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Effects of Light Quantity and Quality on the Decarboxylation of Malic Acid in Crassulacean Acid Metabolism Photosynthesis[1]

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ABSTRACT

The rate of malic acid consumption in the Crassulacean acid metabolism (CAM) plant *Kalanchoë daigremontiana* Hamet et Perrier was found to be more rapid than the rate of photosynthetic oxygen evolution under all levels of irradiation by white light. This accounts for the accumulation of carbon dioxide in CAM tissues in the light.

Action spectra of malate consumption and photosynthetic oxygen evolution in *Kalanchoë* were similar. Experiments using monochromatic photosynthetically active light in addition to a range of narrow waveband irradiations demonstrated that malic acid consumption in the experiments from which the action spectrum of acid consumption was constructed was not limited by the rate of photosynthesis. These data indicate that light involved in the promotion of malate consumption in CAM is absorbed by the same pigments that absorb the light which powers photosynthesis.

The storage as malic acid, of CO_2 acquired from the atmosphere during the night, and, in the following day, its release and photosynthetic utilization behind closed stomata comprise the essentials of CAM.

Although deacidification will occur after prolonged dark periods (10), it is clear that, under normal daylight regimes, light plays an important part in the process which results in the release of CO_2 from malic acid (deacidification) (10). The rate of deacidification has been shown to be irradiance-dependent (4, 5) and it has also been reported that far-red light is of special significance in stimulating the release of CO_2 from malic acid (7). The reversal of this effect by red light suggested the involvement of phytochrome as a light receptor although the reversal of stimulation by cessation of far-red light irradiation indicates that the phenomenon may not be the classic phytochrome response.

Phytochrome has also been implicated in what appears to be a separate aspect of the relationship between light and deacidification, that is the effect of light on the rhythmic variation of the capacity of CAM plants to accumulate and consume acid. In CAM, as in many other rhythmic phenomena, phytochrome appears to be involved in phase-setting (6, 9).

There can be no doubt that the influence of light in CAM is complex and incompletely understood. Accordingly, the present work, which involves a detailed investigation of the effects of light quantity and quality on deacidification and for comparison, photosynthesis, in a CAM plant, was undertaken.

MATERIALS AND METHODS

Plant Material. *Kalanchoë daigremontiana* Hamet et Perrier plants were grown from leaf propagules in Levington Potting Compost (Fisons Ltd., Bramford, Ipswich, United Kingdom) in a greenhouse (minimum temperature 15°C) under natural lighting conditions supplemented by mercury vapor lamps to extend the daylength to 16 h. Between 1 and 3 weeks before the plants were used, at about 6 months old and 40 cm in height, they were transferred to a 12-h day (07:00–19:00 h) under mercury vapor lamps (700 μmol m^{-2} s^{-1} 400–700 nm at the plant tops; day temperature 27 ± 2°C) and watering was reduced. The leaves typically contained 200 μeq total acid g^{-1} fresh weight at the end of the night period and 20 μeq total acid g^{-1} fresh weight at the end of the light period.

Sampling, Irradiation, and Extraction of Organic Acids. Plants were transferred to darkness at approximately 17:30 and kept in the dark overnight at 13 ± 2°C. Individual leaves were removed between 09:00 and 09:45 h, their petioles placed in water and illuminated for 5 h at 21 ± 2°C. Samples for estimation of organic acids were taken at 2, 3, 4, and 5 h with a corkborer (four discs of 3.5-mm diameter) and the holes were plugged by polyethylene inserts to reduce vapor loss from the leaves. The samples were weighed, frozen in liquid N_2, and incubated overnight in 1 ml 0.25% $HClO_4$ in water at room temperature. This extracts at least 98% malic acid in the tissue.

Photon fluence rates were measured at the position of the leaves.

