Photosynthetic Responses of Leaves to Water Stress, Expressed by Photoacoustics and Related Methods

I. PROBING THE PHOTOACOUSTIC METHOD AS AN INDICATOR FOR WATER STRESS IN VIVO

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ABSTRACT

The effect of leaf desiccation on the photosynthetic activities in vivo was probed by the photoacoustic method. The aim of this research was:

(a) To study the photoacoustic signal per se in varied conditions in order to develop this tool as a probe for stress conditions in vivo. (b) To obtain results pertaining to electron transport activities in vivo, and confirm conclusions based on work with isolated chloroplasts, which could otherwise be the result of nonspecific damage occurring during their isolation. Leaf discs from tobacco (Nicotiana tabacum L.) were routinely used, with other species tested also for comparison. Rapid leaf desiccation caused changes in the low frequency photoacoustic signal, attributed both to the mechanism of signal transduction, influenced by changes in the structural parameters of the leaf, and to the direct (nonstomatal) inhibition of gross photosynthesis. The dependence of the photothermal part of the signal on the frequency indicated the presence of two photothermal components, one of which persisted only at low modulation frequencies (below about 100 Hz) and which largely increased with the desiccation treatment. This component was ascribed to a thermal wave which reaches the leaf surface. The other nonvariable photothermal component was ascribed to a thermal wave propagating from the chloroplasts to the surface of the mesophyll cell. Only this component is considered in the ratio of the O₂ signal to the photothermal signal, which is used to estimate the quantum yield of photosynthesis. The specific dependence of the latter ratio on the frequency yielded a comparative quantum yield parameter from its extrapolation to zero frequency, and also indicated stress induced changes in the diffusion of O₂ through the mesophyll cell, reflected by changes in its characteristic slope. The (zero frequency extrapolated) quantum yield was markedly reduced with the progression of the water stress, indicating the inhibition of (gross) photosynthetic electron transport in vivo. This result was expressed even more emphatically by the stronger inhibition of the photochemical energy storage, obtained by photoacoustic measurements at a high modulation frequency.

Water deficit in leaves results in the reduction of the net photosynthetic CO₂ assimilation rate, which in some cases was attributed to stomatal closure (20, 21) and to increased diffusive resistance of the mesophyll cells (17). Boyer et al. and others showed, however, that isolated chloroplasts from water stressed leaves lost activity in their membrane-associated reactions, notably electron transport and photophosphorylation (1, 2, 4, 11, 16), thus putting more emphasis on the nonstomatal component in the reduction of photosynthesis. This conclusion was also achieved indirectly from measurements of leaf conductance (7) and from the lack of influence of CO₂ and temperature on net photosynthesis (2). From the decrease in the quantum yield for CO₂ fixation in vivo which could not be attributed to differences in the rate of either dark respiration or photorespiration, and as the Chl content stayed nearly constant, it was suggested that water stress affects directly the photochemical events (19). Specific changes in the fluorescence induction pattern in leaves have suggested that water stress hampers PSI activity (9).

The above data do not seem conclusive, however. It is possible, for example, that chloroplasts from water deficient leaves were more labile and were inactivated during their isolation. Indeed, the effects of water stress could not be reversed in isolated chloroplasts, while they were rapidly reversed in desiccated leaves (3, and this work). According to Sharkey and Badger (26), uncoupled electron transport is very robust to water stress while photophosphorylation is not. Wong et al. (28) state their impression that “The mechanisms by which water stress affects photosynthetic capacity are unclear.” Certainly part of the conflicting results may be attributed to the various methods that the stress was imposed, and to different responses of different species.

