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Relationships between Stomatal Behavior and Internal Carbon Dioxide Concentration in Crassulacean Acid Metabolism Plants

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ABSTRACT

Measurements of internal gas phase CO_2 concentration, stomatal resistance, and acid content were made in Crassulacean acid metabolism plants growing under natural conditions. High CO_2 concentrations, sometimes in excess of 2%, were observed during the day in a range of taxonomically widely separated plants (*Opuntia ficus-indica* L., *Opuntia basilaris* Engelm. and Bigel., *Agave desertii* Engelm., *Yucca schidigera* Roezl. ex Ortiges, *Ananas comosus* [L.] Merr., *Aloe vera* L., *Cattleya* sp. and *Phalanopsis* sp.) and below ambient air concentrations were observed at night.

Stomatal resistance was always high when CO_2 concentration was high and experiments in which attempts were made to manipulate internal CO_2 concentrations gave data consistent with stomatal behavior in Crassulacean acid metabolism being controlled by internal CO_2 concentration. Exogenous CO_2 applied in darkness at a concentration similar to those observed in the light caused stomatal resistance to increase.

In pads of *Opuntia basilaris* Engelm. and Bigel. subjected to severe water stress internal gas phase CO_2 concentrations exhibited fluctuations opposite in phase to fluctuations in acid content. Stomatal resistance remained high and the opening response to low CO_2 concentration was almost entirely eliminated.

The physiological significance of CAM is believed to result from the temporal separation of photosynthetic CO_2 reduction and gas exchange with the atmosphere. Gas exchange with the atmosphere is restricted to the hours of darkness when the inevitable associated water loss is likely to be minimized. Photosynthetic CO_2 reduction occurs without gas exchange with the atmosphere and hence without water loss. CO_2 acquired at night is temporarily stored in malic acid and is consumed in photosynthetic reactions the following day.

Earlier work (4, 5) has shown that CO_2 may be released to the atmosphere surrounding CAM plants following illumination. This indicates that release of CO_2 from malic acid is not directly caused by photosynthetic consumption of CO_2 and suggests that there might be an accumulation of CO_2 inside illuminated CAM tissues.

Since CO_2 concentration has effects on photosynthesis, respiration, photorespiration, and stomatal behaviour (1, 3, 6–8, 10) and inasmuch as it has been suggested that CO_2 concentration is involved in the regulation of CAM (2), a knowledge of CO_2 concentration inside CAM plants would assist in an understanding of this phenomenon. Analysis of the atmosphere surrounding CAM plants in the light cannot provide information on the levels of gaseous CO_2 inside the tissue because the stomata of CAM plants close in the light. The present work involved measurements of CO_2 concentration in gas samples withdrawn from the air spaces inside the photosynthetic tissues of CAM plants.

The relative rates of the reactions which produce and consume CO_2 in CAM are likely to be affected by environmental factors. To avoid experimental artifacts and in order to obtain realistic values of CO_2 concentration, measurements were made on plants growing under natural conditions at the Philip L. Boyd Deep Canyon Research Center and at the Botanic Gardens, University of California at Riverside.

MATERIALS AND METHODS

Plant Material. Experiments were performed on large plants of *Agave desertii* Engelm. and *Opuntia basilaris* Engelm. and Bigel., growing under natural conditions at the Philip L. Boyd Deep Canyon Desert Research center near Palm Desert California; on *Opuntia ficus-indica* L. and *Yucca schidigera* Roezl. ex Ortgies growing at the Botanic Gardens, University of California at Riverside, and on *Ananas comosus* (L.) Merr., *Aloe vera* L., and orchids of the species *Cattleya* and *Phalanopsis* growing in the greenhouses of the University of California at Riverside.

