/Fm’ against light intensity showed that the afternoon data coincide with those of the morning in the well-watered plant (Fig. 8C). For the stressed plants, however, ΔF/Fm’ was lower in the afternoon than in the morning for the same light intensity, as in the experiment with natural light. Moreover, at high light intensities, ΔF/Fm’ values were lower than for irrigated plant (Fig. 8D).

DISCUSSION

Water Stress Effects on Leaf Photosynthesis

It is shown that under well-watered conditions, ETR, A, and g followed the diurnal time course of PPFD during the whole morning. During the afternoon, however, there was a consistent decrease in A that matched a decrease in g. It is well established that even for irrigated plants some degree of water stress is achieved at midday.
Figure 6. Averaged values±standard error of electron transport rate (ETR, solid circles), CO₂ assimilation (A, empty squares) and stomatal conductance (g, solid squares) at 200 μmol photon m⁻² s⁻¹ during the days of water stress development. This PPFD was chosen to include comparable data of both sunny and cloudy days: (A) represents morning data; (B) represents afternoon data.

as a consequence of excess atmosphere water demand (Chaves, 1991). Down-regulation of A by photosynthetic accumulation has also been claimed to take place (Azcón-Bieto, 1983), although it has been reported by Chau- mont et al. (1994) and Downton et al. (1987) that such an accumulation does not occur in grapevines. Under our experimental conditions, an evaporative demand exceeding the water flux into the leaf seems to be the cause of the decrease in g and A. This is in agreement with the observed decreases in g, concomitant to an increase of A/g during the afternoon, and also such an afternoon depression did not appear in cloudy days, when leaf-to-air vapor pressure deficit had been lower during the morning.

Even when decreases in A were measured, ETR remained unaltered. Such an imbalance between electron transport rate and CO₂ assimilation as a response to water stress has already been reported for the C₃ plants *Vicia faba* and *Hordeum vulgare* (Lal et al., 1996), as well as for grapevines (Flexas et al., 1998; Flexas et al., 1999a) and has been associated with relative increases in photorespiration and/or Mehler reaction rates, which might help to maintain PSII stability under conditions of drought and excess light (Kozaki and Takeba, 1996; Park et al., 1996; Takeba and Kozaki, 1998). The results given here show that when net CO₂ assimilation is close to zero under severe water stress, ETR is still about 75% of control values (see Fig. 6A). This is exactly the percentage of maintained ETR expected at the compensation point, that is, when the only CO₂ assimilation corresponds to recycling of internally produced CO₂, with no net exchange between the leaf and the atmosphere (Takeba and Kozaki, 1998). These results, together with the proven importance of both photorespiration and the Mehler reaction as electron consumers in chilled and water-stressed grapevines (Flexas et al., 1999c), makes us
assume that the ETR calculation is quite accurate even under water stress, in contrast to that suggested by Roesma et al. (1998). An important implication of this is that the imbalance between A and ETR is due to real, physiological events, and not to an invalidation of the Genty model (Genty et al., 1989) for PSII photochemistry under water stress. Thus, it will not be possible to estimate actual CO₂ assimilation from ETR measurements.

Under water stress, almost all measured parameters showed marked decreases throughout the day. Especially remarkable was the decline in g, which reached values near zero only a few hours after dawn. Also, a large decline in A was found, but the ratio A/g increased dramatically. In addition ETR/A strongly increased as a consequence of the low reduction in ETR. These results confirm that water stress does not cause important inhibition of the photochemical mechanism (Cornic et al. 1989; Genty et al., 1987). According to Cornic and Bmittaiais (1991), electron transport to O₂ should be relatively increased during the desiccation of the leaf. This alternative sink for electrons should be large enough to maintain high rates of electron transport during most of the day. In the afternoon, a slight decrease of ETR was observed. By contrast to what happened under irrigated conditions, such an increase in electron transport to O₂ was not able to protect leaves from photoinhibition during drought stress, as witnessed by only partial recovery of the afternoon quenching during the night. This is consistent with recent reports of Brestic et al. (1995). However, photoinhibitory effects did not appear until the afternoon, whereas photosynthesis was almost totally inhibited since early morning, indicating that electron transport to oxygen could help mitigate the damage of photosystem II at least during a large part of the day (Kozaki and Takeba, 1996; Park et al., 1996).