We have developed the application of a new method, photoacoustics, to measure gross photosynthesis, by microphone detection of sound, emitted from leaves when irradiated with periodic intensity-modulated light (5, 22). It seems that this method has certain unique features which could be helpful in the research on the effect of water stress on leaves. It was therefore thought advantageous to supplement the existing data with information obtained from the photoacoustic method. This method allows the performance of direct and extremely fast measurements on intact leaves of the quantum yields of O₂ evolution and the extent of energy storage in the photochemical reactions of photosynthesis. It is applied at physiological conditions, is nondestructive, and is capable of continuous monitoring. Photoacoustics involves the detection of heat and O₂ evolution pulses resulting from the absorption of intensity modulated light by the leaf. Heat and O₂ diffuse from the chloroplasts through the mesophyll cell towards the inner air phase. There, due to the periodic expansion and contraction, a sound wave is generated. The sound propagates through the air spaces inside the leaf, exciting vibrations in all its parts, including the epidermis, which can be considered as a flexible membrane. The acoustic wave is thus transmitted to the outside of the leaf where it is detected. This mechanism of signal transduction and transmission is inferred considering possible diffusion paths of O₂ and heat (14) and from the fact that the photoacoustic signal is totally insensitive to stomatal opening or closure (unpublished data). Because photoacoustics is a modulation method, it measures gross photosynthesis, and is insensitive to respiration or photorespiration.

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By virtue of the small volume of the photoacoustic cell it appears that atmospheric CO₂ is very quickly depleted and that the signals are indicative of photosynthesis at a compensation point. Other features of the photoacoustic method include the possibility of monitoring the Emerson enhancement, which allows us to follow changes in the relative activities of the two photosystems (6).

In this work, we have employed photoacoustics to examine the effects of rapid desiccation on the photochemical reactions of photosynthesis in detached leaves. The main purpose of this work was to examine the capability of the photoacoustic method in answering questions related to the effect of water stress, and to develop general procedures. It was found that both gross O₂ evolution and photochemical energy storage were severely inhibited during the stress period. The effects of water stress on the diffusion parameters of O₂ and heat through the cell matrix were analyzed comparatively in several plant species and were found to reflect changes specific to each leaf morphology, caused by the water stress. These factors had to be considered for the evaluation of the photoacoustic signal, as a probe for photosynthesis in vivo.

MATERIALS AND METHODS

Plant Material. Tobacco leaves (Nicotiana tabacum L., var Xanthi) of about 25 cm long were harvested from mature plants grown in a greenhouse. Other species were used for comparison, including Phaseolus vulgaris, Casimiroa edulis, and Passiflora edulis.

Stress Treatment. Water stress was imposed as described previously (10). Leaf discs of 1 cm in diameter were punched out, placed in Petri dishes, and exposed to an atmosphere of 53% RH under a light intensity of 11 W m⁻² at room temperature (24-27°C). Control discs were kept on moist filter paper under the same illumination and temperature conditions. Recovery experiments were performed by placing the stressed discs in water for 2 h. Relative water content determinations were done according to the following formula: (fresh weight-dry weight)/(water saturated weight-dry weight) as described in Turner (27). This form of presentation of water stress, rather than the customary water potential, is preferred here as equipment to make measurements of the water potential, particularly in the small leaf discs, was not routinely available. Furthermore, it has been claimed (24) that generally the stress effects are better correlated to the relative water content rather than to water potential. Nevertheless, the two parameters were compared in one of the samples, with measurements of water potential done with a borrowed thermocouple psychrometer (Decagon Devices).

The Photoacoustic Method. The photoacoustic measurements, techniques and apparatus, as applied to leaves, were described previously (5, 15, 22). Modulated light was supplied by a 450 W Xenon lamp and a monochromator (Bausch and Lomb). Modulation was achieved by a mechanical chopper (Laser Precision) allowing a convenient range of frequencies (5–1000 Hz). Non-modulated background light (400–680 nm) was obtained from a d.c. operated quartz-iode 250 W projector fitted with an interference shortpass filter (Diric-Optic). The modulated and background lights were combined onto the leaf by means of fiber optic light guides. The acoustic signal was detected by a microphone (Knowles) and analyzed, after pre-amplification, by a lock-in amplifier (Brookdeal 9502) in the two-phase mode, allowing simultaneous recording of the (vectorial) in-phase and quadrature components (22).