Gas Sampling. Disposable hypodermic syringes (2.0-ml capacity, 22-gauge needle) were used to withdraw gas samples (0.5–2.0 ml) from photosynthetic tissue. The syringe needle was inserted into the tissue through a globule of water standing on the surface of the plant or contained in a cup of Plasticine adhering to the plant. Taking samples by this method ensured, providing that stomata were closed, that any gas entering the syringe came from inside the plant and not from leakage around the needle. Furthermore, as the syringe was withdrawn from the plant this procedure ensured that any partial vacuum in the syringe was filled by water and not by gas from the atmosphere. If the syringe was sealed by plunging the needle deeply into a rubber stopper the sample could be stored for periods of up to 30 min without significant change in CO_2 concentration. For the longer periods of storage required for field experiments (up to 30 h) gas samples, usually 1.0 ml, were injected through a serum bottle stopper into small glass vials which contained CO_2-free N_2 and also sand to reduce the internal gas space.

Measurement of CO_2 Concentration. CO_2 was separated from other atmospheric gases by GC of 0.5-ml samples at 70 C on a 2.15-m Porapak Q column using helium at a flow rate of 40 ml/min as carrier gas. The thermal conductivity detector used was calibrated against a 2% CO_2 in air reference standard. For samples stored in vials, corrections were made for variations in volume between vials, on the basis of analysis of aliquots taken from each vial following the injection of a standard amount of CO_2.

Stomatal Diffusion Resistance. All measurements were made using a Lambda Instruments autoporometer. Temperature corrections were applied on the basis of measurements made using the

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thermistor built into the sensor probe (Lambda Instrument Corp., Lincoln, Neb.).

Acid. At each sample time three replicate cores of 1.5-cm diameter were crushed and extracted for 20 min in boiling distilled H_2O. The extracts were titrated to pH 8.0 with 0.01 M NaOH and their acid content expressed as μeq acid/g fresh weight. Samples taken in the field were frozen immediately in solid CO_2—care being taken that the insulated box containing the samples was kept away from the experimental plant and downwind of it.

RESULTS AND DISCUSSION

Diurnal Measurements of Stomatal Resistance, CO_2 and Acid. These parameters were measured in a large plant of *A. desertii* growing on Agave Hill in the Deep Canyon Desert Research Center. A different leaf was used for each set of samples. The plant accumulated acid at night and consumed it the following day (Fig. 1). In general, during the period of acid synthesis the internal gas phase CO_2 concentration was the same as that of ambient air or less. The analytical methods used did not permit accurate measurements to be made at CO_2 concentrations below about half that of ambient air. During acid consumption CO_2 concentration initially rose to around 0.8% (about 25 × ambient air concentration). As acid content approached its minimum level, as occurred toward the end of the day, CO_2 concentration also declined and eventually fell to the minimum detectable level. Stomatal resistance varied with CO_2 concentration being high when CO_2 level was high and low when CO_2 level was low. The transient decrease in resistance after 2 h darkness may have been caused by dew formation interfering with the function of the autoporometer. Similar experiments were performed with *O. ficus-indica* and substantially similar results were obtained except that maximum CO_2 concentrations in excess of 2% were observed.

CO_2 Levels in the Light in a Variety of CAM Plants. The finding that the internal gas phase CO_2 concentrations during daylight were high in both *A. desertii* and *O. ficus-indica* led to the investigation of CO_2 levels in the light in a range of taxonomically widely separated CAM plants. The values obtained (Table I) indicate that a higher than ambient air internal gas phase CO_2 concentration in the light is a general characteristic of CAM plants. The values given are from duplicate samples taken around noon and do not necessarily represent maximum values likely to be observed in a particular plant.

Stomatal opening at night and closure during the day are essential characteristics of CAM. In general, stomates close in response to high and open in response to low CO_2 concentration. The experiments described below were performed to investigate the possibility of a causal relationship between internal CO_2 concentration and stomatal resistance in CAM plants.

