Photosynthesis Research **24:** 63–73, 1990.

Regular paper

Photothermal beam deflection: a new method for *in vivo* measurements of thermal energy dissipation and photochemical energy conversion in intact leaves

Michel Havaux[1], Lucie Lorrain & Roger M. Leblanc
Centre de Recherche en Photobiophysique, Université de Québec à Trois-Rivières, Trois-Rivières (Québec), G9A 5H7, Canada; [1]Present address and address for reprint requests: Dr M. Havaux, Laboratoire de Physiologie végétale (CP 169), Université Libre de Bruxelles, 28 avenue Paul Heger, B-1050 Bruxelles, Belgium

Received 23 June 1989; accepted 20 September 1989

Key words: Heat emission, mirage effect, photoacoustics, photothermal deflection spectroscopy

Abstract

A novel photosynthetic technique, photothermal deflection spectroscopy, is presented which is based on the 'mirage effect' and allows the rapid measurement of thermal deactivation of excited pigments in leaf samples placed in an open cell. Modulated heat emission from leaves illuminated with intensity-modulated light was measured via the detection of the periodic deflection of a laser beam parallel to the sample surface. Photothermal deflection signals can be monitored *in vivo* in leaves placed in various, liquid or gaseous, environments with a satisfactory signal-to-noise ratio close to 60–80 (in distilled water) at low modulation frequencies (below 50 Hz). Using this new and simple photothermal method, it was possible to easily obtain useful information on the leaf photochemical activity and its light-saturation characteristics under normal or stress conditions, suggesting that *in vivo* deflection signals could be used for assaying the photosynthetic state of health of crop plants. The beam deflection method presented in this paper appears to be a potentially useful photosynthetic tool complementary to the related photoacoustic technique.

Abbreviations; DCMU – dichlorophenyldimethylurea, PD – photothermal deflection, PL – photochemical energy storage, S/N ratio – signal-to-noise ratio

Introduction

Light energy absorbed by photosynthetic pigments in green plants is only partially converted into chemical energy by the photosynthetic processes. A substantial part of the absorbed excitation energy is indeed dissipated as heat and, to a lesser extent, as light (predominantly chlorophyll fluorescence which represents less than 5% of the absorbed light). As the different ways of pigment deexcitation are interdependently connected, important information concerning the efficiency of photon utilization in photosynthesis can be derived from measurements of heat and light emissions. Although *in vivo* chlorophyll fluorescence has become a widely used tool in photosynthesis research (see, for example, review by Krause and Weis 1984), relatively much less studies have been devoted to the thermal energy dissipation mode. However, the rediscovery of the photoacoustic technique and its recent application to the field of photosynthesis have brought new perspectives in this field (for review, see Braslavsky 1986). Most of the photoacoustic systems use tightly sealed microphone/gas cells in which modulated heat emission from an absorbing sample illuminated with intensity-modulated light is converted to pressure waves in the surrounding gaseous phase in contact with the sample. These

pressure waves are sensed by a sensitive microphone. In the case of O_2-evolving photosynthetic systems, the interpretation of the *in vivo* photoacoustic signals is complicated by the fact that there is an additional component related to photosynthetic O_2 evolution which, at sufficiently low modulation frequencies, follows the light intensity modulation (Bults et al. 1982, Poulet et al. 1983). Using adequate methodologies, photoacoustic spectroscopy has proved to be a useful tool to probe several aspects of photosynthesis *in vivo* and in *vitro* (Carpentier et al. 1983, Buschmann and Prehn 1983, Canaani and Malkin 1984, Havaux et al. 1986, Canaani et al. 1988, Havaux 1989).

Boccara and co-workers have introduced a different means of carrying out photothermal measurements which is based on the 'mirage effect' (Boccara et al. 1980a and b, Jackson et al. 1981). In this method, temperature-induced changes in the index of refraction of the fluid in contact with the sample are sensed through the deflection of a laser beam placed close to and parallel to the sample surface. This photothermal deflection (PD) technique has been successfully used to characterize some physical and optical properties (absorption spectra, thermal diffusivity, ...) of various (solid, liquid or gaseous) materials (Boccara et al. 1980 a and b, Jackson and Amer 1982, Rousset and Lepoutre 1982, Decker et al. 1987). Here, for the first time, the beam deflection method has been applied to the *in vivo* measurement of thermal energy dissipation and photochemical energy storage in intact plant leaves. A brief account of our PD system has been presented in a previous short report (Havaux et al. 1989). The present paper is a comprehensive extension of our preliminary work, with a detailed study of the *in vivo* PD signals and their variation under various conditions (changes in light intensities, heat stress, herbicides, . . .).

Materials and methods

Plant material

Leaves of pea (*Pisum sativum* L.), sugar maple (*Acer saccharum* Marsh.) and durum wheat (*Triticum durum* Desf., cv. Durelle and Mohamed Ben Bachir) were used in this study. Plants were grown in a growth chamber under controlled temperature, relative air humidity and light conditions.

Heat stress was induced by placing the leaf samples in a thermostatized water bath (Haake 000-5724) as outlined by Havaux et al. (1987).