In general, the experiment with artificial light
yielded very similar results, confirming that the observed effects were due to water stress and not to the heterogeneity of experimental conditions. These results confirm that water stress-induced decreases in CO₂ assimilation were mainly due to stomatal closure and not to decreased photochemical efficiency of PS II (Cornic et al., 1989; Cornic and Briantais, 1991; Cornic, 1994; Lal et al., 1996). However, some down-regulation of PS II activity occurred as a consequence of the decreased CO₂ availability (Foyer et al., 1990), since the rates of electron transport under severe water stress were slightly lower than those of the irrigated one.

Night and Dawn Quenching of Chlorophyll a Fluorescence

The possibility offered by the FIPAM to determine chlorophyll fluorescence without interfering with the light climate of the leaf lead us to apply saturating pulses day and night during several days, over the course of the first experiment (“natural illumination”). As a result, problems that may not appear under short duration measurements became of nonnegligible importance. The clearest consists in a decrease of Fv/Fm at night (Figs. 2–4). This effect was observed in the first experiment, but not in the one with artificial light, after changing two laser conditions (Fig. 7) (i.e., the focalization and the frequency of saturating pulses). This suggests that it was caused by excessive intensity and frequency of repetition of the saturating light pulses. It is important to note that in the set of experiments presented in this work, this quenching is reversible under normal daylight conditions, as the same Fv/Fm value is observed after 24 h (see Figs. 2 and 3, which correspond to two consecutive days). This quenching results from two different effects: an increase in Fo and a decrease in Fm.

The increase of Fo through the night seems to be due simply to a cumulative noncomplete relaxation between saturating pulses. Indeed, 10 minutes should not be enough for complete relaxation in dark-adapted samples, due to slow reoxidation of plastoquinone (Bukhov et al., 1996), especially if there is an accumulation of Q₀-nonreducing centers under water stress, as recently suggested (Lu and Zhang, 1998; Lu et al., 1998). It is likely
that the height of the “comb” effect of night reduction of Fo could be an indicator of the relative abundance of O$_{b}$-nonreducing centers. Also, there was an increase of Fs at dawn because of a lack of activation of photosynthetic enzymes after several hours darkness (Figs. 2–4).

A decrease of Fm through the night was also observed, as well as soon after dawn. As a result the $\Delta F/Fm$ curve exhibited a sort of “hole” during ca. 1 hour to 2 hours at the beginning of the day (Figs. 2–4). This phenomenon could be tentatively related to a State1–State2 transition (that is, a disconnection of a part of PSII antennas that are transported and coupled to PSI reaction centers), as suggested under similar conditions to those encountered here (Bukhov et al., 1996).

In addition to those effects, the Fo level tended to increase through the days of continuous recording on the same part of the leaf, together with a progressive decrease of the maximum Fv/Fm achieved during the night (not shown).

We have recently studied these phenomena using several plant species, and we have confirmed that they were entirely due to excessive frequency of saturating pulses (Apostol et al., 1999). In any case, it is important to stress that although these effects lowered the Fv/Fm to a value of only 0.6 after 15 days of continuous recording over the same leaf, they did not change the main photosynthetic responses to water stress, as demonstrated by the similarity of results between the first experiment and the second one, as well as previous results (Flexas et al., 1998; Flexas et al., 1999a).

The Importance of Fluorescence Parameters for Water Stress Assessment

The present results show that it is not possible to estimate the rate of CO$_{2}$ assimilation from chlorophyll fluorescence measurements in grapevines, at least in the absence of complementary approaches. A similar conclusion has been recently pointed out by Rosema et al. (1998). Even in irrigated plants there was an impairment between electron transport and CO$_{2}$ assimilation, consistent with previous reports (Flexas et al., 1998; Flexas et al., 1999a; Lal et al., 1996), which is likely due to an increase in alternative ways for electron consumption, such as photorespiration during the afternoon, and to an incorrect determination of PSII ETR, as suggested by Rosema et al. (1998). In drought plants there was a generalized lack of relationship between these two parameters.