Kinetics of Changes in CO_2 Concentration and Stomatal Resistance. In Figure 1 there appears to be a close correlation between the increase and decline in CO_2 concentration and stomatal resistance but the experimental samples were too widely spaced over the critical opening and closing phases to be useful in determining causality. Experiments were performed in which samples were taken at approximately 15-min intervals in an attempt to obtain more precise information. All samples (0.5 ml) were taken from the same large pad to avoid problems associated with differences between pads. By the end of the experiment a total of 4.0 ml was withdrawn from the pad. An estimate of the total internal gas space of the pad, based on the difference between the weight of the pad and the weight of an equal volume of water, indicates that it was in the region of 40 ml. Thus, only about 10% of the total volume was removed in the course of the experiment and could not have accounted for more than a small proportion of the observed fall in CO_2 concentration.

As Figure 2 shows, the decline in CO_2 concentration preceded the decrease in stomatal resistance, and although in no way conclusive, the data are consistent with stomatal response being controlled by internal CO_2 concentration.

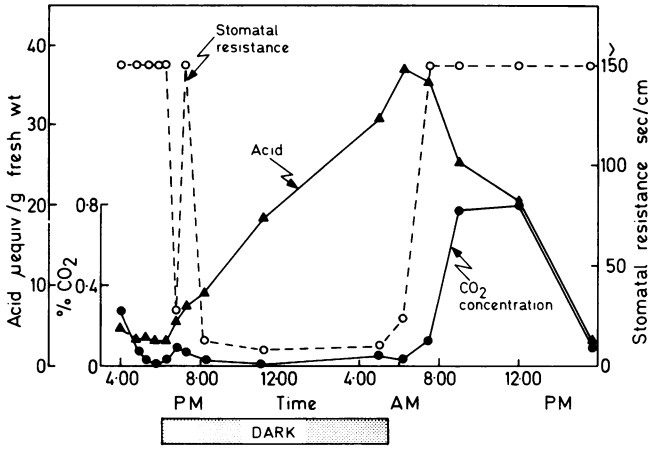

FIG. 1. Diurnal changes in internal gas phase CO_2 concentration, stomatal resistance to diffusion of water vapor, and acid content in *A. desertii*.

Table I. Internal gas phase CO_2 concentration in illuminated CAM plants

Species	Family	%CO_2
Opuntia ficus-indica	Cactaceae	1.30
Opuntia basilaris	Cactaceae	2.50
Agave desertii	Agavaceae	0.80
Yucca schidigera	Agavaceae	0.40
Aloë vera	Liliaceae	0.60
Ananas comosus	Bromeliaceae	0.50
Cattleya sp	Orchidaceae	0.15
Phalanopsis sp.	Orchidaceae	0.23

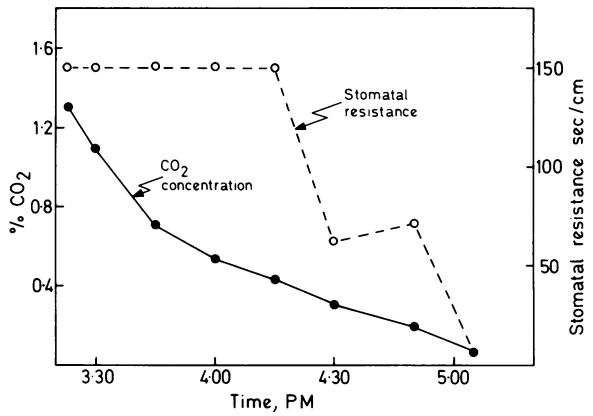

FIG. 2. Kinetics of decline in internal gas phase CO_2 concentration and stomatal resistance to diffusion of water vapor at the end of the day in *O. ficus-indica*.