Experimental set-up

Our laboratory-constructed PD system is schematically depicted in Figure 1. A detailed description of the equipment will be presented in a separated technical paper. Small rectangular pieces of leaves (around 0.5 cm × 1 cm were stuck on a small glass slide (0.9 cm × 2.5 cm) which was mounted on a translational displacement system allowing the precise alignment of the sample with respect to a laser beam propagating along its surface. The laser beam was supplied by a lower power (4 mW) He–Ne laser (Uniphase). The intensity of the probe laser beam was reduced by using a 2%-transmittance red filter. The beam, focused above the sample using a lens, had a diameter of around 40 μm and was placed close to the sample, at a distance of around 20 μm from its surface. Although PD measurements can be performed in both liquid and gaseous environments, all the experiments reported in the present study were done with the PD cell filled with filtered distilled water (around 50 cm^3). A crucial point of the technique is the fact that the sample surface has to be very flat. To this end, the leaf pieces were slightly stretched on the glass slide. The leaf was

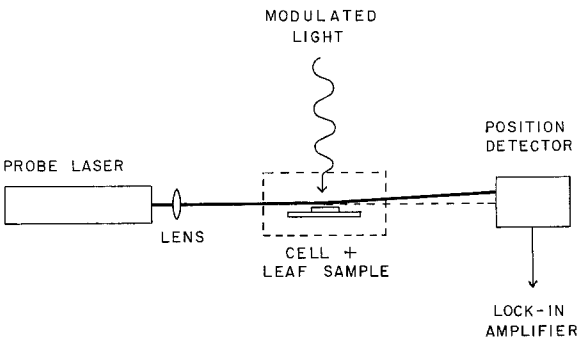

Fig. 1. Scheme of the photothermal deflection (PD) system used to measure thermal energy dissipation in intact leaves. Absorption of intensity-modulated light by a leaf results in the production of heat waves which create a refractive index gradient in the fluid above the sample. A laser beam propagating along the leaf surface is then periodically deflected ('mirage effect'). The beam deflection is measured by a position sensor.

illuminated with a modulated light provided by a 1000-W xenon arc lamp (Schoeffel Instrument Corp.) combined with a monochromator (Schoeffel) and a mechanical chopper (Scitec Instruments). The modulated heat emission from the leaf, resulting from the absorption of the modulated light, generated an index-of-refraction gradient which periodically deflected the probe laser beam. The beam deflection was monitored by the use of a position sensor (Optikon SD 380-23-21-051) shielded with a 10%-transmittance neutral density filter. The signals from the detector were fed into a lock-in amplifier (Ithaco, model 393) coupled to a chart recorder. The background (actinic) light, supplied by a d.c. operating 150-W halogen lamp, was transmitted onto the leaf sample using a fiber-optic light guide. The maximal intensity of this background light was 270 $W m^{-2}$. Light intensities were measured using a calibrated lightmeter (United Detector Technology, model 1223). Signal-to-noise ratios (S/N) were calculated according to Ducharme et al. (1979): the amplitude of the PD signal (measured with a time constant of 1.25 s) was divided by the fifth of the peak-to-peak fluctuations of the signal recorded with a time constant of 125 ms.

PD signals were compared to photoacoustic signals. *In vivo* photoacoustic measurements were performed using a home-made photoacoustic spectrometer which has been described in detail elsewhere (Ducharme et al. 1979, Carpentier et al. 1983). The modulated and non-modulated light sources were the same than those used for the PD measurements.

Results and discussion

The beam deflection signal

Figure 2 shows a typical PD signal generated by a pea leaf illuminated with a 680-nm light modulated at 18 Hz. It can be seen that, upon illumination, the *in vivo* deflection signal rapidly rose to a constant and stable level with a satisfactory signal-to-noise ratio (S/N) which was calculated to be around 80. This latter value is slightly lower than the S/N ratios of *in vivo* photoacoustic signals obtained in the same frequency range with our photoacoustic spectrometer (*ca.* 100). As a comparison, the PD signals obtained at 18 Hz with carbon black had a

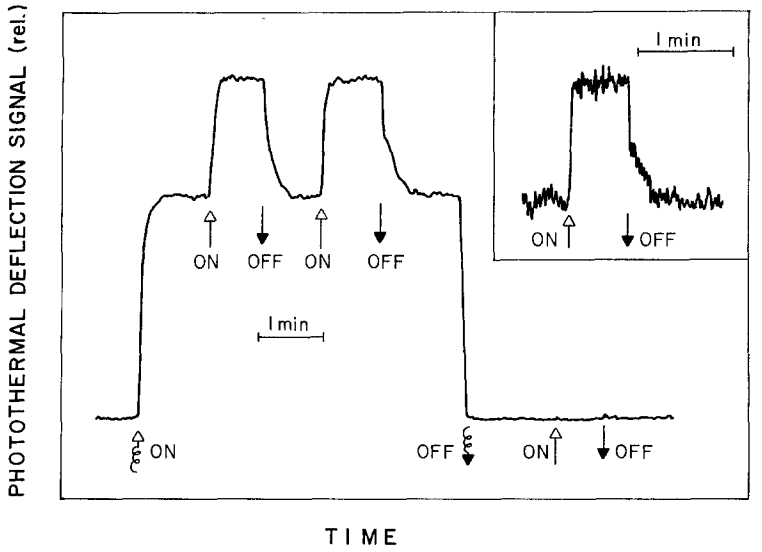

Fig. 2. Typical PD signal measured in pea leaves illuminated with a 680-nm light (14 $W m^{-2}$) modulated at 18 Hz. The time constant of the lock-in amplifier was 1.25 s. Saturation of the photochemistry with a strong, non-modulated, white light (270 $W m^{-2}$) resulted in an increase in the amplitude of the PD signal to its maximal level, allowing the determination of the photochemical energy storage (PL). PL is the percentage difference between the PD signal measured in the presence and in the absence of the saturating background light (SL); PL = (signal (+SL) − signal (−SL))/signal (+SL). No phase shift was observed upon application of the saturating background light. ⇞ and ⇟, modulated light on and off; ↑ and ↓, saturating background light on and off. Insert: the effect of the saturating white light was measured with a better time resolution (400 ms).