The photoacoustic signal was measured at two modulation frequencies: 'low' (22 Hz) and 'high' (about 400 Hz). A major part of the photoacoustic signal at low frequency arises from modulated photosynthetic O₂ evolution (5, 22). With addition of nonmodulated background light of high intensity the periodic modulation of the O₂ evolution rate is eliminated due to photo-

\[ \text{synthesis saturation.} \]

The resulting signal (photothermal signal) is then solely due to the maximal conversion of light to heat. The level of the photothermal signal serves as a zero base line for the O₂ evolution signal, except for a correction to account for the photochemical energy storage when the background light is absent. This correction, termed the 'photochemical loss' (14, 15), is the fractional change of the photothermal signal from its maximum obtained in the presence of saturating background light. The PL is estimated separately from experiments at high frequency where only the photothermal signals exist.

In all the above the photoacoustic signals are treated as (mathematical) vectors in the two dimensions, by using the readings from the two phase channels of the lock-in amplifier as two vector components (amplitude/phase or sine/cosine components). The total (low frequency) photoacoustic signal is therefore considered as a vectorial combination of the O₂ and photothermal signal vectors (22). The total amplitude of each is calculated as the square root of the square sum of its components. A_PT is directly proportional to the total energy absorbed by the leaf and therefore, to a first approximation, the ratio of A_Ox to A_PT can be used as a parameter indicating the relative quantum yield for O₂ evolution.

The PL is considered to be a unique and valuable parameter per se, which is a direct measure of the efficiency of storage of light energy in the form of chemical energy due to the intermediate steps of electron transport and photophosphorylation (14, 15).

RESULTS

When small discs of tobacco leaves were exposed to air, they rapidly dehydrated (Fig. 1). After 4 h of this desiccation treatment, the relative water content of leaf samples was reduced to around 30%, whereas in control leaf discs kept on moist filter paper the water content was maintained at about 85%. In Figure 1, we give also the calibration of the water potential in terms of the relative water content done in a separate experiment.

Raw data for the photoacoustic signals at low modulation frequency are shown in Figure 2. Water stress causes two main effects: a decrease in the amplitude of the O₂ signal and a strong enhancement of the amplitude of the photothermal signal.

The increase of A_PT at low frequency at various stress levels is depicted in more detail in Figure 3. Another way to present this \[ \text{Abbreviations: PL, photochemical loss; A_PT, photothermal signal vector amplitude; A_Ox, oxygen evolution vector amplitude.} \]
FIG. 2. A record of the in-phase vectorial component of the photoacoustic signal emitted from control (A) and dehydrated (B—-for 4 h) tobacco leaf discs. The photoacoustic signal is composed of two contributions: the modulated heat (---) and the modulated O₂ evolution (—). Modulated light at 680 nm: 24 Hz: 6.5 W m⁻². Saturating light (S.L.): 460 W m⁻². A separate check (not shown) was made to ascertain that the phase angle between the photothermal and O₂ evolution vectors was not modified by the stress, at this frequency.

FIG. 3. Changes in the amplitude of the photothermal signal (A РФ) at low frequency (24 Hz) during dehydration in air of tobacco leaf discs. Other conditions as in Figure 2.

The phenomenon is to plot the ratio of A РФ to the energy flux of the incident light (I) measured separately as a function of the wavelength, which gives the light absorption spectrum in relative percent (Fig. 4A). The photoacoustic spectrum of the stressed leaf has higher intensity and shows a better spectral resolution. The spectral shapes were similar in their qualitative features to those obtained conventionally by diffuse reflectance, except for the lower spectral resolution (Fig. 4B). Since water stress induced relatively insignificant changes in the diffuse reflectance spectra of the leaves, one may conclude that the leaf's main optical properties and the extent of its light absorption were not modified significantly by drought. Similar conclusions were also drawn by Mohanty and Boyer (19). Their spectra for the absorption of well watered and desiccated leaves appear almost identical to those shown here by diffuse reflectance. One must seek a different explanation for the large stress-induced change in A РФ.