Malate Level and Stomatal Resistance. Since the CO_2 released in the light in CAM plants has its origin in malate it might be expected that the internal CO_2 concentration in the light would fall when malate reserves are fully consumed. An attempt was made to reduce the amount of malate accumulated to such an extent that it would be fully consumed in the course of a natural day period. This was achieved by restricting gas exchange during the night between the CAM plant and the atmosphere. Just before sunset large pads of *O. ficus-indica* were covered by aluminum foil-lined plastic bags tied tightly at the junction between the pad

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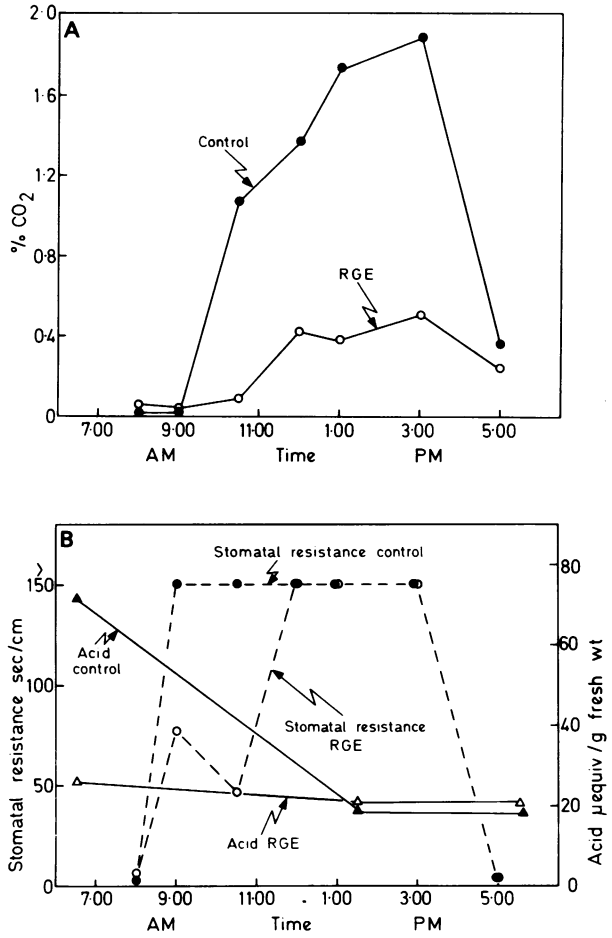

FIG. 3. A: internal gas phase CO_2 concentration in *O. ficus-indica* in daytime following RGE with the atmosphere the previous night. B: acid content and stomatal resistance to diffusion of water vapor in *O. ficus-indica* in daytime following RGE with the atmosphere the previous night.

and the pad below. The following morning samples for acid measurement were taken before dawn and it was found that whereas control pads contained 72 μeq/g of acid, the RGE[1] pads contained 26. Measurements of internal CO_2 concentration, stomatal resistance, and acid were then made throughout the day (Fig. 3, A and B). During the day the CO_2 concentration in the control pads rose more rapidly than in the RGE pads which reached a maximum value of 0.5% compared with 1.9% in the control. A decline in CO_2 concentration was observed at the same time in the RGE and control pads toward the end of the day. Stomatal resistance, like CO_2 concentration, increased more slowly in the RGE pads and the decline in stomatal resistance at the end of the day occurred simultaneously in both the RGE and control pads. These data are consistent with the regulation of stomatal resistance by CO_2. It also seems that there is a mechanism in this cactus which maintains a high internal CO_2 concentration (and therefore high stomatal resistance) in the light even when malate reserves run low. Such a mechanism could conceivably be of value in the field of environmental conditions, such as high night temperature, occasionally prevented a massive nighttime accumulation of malate.

Effects of Exogenous CO_2 on Stomatal Resistance. All of the experiments so far described gave results consistent with the possibility that the daytime stomatal closure which is characteristic of CAM plants is caused by high internal CO_2 concentrations.

Figure 4 shows the results of an experiment designed to investigate this possibility further by studying the effects of exogenous CO_2 on stomatal resistance in darkness—that is when the stomatal resistance is low. A large pad on a large plant of *O. ficus-indica* was enclosed after sunset in a plastic bag fitted with inlet and outlet tubes and a sealable flap to allow access for stomatal resistance measurements. An air flow of about 200 ml/min was maintained during which measurements of stomatal resistance confirmed that the plant had entered the acid accumulation phase of CAM shown in the earlier experiments to be characterized by a low internal CO_2 concentration. The gas stream was then changed from air to 2% CO_2 in air following which the stomatal resistance increased rapidly to typically daytime values. The return to air after 50 min was followed by a decline in stomatal resistance to nighttime values. The stomatal resistance of an untreated control pad remained low throughout the experiment. Similar experiments were performed with *A. desertii* and substantially similar results were obtained.