S/N ratio of around 800 (not shown). Qualitatively similar *in vivo* PD signals were obtained with leaves of a variety of plant species (including wheat, sugar maple and Virginia creeper) as well as in algae (*Dunaliella tertiolecta*) deposited on paper filter. The most important requirement for recording good signals is the necessity to use samples with a very flat surface in order to ensure an optimal alignment of the sample and the probe laser beam. Up to now, we were unsuccessful in measuring PD signals with a tolerable S/N ratio from rough (bean) or slightly pubescent leaves (maize). In Figure 2, the deflection of the laser beam was measured in a liquid medium (filtered distilled water). PD signals can also be easily monitored in air. However, as the temperature-induced changes of the index of refraction are noticeably smaller in air than in water, the amplitude of the *in vivo* PD signals measured under those gaseous conditions was markedly reduced, consequently decreasing the accuracy of the measurements: at a low frequency of 30 Hz, the S/N ratio in air was observed to be around half of that measured in water (not shown).

In order to get useful, physiologically relevant, information from the *in vivo* PD signals, a strong, photosynthetically saturating, non-modulated light (*ca.* 270 $W m^{-2}$) was added to the modulated measuring beam. Being non-modulated, this strong background light did not induce any measured deflection signal. When the background light was switched on, the PD signal increased to a higher level, with the phase angle of the signal remaining unchanged. This increased photothermal signal can be attributed to the saturation of the photochemistry and the resulting maximal dissipation of almost all the absorbed modulated light energy as heat; radiative energy dissipation (*i.e.* chlorophyll fluorescence) is very small in comparison to heat emission and can be neglected. By comparing the amplitude of the PD signal recorded in the presence of the saturating light (in other words, when photosynthesis was light-saturated) with that obtained in its absence, it is then possible to estimate the proportion of absorbed light energy which was stored in the intermediates of the photochemical processes. The amount of stored energy (in % of the maximal heat emission level) is denoted PL ('photochemical losses'). Although this term is somewhat confusing (indeed, it is not a measurement of loss from the point of view of the plant but rather it is the amount of usefully stored energy), it is however used in the present study in order to follow the nomenclature used in previous photoacoustic works (see, for example, Malkin and Cahen 1979, Malkin et al. 1981, Bults et al. 1982, Kanstad et al. 1983, Poulet et al. 1983). The above procedure employed to self-reference a photochemically active sample by light saturation was originally developed by Malkin and co-workers for the analysis of high-frequency photoacoustic signals of plant leaves (Bults et al. 1982, Kanstad et al. 1983). The insert of Figure 2 displayed the background light-induced changes in the PD signal amplitude measured with a much better time resolution (time constant of the lock-in = 400 ms). It can be seen that the background light saturates the photochemistry almost instantly whereas the return to the initial level after the background light was switched off was substantially slower, with two kinetically

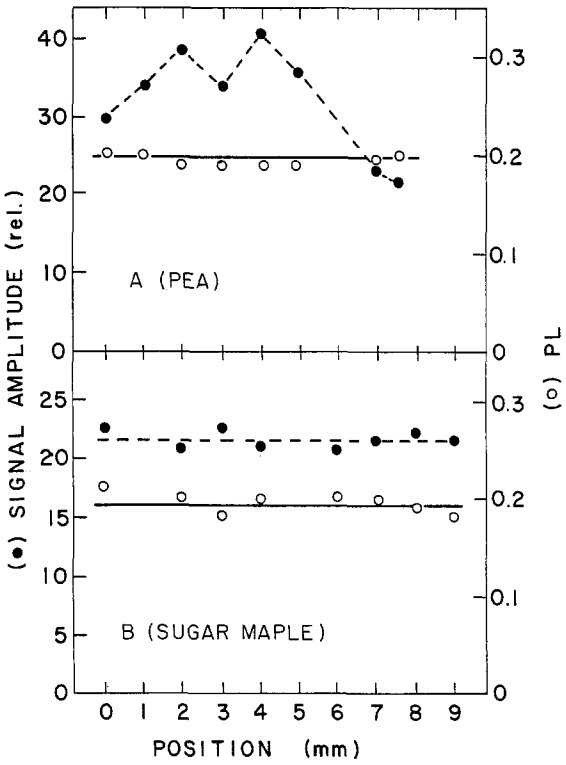

Fig. 3. Relative amplitude of the PD signals and PL values measured at different positions in A) a young pea leaf and B) a sugar maple leaf. The PD signal was first measured at position 0 and then at different distances (1 mm, 2 mm, ...) from that position by displacing transversally the sample with respect to the probe laser beam. Modulated light: 18 Hz, 14 $W m^{-2}$, 680 nm. Background light: 160 $W m^{-2}$.

different phases. There was first a fast decrease in the signal followed by a second, much slower component, the total recovery taking as long as around 25 seconds. Although we did not study this delay in detail, it seems reasonable to think that it is related to the pool size capacity of the rate-limiting step of the photosynthetic electron transport chain.