Estimation of Organic Acids. Samples were centrifuged (2 min in a high speed bench centrifuge) or filtered (0.2 μm cellulosic disc filters) and the supernatant was either assayed immediately or following storage at −4°C. Malic, citric, and isocitric acids were separated by HPLC (25 cm × 5 mm i.d. Spherisorb 5 ODS column with 10 cm × 5 mm i.d. Co:pell ODS guard column using 0.25% $HClO_4$ in water as solvent, flow rate 1 ml min^{-1}). These acids are known to be the major acid components of aqueous *K. diagremontiana* extracts and each peak co-chromatographed with the authentic acids obtained from Sigma. The eluent was monitored at 210 nm and the peaks were quantified against standards. Amounts of acid were expressed on a fresh weight basis and rates of acid loss were calculated from the time course of acid loss.

Total acidity of leaf material was estimated by titration of boiled samples with 10 mM NaOH to the phenolphthalein end point.

Estimation of Oxygen Evolution. Oxygen evolution from leaf tissue which contained less than 25 μeq total acid g^{-1} fresh weight was measured polarographically (2). Leaf lamina (0.50 g) was chopped finely with new razor blades and incubated at 20°C in a Clark-type oxygen electrode (Rank Bros., Bottisham, Cambridge, United Kingdom) containing 2.0 ml 100 mM Hepes-KOH and 5 mM $NaHCO_3$ at pH 7.0. Fresh tissue was used for the measurement of oxygen evolution at each wavelength studied. The effects of three fluence rates were investigated at each wavelength and a single leaf provided sufficient tissue for the 10 interference filters used. Photon fluence rates were measured at the face of the electrode and the data refer to the mean of three separate experiments.

Light Sources. Narrow waveband light which contained 90% of

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the photon fluence rate within a 15-nm half-bandwidth was obtained using interference filters (Balzers Ltd, 719496 Furstentum, Liechenstein). Tungsten halogen bulbs (300 w) and tungsten bulbs (500 w) were used as light sources for most of the deacidification studies and a Xenon-arc lamp (1 kw, Oriel Scientific Ltd, PO Box 136, Kingston upon Thames, Surrey, United Kingdom) was used for the higher fluence rates and for all experiments with the oxygen electrode. Water filters were used in all cases. Photosynthetically active monochromatic background light was provided by a SOX sodium lamp (135 w). In all experiments used in the construction of the action spectra, photon fluence rates employed did not saturate the process under investigation.

All photon fluence rates were measured with a spectroradiometer (Gamma Scientific Ltd., 3777 Ruffin Rd., San Diego, CA 92123).

Construction of Action Spectra. For each wavelength, a dose-response curve was plotted using regression analysis and the values at 200 μmol m^{-2} s^{-1} of light at each wavelength were plotted against the wavelength.

RESULTS AND DISCUSSION

Effects of Photon Fluence Rate of White Light on Photosynthesis and Deacidification. Figure 1 shows saturation curves in white light of malic acid consumption in acidified leaf material and photosynthetic oxygen evolution in deacidified leaf slices. Deacidification saturated at a much lower photon fluence rate (about 500 μmol m^{-2} s^{-1}) than oxygen evolution (above 2,000 μmol m^{-2} s^{-1}). The highest rates of deacidification observed were around 70 μmol malic acid mg Chl^{-1} h^{-1} (presumably equivalent to CO_2 released) and the highest rates of oxygen evolution were about 40 μmol O_2 mg Chl^{-1} h^{-1} (presumably equivalent to CO_2 uptake). These values are of the same order of magnitude as those observed by Day (3) and Usuda (11), respectively. At all photon fluence rates, including the higher values which were comparable to noon summer sunlight, maximal rates of malic acid deacidification were substantially greater than those of oxygen evolution.

It has been shown previously (1) that CO_2 accumulates within the internal gas phase of CAM plants during illumination following malic acid accumulation in darkness. This CO_2 accumulation is believed to be involved in the daytime stomatal closure characteristic of and essential to CAM. The present finding that CO_2 release is likely to be more rapid than CO_2 fixation under all conditions of irradiation readily accounts for this accumulation.