FIG. 4. A, Relative photoacoustic absorption spectrum of control and dehydrated tobacco leaf discs. Control leaf (——). Leaf dehydrated in air for 4 h (---). The photoacoustic spectrum is obtained by dividing the photothermal signal (i.e. in presence of saturating background light) by the incident light energy flux (A РФ/I) and scanning over the wavelength. Conditions similar to those in Figure 1. B. Diffuse reflectance spectra of the above stressed (---) and nonstressed (——) tobacco leaf discs. The spectra were obtained using a Cary 16 spectrometer with an integrating sphere attachment.

Under conditions where the specific thermal diffusion length³ is much larger than the specific or the actual light absorption length, one may anticipate that the photothermal signal amplitude would be inversely proportional to the modulation frequency f. Under this condition the thermal waves generated along the depth where light is absorbed arrive unattenuated to the surface and are proportional to the total light absorption. The temperature modulation amplitude is then proportional to the integrated light energy input during one chopping cycle (i.e. proportional to the cycle time) and hence to the inverse of the modulation frequency. When, however, the thermal diffusion length significantly decreases, the signal becomes progressively smaller than predicted from this relation, as photothermal waves.

³ Thermal waves generated by the modulated light absorption have the property that their amplitude decreases exponentially along their path, even for one-dimensional propagation (23). The specific thermal diffusion length (μ) is defined as a characteristic distance, which when traveled by a thermal wave, results in its attenuation by a factor 1/e (about 0.37) of the original amplitude. From the diffusion equation μ is equal to (2D/ω)², where ω is the angular frequency (=2πf) and D the thermal diffusivity (=κ/Cₚ, where κ is the heat conductivity, C the specific heat, and ρ the density). μ thus depends linearly on the inverse of the square root of the frequency.
arising from deeper regions of the light path become significantly attenuated at the surface and only part of the absorbed light contributes to the generation of the photoacoustic signal. In Figure 5, we tested the frequency dependence of the photothermal part of the photoacoustic signal. In control, unstressed, leaves the signal was largely linear with 1/f over a wide range of frequencies (5–1000 Hz), with only small deviations at low frequency (large 1/f). In stressed leaves it is also possible to distinguish a low frequency range in which the photothermal signal is proportional to 1/f and with a slope higher compared to the control. However, as the frequency increases, there is increasing deviation of the photothermal signal towards lower values, finally reaching a new proportionality between the photothermal signal and 1/f which has a smaller slope. It appears from Figure 5 that, in the stressed leaf the photothermal signal is composed of two contributions: one which is damped completely at the higher frequencies and one which persists over the whole frequency range of the experiment. Actually, in the control leaves it is also possible to distinguish the same trend in similar frequency ranges, but in this case the relative magnitude of the additional low frequency contribution is much smaller and only slightly noticeable. Most importantly, the photothermal signals at high frequency are essentially equal for both stressed and unstressed leaves (Fig. 5, inset). Thus, only that photothermal contribution, which persists at low frequencies but which is eliminated at high frequencies is affected by the stress.

The above result might be in accord with the suggestion of Larue et al. (13) of two photothermal components, arising from 'internal,' \( T_n \), and 'surface reaching,' \( T_o \), thermal waves. \( T_o \), follows an actual diffusion path as the \( O_2 \) evolution component—a short distance (less than about 1 \( \mu m \)) from the photosynthetic membranes to the internal air spaces, where the acoustic signal is produced. \( T_n \) travels all the way from the light absorbing chloroplasts to the leaf surface. The effective average path of thermal diffusion in this case is governed by the depth where most of the light is absorbed, which is of the order of several tens of microns. \( T_n \) is thus expected to be damped much more before \( T_o \), at increasing frequencies. At the highest frequency used (about 800 Hz), at which the specific thermal diffusion length in water is around 7.5 \( \mu m \), one still expects only a small attenuation by a factor \( \exp(-1/7.5) = 0.88 \) of the original value of \( T_n \), while \( T_n \) should be almost completely damped at this frequency (by a factor \( \exp(-30/7.5) = 0.02 \) for light penetration path of 30 \( \mu m \)).