In order to examine the reproducibility of the *in vivo* measurements performed with our beam deflection system, we monitored thermal dissipation positions in a pea leaf (Fig. 3A). A marked spatial variation was observed in the relative amplitude of the PD signals. The reason for this high variability has probably to be found in the fact that the surface of a leaf is not perfectly flat and in consequence, the alignment of the laser beam with respect to the leaf surface can change when different parts of the leaf are investigated, leading to variations in the signal amplitude. It is also possible that the PD measurements are highly sensitive to local changes in pigment composition and absorption properties of the leaf, since the laser beam senses heat waves produced by extremely small leaf areas. These problems were however not observed in leaves of other species such as sugar maple for example (Fig. 3B). In contrast to the relative signal amplitude, the measured PL values in both pea and sugar maple leaves were remarkably constant (Fig. 3A and 3B), confirming the validity of the PD technique for the rapid estimation of leaf photochemical activity (through the PL) and also the necessity to self-reference the samples using the saturation method described above.

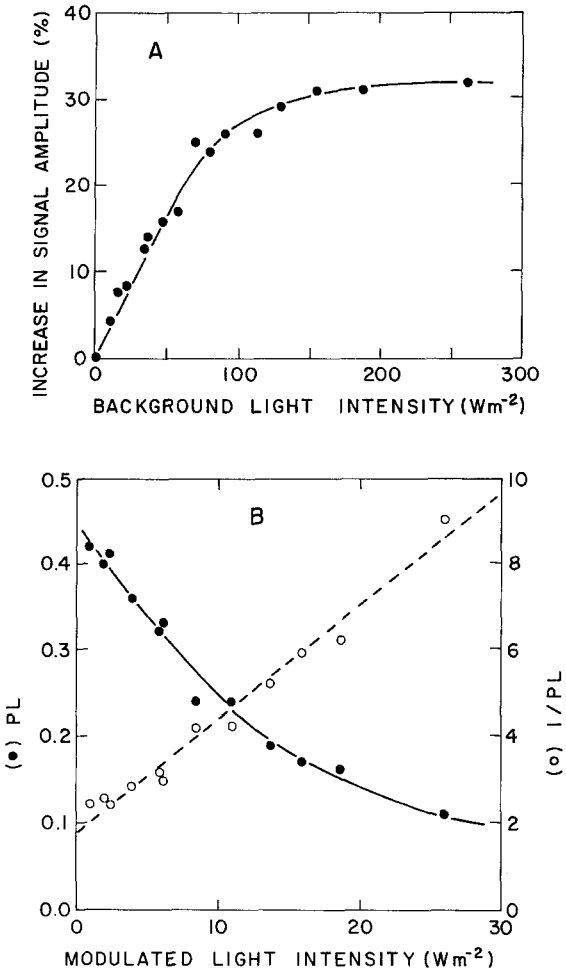

Fig. 4. Effects of the intensity of the background and modulated lights on the PL measured in pea leaves with the PD technique. A) Increase in the PD signal induced by different intensities of the background white light. The intensity of the modulated light (680 nm, 18 Hz) was 14 W m^{-2}. B) Pl and 1/PL measured at different intensities of the modulated measuring light (680 nm, 35 Hz). The intensity of the background light was 160 W m^{-2}. Although the results presented in this figure are data from single leaves, the experiments were performed three times in order to ensure repeatibility.

Effects of light intensity

By varying the intensity of the modulated and non-modulated lights, the saturation profile of leaves can be investigated. In Figure 4A, photochemistry was progressively saturated (as reflected by the increased heat emission yield) by adding increasing amount of background light. A typical saturation curve was obtained, with a linear relationship in the low light intensities range (0–80 W m^{-2}) and a plateau at saturating intensities above around 150 W m^{-2}. The data of Figure 4A were obtained using a modulated measuring light of relatively high intensity (14 W m^{-2}) which can cause partial (steady-state) closure of the reaction centers, thus resulting in a decrease in the apparent PL. This was checked by examining the effects of the *modulated* light intensity on the magnitude of PL (Fig. 4B). PL was shown to be strongly dependent on the intensity of the modulated light. In agreement with theoretical models relating steady-state rate of electron transfer and light intensity (Farquhar and von Caemmerer 1981), the two parameters were related by a hyperbolic relationship. When the reciprocal

of PL was plotted as a function of the modulated light intensity, a linear relationship was obtained. The extrapolation of 1/PL to a modulated light intensity of zero gave an estimation of the *maximal* efficiency of photochemical energy storage. In the case of pea leaves (Fig. 4B), a maximum of around 55% of the absorbed light energy appeared to be stored as products of photosynthesis. Such a high value is somewhat surprising. It has to be kept in mind that the apparent extent of energy storage is dependent on the modulation frequency and is supposed to correspond to intermediates found at various times for the photoact (Malkin and Cahen 1979). At the low frequencies used in the experiments shown in Figure 4, the monitored energy storage is related to a time interval in the ms range (reciprocal of the angular frequency). We cannot exclude the possibility that exothermic dark photosynthetic reactions occuring on the ms time scale also contribute to the signal. This possibility points out the fact that the measured PL has to be considered as a qualitative indicator of photosynthesis rather than an absolute measure of the efficiency of photochemical energy storage.

The measured PL was observed to be dependent on the physiological state of the leaf. For example, the extrapolated PL was substantially lower in young pea leaves as compared to mature leaves (not shown) and was very sensitive to stress conditions (cf. below). The above results (Fig. 4B) also imply that only very low intensities of the modulated light can provide a proper measurement of the photochemical energy storage.