Action Spectra of Deacidification and Photosynthesis. The relationship between wavelength, photon fluence rate, and rate of deacidification is shown in Figure 2, and oxygen evolution is shown in Figure 3. The action spectra in Figure 4 relate to data drawn from Figures 2 and 3 at a photon fluence rate of 200 μmol m^{-2} s^{-1} which was sufficient to cause a readily measureable rate of deacidification and photosynthesis, but did not saturate the process in the most effective wavelengths.

The deacidification action spectrum experiments utilized fully acidified leaf material containing approximately 200 μeq total acid g^{-1} fresh weight. Although changes in malic acid were readily measurable, no consistent changes in the levels of citric or isocitric acids were observed during the 5-h experimental period.

In the case of the photosynthetic action spectrum, it was necessary to ensure that oxygen evolution was not limited by the rate of release of carbon dioxide from malic acid or perhaps influenced

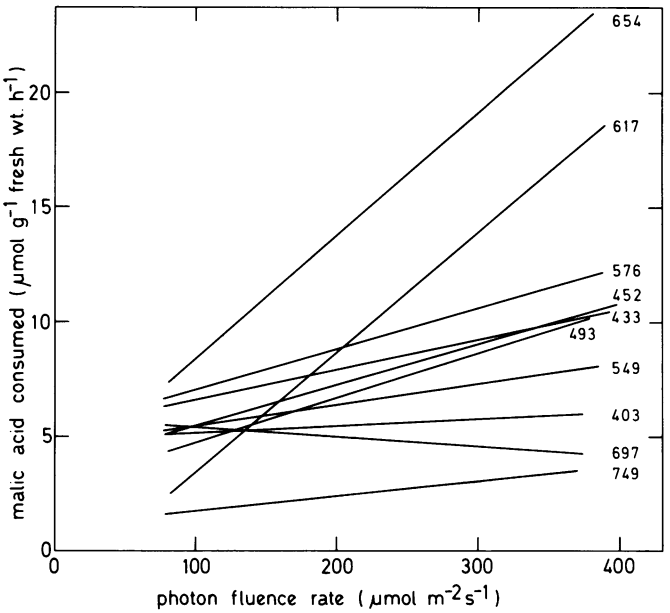

FIG. 2. Relationship between malic acid consumption in acidified *Kalanchoë* leaves and photon fluence rate of narrow waveband lights of 403, 433, 452, 493, 549, 576, 617, 654, 697, and 749 nm.

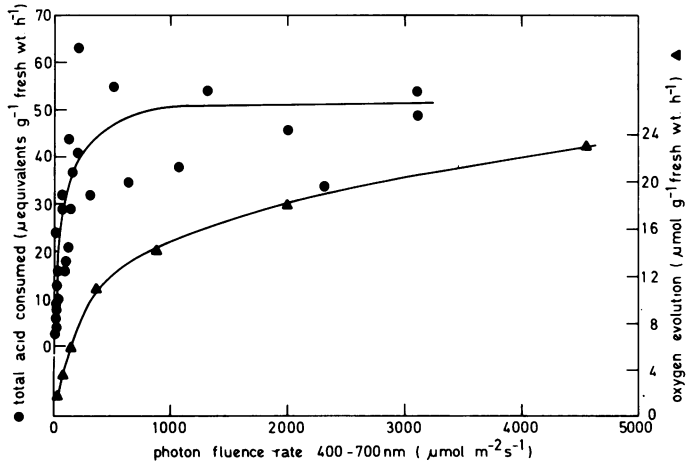

FIG. 1. Relationship between photon fluence rate of white light and rate of malic acid consumption in acidified *Kalanchoë* leaves ([●], each point relates to an independent experiment) and rate of photosynthetic oxygen evolution in deacidified *Kalanchoë* leaf tissue. ([▲], each point is the mean of three independent replicate experiments).