A more quantitative inspection of the data shows that this theory is too simplified. Thus, the whole experimental attenuation pattern of \( T_o \), with respect to the frequency seems to be much too sharp, compared to the expected moderate frequency dependence of the thermal diffusion length (proportional to the reciprocal of its square root). For example, from Figure 5, \( T_o \) is effectively damped at about 200 Hz, while from the specific thermal diffusion length for water at this frequency (28 \( \mu m \)) and the average light penetration length (around 30 \( \mu m \)) one expects an attenuation of the signal by about 1/e, i.e. to 30%. It is clear that one has to consider various other factors which contribute to greater damping of the surface-reaching photothermal signal. For example, there may be further resistances to thermal diffusion at the contact regions between cells. This may have bearing on the question why \( T_o \) increases in amplitude in dehydrated leaves, as drought is known to cause changes in the internal structure (12) and the leaf thickness (18). We observed extensive leaf shrinkage and considerable reduction of extracellular air spaces during drying of tobacco leaf discs (data not shown), probably resulting in better cell to cell contact and increase of the probability that the thermal waves will reach the leaf surface.

Clearly the problem of photoacoustic signal generation in leaves is quite complex. It should be particularly emphasized in this context that the simple approach of Rosencwaig and Gersho (see Ref. 23), applicable to matter in bulk, cannot be applied to the leaf as a whole, which unfortunately was done in several cases.

The most important observation however is that at high modulation frequencies there was no change in the amplitude of the photothermal signal between stressed and nonstressed leaves. At these frequencies, only \( T_n \) remains which presumably follows the same diffusion path as \( O_2 \) (from the chloroplasts to the mesophyll cell surface). This path is very small compared to the thermal diffusion length and only very insignificant attenuation is expected.

This estimation for the light penetration is extrapolated from the work of Terashima and Saeki (25). At a Chl surface density of 30 \( \mu g/cm^2 \) and at 680 nm, there is an attenuation to the extent of 1/e of the light intensity at 40 \( \mu m \) below the surface. In our case where the Chl surface density is higher (around 40 \( \mu g/cm^2 \)), the attenuation of the light should be correspondingly stronger by a factor 4/3.

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**FIG. 5.** The amplitude of the photothermal signal \( A_{PT} \) versus the reciprocal of the modulation frequency in a control leaf (\( \Delta \)) and in leaves which were water-stressed for 2 (\( \bullet \)) or 4 h (\( \bigcirc \)). Modulated light at 650 nm: 11 W m\(^{-2} \). Saturating light: 400 W m\(^{-2} \). Inset, an expansion of the high frequency region, showing equivalent results for all samples.
phosphorylation, it was invariant, it was found to be a significant rather than total photothermal signal. Since \( T \) was invariant, it was found convenient to express the quantum yield by dividing the \( O_2 \) evolution signals (in both stressed and control leaves) by the photothermal signals of the control (mostly \( T \)), obtained at the same frequency.

**Effect of Leaf Dehydration on its Photochemical Activities.**

Taking the apparent \( O_2 \) evolution quantum yield to be expressed by the ratio of \( A_{ox} \) to \( A_{pt} \) of the control, the results of Figure 2 are straightforward in showing a considerable reduction of the quantum yield with the water stress. Figure 6 gives a complete account of the dependence of \( A_{ox}/A_{pt} \) on the time of stress which shows a decline of up to 15% of the control. Concomitantly, the quantum yield of photochemical energy storage diminished even much more strongly (Fig. 7). The drastic effects of the water stress were completely reversed when the dehydrated leaf discs were transferred to water. Figure 6 shows also that, upon rehydration, \( O_2 \) evolution returned very quickly (within less than 2 h) to the original level even for a very severe stress. A similar fast recovery was also observed for the photochemical energy storage (Fig. 7).