Effects of modulation frequency

Figure 5 shows that the amplitude of the *in vivo* PD signals drastically decreased with increasing frequency of the modulated light. In contrast, the 'noise' of the signals was considerably less dependent on the frequency, so that the S/N ratio also decreased spectacularly, almost in parallel with the signal amplitude. In consequence, useful PD measurements with a tolerable S/N ratio were restricted to a rather limited frequency range from 10 Hz to around 50 Hz. In the experiment reported in Figure 5, we were able, however, to calculate PL with a satisfactory precision over a somewhat larger range of frequencies (up to 120 Hz). We observed that PL did not vary significantly with the frequency (within the limits of the experimental error, $\pm 10\%$). In Table 1, we compared the extent of PL measured at a low frequency using the PD method to the PL values derived time photoacoustic measurements performed in the same leaves at higher frequencies (> 400 Hz) at which the O_2 evolution-related photoacoustic component is completely damped out

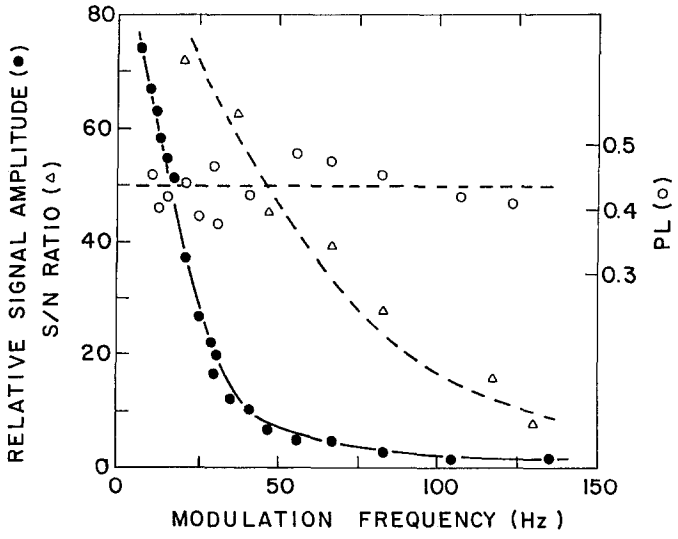

Fig. 5. Effects of the frequency of the modulated exciting light (680 nm, 8.5 $W m^{-2}$) on the relative amplitude of the PD signals, the S/N ratio and the PL measured in pea leaves. Background light: 160 $W m^{-2}$.

Table 1. Comparison of the PL values measured at low modulation frequency with the PD method and at high frequencies with the photoacoustic method. Modulated light: 680 nm, 14 $W m^{-2}$; background light: 270 $W m^{-2}$. Data are mean values ± standard deviation

	PL	
Modulation frequency (Hz)	PD method	Photoacoustic method
25	0.28 ± 0.08	--------
403	--------	0.32 ± 0.04
513	--------	0.33 ± 0.02
603	--------	0.33 ± 0.03

and the photoacoustic signal is purely photothermal (Bults et al. 1982, Poulet et al. 1983). The data indicated that those two related photothermal techniques gave roughly similar results. The fact that PL values measured at high and low frequencies are directly comparable is an interesting result which gives additional support to the vectorial method developed by Poulet et al. (1983) for the calculation of O_2 evolution from low-frequency photoacoustic measurements. Indeed, in this method, the photoacoustically monitored PL is implicitly assumed not to vary with frequency so that it can be used directly in the analysis of low-frequency photoacoustic data. On the other hand, the agreement between beam deflection and photoacoustic data suggested that the extent of energy storage in early and later intermediates in the photosynthetic reaction chain is identical. At 25 Hz, energy storage is supposed to represent the reduced form of an electron acceptor having a lifetime of around 6 ms whereas PL measured at 600 Hz reflects energy storage in intermediates closer to the photoexcitation, with a shorter lifetime of around 0.25 ms (Malkin and Cahen 1979). Then, very little energy loss appeared to occur between about 6 and 0.25 ms. Constancy of PL with modulation frequency has been previously reported in leaves of other plant species using photothermal radiometry (Kanstad et al. 1983) but not in chloroplasts or algal cells (Lasser-Ross et al. 1980, Malkin et al. 1981). In these latter photosynthetic systems, photoacoustically monitored PL was shown to noticeably increase with increasing frequencies. As far as the generation of acoustic waves is concerned, chloroplasts are systems much simpler than leaves whose internal structure can strongly influence the *in vivo* photoacoustic signals (Havaux et al. 1986, Havaux 1989). In particular, according to Rosencwaig (1980), when frequency increases, the signal comes from less and less deep sections of the leaf, which can possibly have different photosynthetic properties (Buschmann and Prehn 1983) and hence different PL. At very high frequencies, the contribution of the epidermis with little photosynthesis may be predominant. This factor possibly interfered with *in vivo* measurements of PL, maybe explaining the discrepancy between the frequency responses of PL *in vivo* and *in vitro*.

Effects of electron transfer inhibition

As PD measurements are performed in water, they seem very suitable for the study *in situ* of the effects of chemicals such as herbicides or pollutants. In Figure 6, the herbicide DCMU was used in order to examine the repercussions of inhibited photosynthetic electron flow on the *in vivo* PD signals generated by pea leaves. Different amounts of DCMU were injected in the PD cell and, after stirring the solution, PL was monitored in the leaves. Following a delay of around 10 min (which probably reflected the uptake of the herbicide by the leaf tissues), the apparent PL progressively decreased. The time course of the PL inhibition strongly depended on the final DCMU concentration in the cell. For example, in leaves incubated for 20 minutes in DCMU 10^{-4} M, the saturating background light had no visible effect on the PD signal (in other words, PL = 0), reflecting the complete blockage of the photosynthetic electron flow. These data illustrate the potential utility of the PD method for studies of herbicide effects and also confirm that the PD measurements presented above are effectively related to photosynthesis.