Exogenous application of CO_2 in darkness of concentrations of the same order as those observed inside the plants during illumination caused stomatal resistance to increase to levels normally found in daylight. This, along with the other data presented here, indicates that the daytime closure in CAM stomata is a response to the high internal CO_2 concentration found during the day. The data are also consistent with the nocturnal stomatal opening being a response to low internal CO_2 concentration caused by consumption of CO_2 in association with acid accumulation.

"Idling" in *O. basilaris*. As shown by Szarek and Ting (9), under severe water stress the stomatal resistance of *O. basilaris* remains

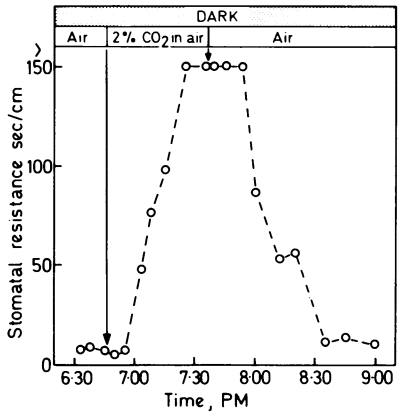

FIG. 4. Effects of CO_2 in darkness on stomatal resistance to diffusion of water vapor in *O. ficus-indica*.

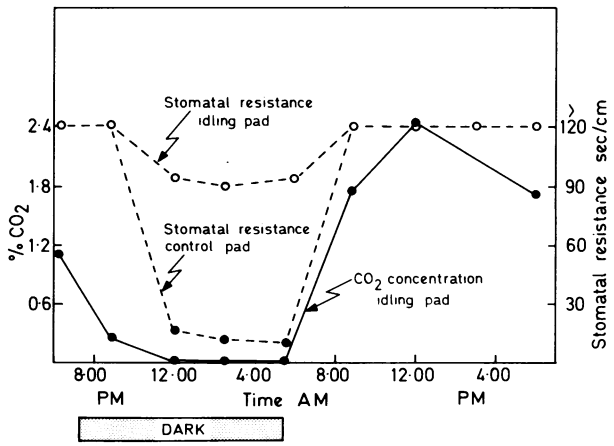

FIG. 5. Stomatal resistance to water vapor and internal gas phase CO_2 concentration in idling *O. basilaris*.

[1] Abbreviation: RGE: restricted gas exchange.

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high at night although diurnal fluctuations of acid content still occur. This was interpreted as indicating that CO_2 is recycled on a diurnal basis through respiration and the photosynthetic and acid-synthesizing reactions of CAM. *O. basilaris* pads were induced to idle in this way by detaching them and keeping them dry on a greenhouse bench. After 3 days, measurements of stomatal resistance, internal CO_2 concentration, and acid were made (Fig. 5). Measurements were also made on the stomatal resistances of control pads growing in pots in the same greenhouse. Acid levels in the idling pads increased during the night and decreased during daytime between limits of about 30 and 55 μeq/g fresh weight—similar to the range observed by Szarek and Ting (9). Internal CO_2 concentration also varied between around 2% during the day and less than the minimum detectable level during the night. Stomatal resistance remained high throughout although it was always lowest when CO_2 concentration was lowest. Stomatal resistance in the untreated control plants was low during the hours of darkness.

Thus, idling in water-stressed *O. basilaris* involves fluctuations in internal CO_2 concentration as well as in acid content. Although there were indications of a response of stomatal resistance to low CO_2 concentration it was very small. It appears that under severe water stress the response of *O. basilaris* stomata to CO_2, which usually results in a nighttime reduction of resistance in CAM plants, is overridden.

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