One must consider, however, that morphological changes caused by the stress would also affect the apparent \( O_2 \) evolution signal by modification of the diffusion path of \( O_2 \). Following the approach of Poulet et al. (22), we plotted the logarithm of the \( A_{ox} \) to \( A_{pt} \) ratio as a function of the square root of the modulation frequency for water-stressed and non-stressed leaf discs (Fig. 8). At a sufficiently low frequency range, such a plot is linear, with a predicted slope of

\[
\sqrt{\pi} \left( \frac{1}{\sqrt{D_{ox}}} - \frac{1}{\sqrt{D_{pt}}} \right)
\]

where \( l \) is the average diffusion path length of heat and \( O_2 \) from the chloroplasts to the boundary of the cell, and \( D_{ox} \) and \( D_{pt} \) are, respectively, the diffusion coefficient of \( O_2 \) and the thermal diffusivity in the aqueous medium of the cell (22). (Actually \( 1/\sqrt{D_{pt}} \) is quite small and might be neglected). The slope of the plot was significantly modified during the first period (about 2 h) of the stress but remained invariant later. Two opposing effects are to be considered: (a) a decrease in the \( O_2 \) diffusion path, \( l \), due to cell and chloroplast shrinkage, affecting a smaller slope; (b) an increase in the interior cell viscosity and hence a decrease of the \( O_2 \) diffusion coefficient tending to increase the slope. The steeper slope observed in stressed tobacco leaves indicates that the fast effect is much more important.

We repeated the above experiments to compare with other plant species (Fig. 9). In all the examined species, the relative water content of the leaves was reduced to around 40% and \( A_{ox} \) was drastically reduced. However, the drought-induced change in the slope of the linear plot of \( \ln A_{ox}/A_{pt} \) versus the square root of the frequency was highly dependent on the nature of the leaves. In the thin leaves of Phaseolus vulgaris, the absolute magnitude of the slope was noticeably increased, as in tobacco. On the other hand, for the thick leaves of Casimiroa edulis, the slope remained unchanged, and in Passiflora edulis leaves there was even a net decrease in the absolute magnitude of the slope, indicating that the change in the diffusion distance was more important there.

The above results show that one has to consider that other factors besides the inhibition of photosynthetic activity are involved in the photoacoustic signal changes. To eliminate these additional factors, one should use the extrapolated intercept of the linear segment shown in the plots of Figures 8 and 9. The extrapolated value is a better relative measure of the \( O_2 \) evolution quantum yield than any particular value of \( A_{ox}/A_{pt} \) because the dependence of the signal on the diffusion parameters and anatomical characteristics of the leaf tissues is then eliminated. Table I summarizes the extrapolated value of \( A_{ox}/A_{pt} \) in tobacco and demonstrates that the true degree of inhibition of \( O_2 \) evolution is less severe than the apparent inhibition as measured at 24 Hz (Fig. 6), but nevertheless progressively increases with the time of desiccation, in contrast to the effect on the slope, which is already terminated after about 2 h.

Figure 10 shows the fast recovery from water stress as is reflected in the plots \( \ln A_{ox}/A_{pt} \) as a function of the square-root of the frequency, at various recovery times. Well watered previously dehydrated leaves demonstrate complete recovery of both

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**Fig. 6.** Relative quantum yield for \( O_2 \) evolution from tobacco leaf discs exposed to dehydration in air. At the times indicated by the arrows, leaf discs were transferred back to water for recovery. Other conditions as in Figure 2.

**Fig. 7.** Photochemical energy storage of tobacco leaf discs submitted to dehydration in air, as a function of time. At the time indicated by the arrow, leaf discs were transferred to water. Other conditions as in Figure 2.
photosynthetic activity and diffusion parameters, as exhibited by the identical slope and zero frequency extrapolation as an untreated leaf. Recovery was observed even in severely stressed discs in which an 80% inhibition of quantum yield for O₂ evolution was obtained.

**DISCUSSION**

The results reported here confirm in general the results of Boyer *et al.* in sunflower leaves, who showed inhibition of

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**Table 1. Relative Quantum Yields (in Percent of Control), for Tobacco Leaf Discs Exposed to Dehydration in Air**

The quantum yield is determined from the extrapolation to zero frequency of the plot of the logarithm of the A_{ox} to A_{pt} ratio versus the square root of the modulation frequency, f (data of Fig. 8). O₂ diffusion is expressed by the slope of this plot.