Another way to inhibit electron transport (in photosystem II) is to subject leaves to heat stress (Quinn and Williams 1985). In Figure 7, PL was monitored at room temperature in pea leaves previously heated for a short time (15 minutes) at different temperatures ranging from 36°C to 47°C. A sharp temperature dependence of the photochemical energy storage was observed, with the temperature for 50% inhibition being around 41.5°C. Above 45°C, the d.c. light had no apparent effect on the PD signal. The time course of the heat-induced reduction of PL is shown in the insert

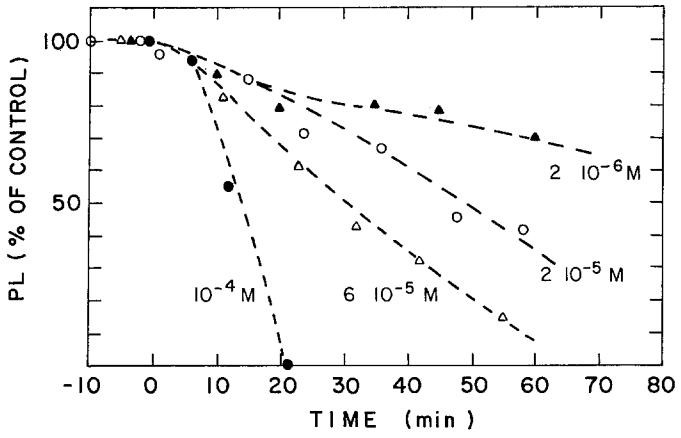

Fig. 6. Effects of the herbicide DCMU on the PL measured with the PD method in pea leaves. At time 0, DCMU was injected in the PD cell so that the final concentration was $2\ 10^{-6}$ M, $2\ 10^{-5}$ M, $6\ 10^{-5}$ M or 10^{-4} M (in less than 2% alcohol). Modulated light: 680 nm, 15 Hz, 14 W m^{-2}; background light: 270 W m^{-2}.

of Figure 7, indicating a rapid and linear decrease in the photochemical activity with increasing length of the stress treatment. The temperature for complete abolition of PL was shown to be related to the degree of heat resistance of the plants. In Table 2, two different durum wheat varieties, with known and contrasting heat tolerance characteristics, were compared. Exposure of leaves to a temperature of 40.5°C for 15 minutes resulted in a complete inhibition of PL in the heat-sensitive French variety (Durelle) but not in the resistant Algerian genotype (Mohamed Ben Bachir) in which more than 50% of the apparent energy storage was preserved. From this result, it appears that it would be possible to use PD measurements as the basis for the development of rapid screening methods for stress toler-

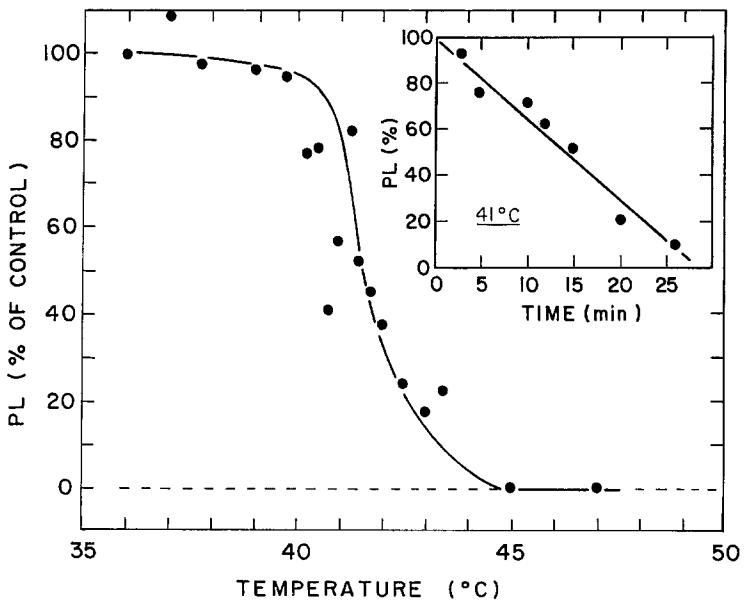

Fig. 7. Effects of a short heat stress on the PL measured in pea leaves with the PD technique. After incubation of leaf samples at different temperatures ranging from 36°C to 47°C for 15 minutes, PL was measured at room temperature. PL was expressed as a percentage of the value measured in the same sample before the heat treatment. Insert: time course of the inhibition of PL in pea leaves incubated at 41°C. Modulated light: 28 Hz, 14 W m^{-2}, 680 nm. Background light: 160 W m^{-2}.

Table 2. Effect of a short heat stress (15 minutes at 40.5 °C) on the PL measured at room temperature with the PD technique in leaves of two durum wheat varieties (Durelle (heat sensitive) and Mohamed Ben Bachir (heat resistant)). Modulated light: 14 W m^{-2}, 680 nm, 18 Hz; Background light: 160 W m^{-2}. Data are mean values ± standard deviation

	PL	
	Mohamed Ben Bachir	Durelle
Control	0.290 ± 0.06	0.154 ± 0.07
Heat stressed	0.157 ± 0.09	0
% inhibition	46	100

ance in crop plants. The method could also be useful for assaying the photosynthetic state of health of a crop.