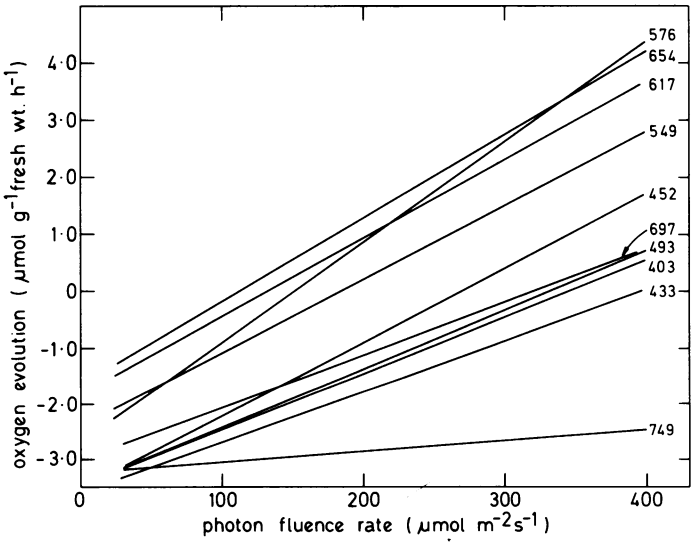

FIG. 3. Relationship between photosynthetic oxygen evolution in deacidified *Kalanchoë* leaf tissue and photon fluence rate of narrow waveband lights (wavelengths as in legend to Fig. 2).

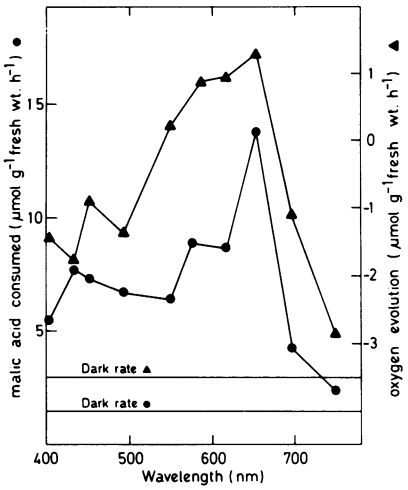

FIG. 4. Action spectra of malic acid consumption in acidified *Kalanchoë* leaves and photosynthetic oxygen evolution in deacidified *Kalanchoë* leaf tissue at a photon fluence rate of 200 μmol m^{-2} s^{-1}.

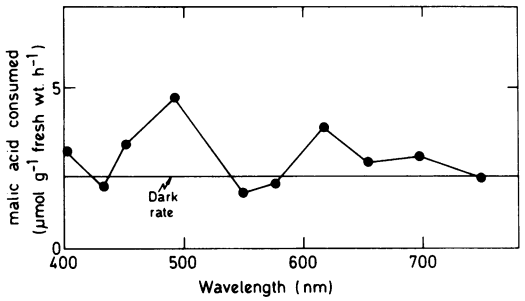

FIG. 5. Effect of low photon fluence rates (1–12 μmol m^{-2} s^{-1}) of narrow waveband light (wavelengths as in legend to Fig. 2) on rate of malic acid consumption.

in some other way by the deacidification process. For this reason, the experiments shown in Figure 3 from which the action spectrum of photosynthetic oxygen evolution (Fig. 4) is derived were performed with leaf tissue containing minimum amounts of acid (less than 25 μeq total acid g^{-1} fresh weight). Saturating levels of sodium bicarbonate were supplied as carbon source.