<table>
<thead>
<tr>
<th>Time of Dehydration</th>
<th>Extrapolated Value of A_{ox}/A_{pt}</th>
<th>Slope of ln A_{ox}/A_{pt} versus ( \sqrt{f} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (control)</td>
<td>100</td>
<td>-0.115</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>-0.190</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>-0.210</td>
</tr>
<tr>
<td>5.5</td>
<td>31</td>
<td>-0.220</td>
</tr>
</tbody>
</table>

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**Fig. 8.** Logarithm of the ratio of A_{ox} to A_{pt} versus the square root of the modulation frequency for tobacco leaf discs submitted to dehydration (2 h [ ], 4 h [ ], 5.5 h [ ], control [ ]). Modulated light at 680 nm: 16 W m⁻². Saturating light: 450 W m⁻².

**Fig. 9.** Frequency dependence of the relative quantum yield for O₂ evolution in three different plant species. *P. vulgaris* (A), *C. edulis* (B), and *P. edulis* (C). Nondesiccated leaf discs ( ), leaf discs dehydrated for 4 h ( ). Modulated light at 650 nm: 19 W m⁻². Saturating light: 500 W m⁻².

**Fig. 10.** Effect of rehydration on the frequency dependence of the quantum yield for O₂ evolution from water-stressed young (around 18 cm long) tobacco leaf discs. Discs dehydrated for 2 ( ) and 4.5 h ( ) were rehydrated for 2 h ( ) and O₂, respectively. Nondesiccated discs ( ). Other conditions as in Figure 6.

One of the outcomes of this work is the demonstration of the value of photoacoustic studies and their manipulation for monitoring drought injury to the photosynthetic apparatus. In an intermediate stress situation, primary photochemistry, enzymic activity, CO₂ diffusion and leaf morphology are contributing to the limitation of photosynthesis. It is difficult to separate these limiting factors, particularly in a quantitative way, with methods that preserve leaf integrity. The photoacoustic method, to some extent, provides such a means: the modulated O₂ evolution expressed by the corresponding acoustic wave is insensitive to CO₂ diffusion rate from the outside, as it is formed inside the leaf, presumably at a CO₂ compensation point, and therefore independent of the state of the stomata. Moreover, photoacoustic measurements are related to gross photosynthetic O₂ evolution, eliminating the need to correct for the effects of drought on either light or dark respiration. Another advantage of the photoacoustic method is the possibility of fast monitoring (time...
resolution about 1 s). The potential for field studies and rapid screening of drought tolerant plants is now being investigated.

The observed rapid recovery from the stress points out that there is probably no extensive degradation of the thylakoid membranes. Such a case probably requires a longer time for de novo synthesis and organization. More likely one deals with reversible changes of conformation and rearrangement of the functional membrane proteins. In the next article of this series we show, by combining also fluorescence measurements, that a possible site for the rapid water stress effect is in the electron donation side of PSII, probably close to the O₂ evolution site itself. In addition, Fellows and Boyer (8) pointed out that the drought-induced inhibition of photosynthesis correlated well with changes in the thickness of the lamellar membranes of the thylakoids, and it has been suggested (29) that the molecular basis of these changes stems partly from changes in the conformation of the chloroplast coupling factor CF₁. The above conclusions are corroborated also by the changes in energy storage (by measurements of the PL) observed in this study. At the frequency range used here (around 400 Hz), the monitored photochemical energy storage is related to a time interval of about 0.4 ms after the primary photact (14). In general, the photochemical energy storage was inhibited much more strongly than O₂ evolution, suggesting that perhaps the photophosphorylation activity which is a major energy transducing reaction was rapidly lost. With regard to this explanation, it must be noted also that O₂ evolution need not be coupled to CO₂ fixation, which stores energy but may be either uncoupled or coupled to a less energetically demanding redox reaction, e.g. O₂ reduction through the oxygenase system.

It must be kept in mind that the results reported here are of relevance to rapid water stress (hours) in detached leaves. Separate work was dedicated to water stress of whole plants (days), which points to a much more complex pattern.

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LITERATURE CITED