In Figure 8, the dependence of 1/PL on the intensity of the modulated monitoring beam was examined in control and heat stressed pea leaves. The results indicated that the heat-induced reduction of PL was caused by two simultaneous effects:

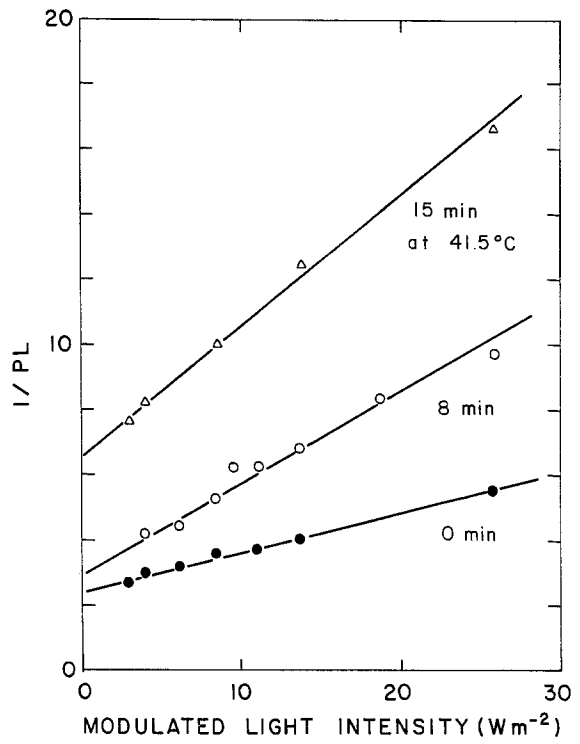

Fig. 8. Plot of the reciprocal of PL as a function of the intensity of the modulated light in pea leaves preincubated for 0, 8 and 15 min at 41.5°C. Measurements were done at room temperature. Modulated light: 680 nm, 28 Hz. Background light: 160 W m^{-2}.

a decreased photochemical efficiency and a modification of the light-saturation characteristics of the photochemistry. After 8 minutes at 41.5°C, the 'true' energy storage (PL extrapolated to an intensity of zero) was slightly reduced (around −20%). Using a modulated light intensity of 20 W m^{-2}, we would measure an apparent decrease of 45%. This much higher apparent inhibition was due to a marked change in the slope of the linear plot of 1/PL *vs* light intensity, indicating a faster light-saturation of the photochemistry in stressed chloroplasts. More severe stress conditions (15 min at 41.5°C) induced a further decrease in PL (around 60%-inhibition at all light intensities), with a small additional change in the slope of the linear plot.

Conclusion

The data presented above clearly demonstrate the possibility of monitoring heat emission in leaves with a simple PD technique. The *in vivo* PD signals contain relevant information on the photochemical energy conversion which can be easily determined using an 'internal' reference conveniently obtained by saturation of the photochemical processes with a strong continuous light (Bults et al. 1982). Non-stressed, well developed, leaves of the few plants species studied so far with our method were characterized by an apparent energy storage approaching around 55% at very low light intensities (see, for example, Figures 4B and 7). Following the example of the photoacoustic technique, PD spectroscopy could provide a new, powerful photosynthetic tool with various potential applications in plant physiological/biochemical work, in particular in the study of the regulation of leaf photosynthesis by the physico-chemical environment as illustrated in Figures 7 and 8.

PD and photoacoustic spectroscopies are two related photothermal techniques. Although both of them have their own merit, PD spectroscopy has several obvious advantages over the photoacoustic method. First, in contrast to photoacoustics, PD measurements do not require an hermetically closed cell, allowing to work in less artificial gaseous conditions. Due to the small cell volume, *in vivo* photoacoustic measurements are indeed performed at a very low CO_2 concentration, probably close to the CO_2 compensation point, since CO_2

should be rapidly depleted upon illumination. The environment of the sample in the PD cell can be more easily modified as compared to the photoacoustic method, allowing photosynthetic inhibitors to be studied *in situ* (Fig. 6).

On the other hand, as gas-exchange phenomena do not interfere with the *in vivo* PD measurements, PL can be estimated at very low modulation frequencies at which the signal amplitude is high and the S/N ratio favorable (Fig. 5). Purely photothermal signals can be photoacoustically measured in leaves at high modulation frequencies only (above around 400 Hz), reducing the accuracy of the PL measurements. For example, the S/N ratio of our photoacoustic cell was around 5–10 (for a leaf sample) at frequencies close to 500 Hz. This value is much lower than the S/N ratio of the *in vivo* PD signals obtained at low frequencies (*ca.* 80). In this connection, Figure 4B shows that it was possible to measure PL in the very low light intensities range ($1–2\ W\ m^{-2}$) which, up to now, was very difficult to explore by high-frequency photoacoustic measurements. Under optimal conditions, we were also able to monitor *in vivo* PD signals with a fast time resolution (*ca.* 125 ms), suggesting that the technique could be adequate to follow rapid transitory phenomena such as, for example, those occurring during the induction of photosynthesis in dark-adapted samples. The PD technique provides then a useful photosynthetic tool complementary to the photoacoustic technique in particular at low modulation frequencies. This new technique has however some disadvantages: it requires very flat surface of the leaf which is often not possible to get and it samples a very small area in the leaf.

Our preliminary study (see Havaux et al. 1989) revealed that the spectral resolution of the PD technique is noticeably superior to that of photoacoustic spectroscopy. Our current work is centered on the use of the beam deflection method to probe the spectroscopic properties of various *in vivo* and *in vitro* photosynthetic systems.