The measurement of photosynthetic rates in CAM tissue is complicated by the daytime closure of stomata characteristic of this phenomenon and by the endogenous supply of CO_2 by malate decarboxylation. Measurement of oxygen evolution circumvents the problems related to malate decarboxylation. The possibility of light-induced closure of stomata influencing the apparent rate of oxygen evolution was avoided by using fine slices of leaf material, a mode of preparation which was also consistent with the requirements of the oxygen electrode. Although every attempt was made to maximize the rates of photosynthetic oxygen evolution, the possibility remains that the rates observed in the sliced tissue may not necessarily equal rates achieved in intact tissue.

There is a marked correspondence between the two action spectra. In both deacidification and photosynthesis, light of around 650 nm is used most efficiently. Above 700 nm, there is a marked decline in the efficiency of light utilization.

The close similarity between the two action spectra strongly suggests that the same pigments are responsible for the acquisition of light energy in both photosynthesis and deacidification.

Previous work (7) which indicated a special role for far-red light was carried out using fluence rates much lower than the 200 μmol m^{-2} s^{-1} to which the data displayed in Figure 4 refer. Figure 5 shows the relationship between deacidification at low fluence rates similar to those used in earlier work. No effect was apparent at any wavelength. It is possible that the stimulation of deacidification by far-red light observed by Nalborczyk *et al.* (7) related to the regulation of the rhythmic aspects of CAM rather than to the direct stimulation of deacidification.

Table I. *Effects on the Rate of Malic Acid Consumption of Irradiation of Acidified Kalanchoë Leaves with Narrow Waveband Light in the Presence and Absence of Photosynthetically Active Monochromatic Light*

Narrow waveband light is at 749, 654, 549, and 542 nm; monochromatic light is at 585 nm.

Wavelengths	Total Photon Fluence Rate	Fluence Rate at Each Wavelength	Predicted Rate of Deacidification at Each Wavelength	Predicted Total Rate of Deacidification	Observed Rate of Deacidification
nm	*μmol m^{-2} s^{-1}*		*μmol g^{-1} fresh wt h^{-1}*		
749	154	118	−1.94	−8.66	−8.13
585		36	−6.72		
749	324	276	−2.91	−10.15	−8.28
585		48	−7.24		
749	327	279	−2.91	−10.15	−9.55
585		48	−7.24		
654	166	63	−6.49	−16.27	−16.87
585		103	−9.78		
654	239	98	−8.36	−19.85	−17.01
585		141	−11.49		
654	204	87	−7.76	−18.13	−24.63
585		117	−10.37		
549	268	172	−6.12	−15.52	−15.30
585		96	−9.40		
549	272	134	−5.82	−17.16	−16.27
585		138	−11.34		
542	195	133	−6.12	−14.03	−5.15
585		62	−7.91		
542	193	127	−5.97	−14.03	−16.42
585		66	−8.06		

Simultaneous Irradiation with Narrow Waveband Light and Monochromatic Photosynthetically Active Light. To investigate the possibility that, in the action spectrum experiments, the rate of deacidification was limited by the rate of photosynthesis, experiments were performed to investigate the effects on deacidification of narrow waveband light in the presence of limiting monochromatic photosynthetically active light of 585 nm wavelength. Separate experiments in which leaf tissue was irradiated with either 585 nm light or narrow waveband lights gave information on the rates of acid consumption caused by each individual light treatment. During simultaneous illumination, when the 585 nm light was supplied in addition to the narrow waveband light under test, it was found that the total rate of acid consumption was no more than the total of the rates as measured during the separate illumination experiments (Table I). This demonstrates that, in the single waveband experiments on which the action spectrum in Figure 4 was based, deacidification was never limited by the inability of certain wavelengths to supply adequately quanta to photosynthesis.

It seems clear that the light which promotes deacidification is absorbed by the same pigments which absorb photosynthetically

active light. The finding that diuron, a specific inhibitor of PSII, prevents deacidification (8) indicates that the link between the photosynthetic pigments and the deacidification process is not direct but involves the participation of some aspect of photosynthetic electron transport or carbon metabolism. The nature of this link has yet to be elucidated.

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