In spite of these results, chlorophyll fluorescence assessment can be a very useful tool for stress detection, especially with instruments that allow a continuous recording under natural light conditions, such as the FIPAM tested here. Some fluorescence parameters clearly reflect plant water status, and we will focus on two of them: the relationship between $\Delta F/Fm$ and light intensity, and the diurnal pattern of steady-state chlorophyll fluorescence (Fs). We assume that these two approaches can be useful tools for water stress assessment, although it remains to be tested if other stresses would lead to similar results.

Under irrigation conditions the relationship between $\Delta F/Fm$ and light intensity showed low scatter, and both morning and afternoon points fitted the same relationship. When water stress was present, the points corresponding to morning measurements fitted a curve clearly different from that for afternoon data. For the same light intensity, the values of $\Delta F/Fm$ measured in the afternoon were lower than those in the morning, indicating down-regulation of PS II efficiency after a large period under excess light. When water stress became more pronounced, this difference increased. All these characteristics contribute to qualify the relationships between $\Delta F/Fm$ and light intensity as a robust tool for water stress detection.

The diurnal response of Fs to light intensity could also be a sensitive indicator of water deficit. The inverse correlation between Fs and light intensity is a characteristic signal of water stress, which can be related to a strong increase of the nonphotochemical quenching (Cerovic et al., 1996; Flexas et al., 1998). Although this behavior of Fs under water stress has been reported earlier (Cerovic et al., 1996; Flexas et al., 1999a; Flexas et al., 1999b; Rosema et al., 1998), here we show (thanks to the ability of FIPAM to measure Fs and PPFD from the same leaf area and every second) that the response of Fs to sudden changes in PPFD takes place in seconds, so the light response of Fs should be an accurate and simple signal to detect water stress that can be used even in cloudy days or with heterogeneous structures such as those of the glasshouse used here.

To illustrate the correlation between Fs and PPFD even during short periods of light variation (several minutes), we have depicted such a correlation with data from Figs. 5a and 5c (Fig. 9). We have chosen data from monotonous light transitions since the changes at high PPFD are too rapid and cause high hysteresis. The different response of well-watered and water-stressed plant is quite clear. It is shown that under water stress, there is a saturation of the minimum value of Fs above 400 $\mu$mol m$^{-2}$ s$^{-1}$. This may coincide with the saturation of nonphotochemical quenching. Under irrigation, with these particular plants and conditions, the relationship between Fs and PPFD is poor above 600 $\mu$mol m$^{-2}$ s$^{-1}$, due to the slow development of a high nonphotochemical quenching (not shown in Fig. 9). However, in field-grown plants, this relationship is clear at much higher PPFD values (Flexas et al., 1999a). This technique is especially easy to use with the FIPAM fluorimeter, which allows the measuring of Fs in a same leaf continuously.
and at a distance during long periods without interfering with leaf physiology.

CONCLUSIONS

The present work shows that the new technique discussed here is a very useful tool for remote sensing of vegetation stress.

The present results confirm and extend previous work by Cerovic et al. (1996) and Flexas et al. (1999a). The interest of F’s response to light as a putative indicator of water stress is shown again. This idea has also been proposed by Rosema et al. (1998).

In addition, this work presents novel aspects in respect to our previous work:

1. The possibility of measuring Fm at distance has been shown for the first time thanks to the new concept of saturating fluorescence by changing the frequency of the excitation source.

2. Fs and PPFD are obtained from the same leaf piece with this new instrument. This has allowed us to resolve rapid variations (within seconds) of F’s in response to sudden changes of incident light. Such variations are shown to be a simple and rapid way to detect the dominant type of quenching regulating leaf photochemistry, which is a sensitive indicator of plant stress.

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