Acknowledgments

We are particularly grateful to Alain Tessier for his help and useful suggestions concerning the electro-optics aspect of the instrument. Seeds of heat-resistant and -sensitive durum wheat varieties were kindly provided by Dr M. Ernez (ULB, Brussels). This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR). One of us (M.H.) gratefully acknowledges the U.Q.T.R. for a visiting professorship at the Centre de recherche en photobiophysique.

References

Boccara AC, Fournier D and Badoz J 1980a Thermo-optical spectroscopy: Detection by the 'mirage effect'. Appl Phys Lett 36: 130–132.

Boccara AC, Fournier D, Jackson W and Amer NM 1980b Sensitive photothermal deflection technique for measuring absorption in optically thin media. Optics Lett 5: 377–379.

Braslavsky SE 1986 Photoacoustic and photothermal methods applied to the study of radiationless deactivation processes in biological systems and in substances of biological interest. Photochem Photobiol 43: 667–675.

Bults G, Horwitz BA, Malkin S and Cahen D 1982 Photoacoustic measurements of photosynthetic activities in whole leaves. Photochemistry and gas exchange. Biochim Biophys Acta 679: 452–465.

Buschmann C and Prehn H 1983 *In vivo* photoacoustic spectra of *Raphanus* and *Tradescantia* leaves taken at different chopping frequencies of the excitation light. Photobiochem Photobiophys 5: 63–69.

Canaani O and Malkin S 1984 Distribution of light excitation in an intact leaf between the two photosystems of photosynthesis. Changes in absorption cross-section following state 1–state 2 transitions. Biochim Biophys Acta 766: 513–524.

Canaani O, Malkin S and Mauzerall D 1988 Pulsed photoacoustic detection of flash-induced oxygen evolution from intact leaves and its oscillations. Proc Natl Acad Sci USA 35: 4725–4729.

Carpentier R, LaRue B and Leblanc RM 1983 Photoacoustic spectroscopy of *Anacystis nidulans*. Arch Biochem Biophys 222: 403–415.

Decker F, Neuenschwander RT, Cesar CL and Penna AFS 1987 The mirage effect in electrochemistry. J Electroanal Chem 228: 481–486.

Ducharme D, Tessier A and Leblanc RM 1979 Design and characteristics of a cell for photoacoustic spectroscopy of condensed matter. Rev Sci Instrum 50: 1461–1462.

Farquhar GD and von Caemmerer S 1981 Electron transport limitations on the CO_2 assimilation rate of leaves: a model and some observations in *Phaseolus vulgaris* L. In: Akoyounoglou G (ed) Photosynthesis IV. Regulation and Carbon Metabolism, pp. 163–175. Balaban International Science Services, Philadelphia.

Havaux M, Canaani O and Malkin S 1986 Photosynthetic responses of leaves to water stress, expressed by photoacoustics and related methods. Plant Physiol 82: 827–839

Havaux M, Canaani O and Malkin S 1987 Oxygen uptake by

tobacco leaves after heat shock. Plant Cell Environ 10: 677–683.

Havaux M 1988 Photoacoustic characteristics of leaves of atrazine-resistant weed mutants. Photosynth Res 21: 51–59.

Havaux M, Lorrain L and Leblanc RM 1989 *In vivo* measurement of spectroscopic and photochemical properties of intact leaves using the 'mirage effect'. FEBS Lett 250: 395–399.

Jackson WB, Amer NM, Boccara AC and Fournier D 1981 Photothermal deflection spectroscopy and detection. Appl Optics 20: 1333–1344.

Jackson WB and Amer NM 1982 Direct measurement of gap-state absorption in hydrogenated amorphous silicon by photothermal deflection spectroscopy. Phys Rev 25: 5559–5562.

Kanstad SO, Cahen D and Malkin S 1983 Simultaneous detection of photosynthetic energy storage and oxygen evolution in leaves by photothermal radiometry and photoacoustics. Biochim Biophys Acta 722: 182–189.

Krause GH and Weis E 1984 Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth Res 5: 139–157.

Lasser-Ross N, Malkin S and Cahen D 1980 Photoacoustic detection of photosynthetic activities in isolated broken chloroplasts. Biochim Biophys Acta 593: 330–341.

Malkin S and Cahen 1979 Photoacoustic spectroscopy and radiant energy conversion: theory of the effect with special emphasis on photosynthesis. Photochem Photobiol 29: 803–813.

Malkin S, Lasser-Ross N, Bults G and Cahen D 1981 Photoacoustic spectroscopy in photosynthesis. In: Akoyounoglou G (ed) Photosynthesis III. Structure and Molecular Organisation of the Photosynthetic Apparatus, pp. 1031–1042. Balaban International Science Services, Philadelphia.

Poulet P, Cahen D and Malkin S 1983 Photoacoustic detection of photosynthetic oxygen evolution from leaves. Quantitative analysis by phase and amplitude measurements. Biochim Biophys Acta 724: 433–446.

Quinn PJ and Williams WP 1985 Environmentally induced changes in chloroplast membranes and their effects on photosynthetic function. In: Barber J and Baker NR (eds) Photosynthetic Mechanisms and the Environment, pp. 1–47. Elsevier, Amsterdam.

Rosencwaig A 1980 Photoacoustic spectroscopy. Ann Rev Biophys Bioeng 9: 31–54.

Rousset G and Lepoutre F 1982 Mesures de diffusivites thermiques pa lar methode photoacoustique et par l'effet mirage. Revue Phys Appl 17: 201–207.