Environmental Influences on Open Stomates of a Crassulacean Acid Metabolism Plant, *Agave deserti*[1]

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ABSTRACT

The major short term stomatal response of *Agave deserti* was to temperature; increases in leaf temperature led to decreases in water vapor conductance for stomatal opening during the daytime (C_3 mode) as well as at night (Crassulacean acid metabolism or CAM mode). Hourly changes in the water vapor concentration drop from leaf to air had no significant stomatal effect in either mode. Stomatal responses to external CO_2 levels up to 800 microliters per liter were not significant after 15 minutes and only moderate after a few hours, suggesting that CO_2 effects on open stomates of this succulent were indirect in both CAM and C_3 modes.

The degree of stomatal opening can be influenced by various environmental factors: light, CO_2 level, water vapor concentration drop from leaf to air, and temperature (4). Greater stomatal opening is generally favored at relatively high illuminations, low CO_2 levels, low water vapor concentration drops, and high temperatures. Most research has dealt with C_3 and C_4 plants, which exhibit daytime stomatal opening. The present study deals with a CAM[2] plant, *Agave deserti* Engelm., whose stomates open at night under natural conditions (13). However, this plant can be converted to predominantly daytime stomatal opening and C_3 photosynthesis by increased watering (5). Thus *A. deserti* is an interesting succulent for examining environmental influences on stomatal opening in both the CAM and C_3 modes for the same species. Its stomatal responses to environmental factors differed from the general pattern for C_3 and C_4 plants.

MATERIALS AND METHODS

Mature plants of *Agave deserti* Engelm. (Agavaceae) transplanted from the western Colorado desert (13) were grown in chambers providing 14-hr days with a total of 20 E m^{-2} PAR (400–700 nm) in the planes of the leaf surfaces and a leaf temperature of 26 ± 1 C (15). To induce daytime stomatal opening (5), the weekly watering regime was replaced by daily watering for 6 weeks such that the soil water potential 10 cm below the surface was always above −0.05 MPa (1 MPa = 10 bar), and the nighttime leaf temperature was raised from 15 ± 1 C to 20 ± 1 C (± indicates range). Allowing the soil to subsequently dry caused the plants to revert to a predominantly CAM mode over a period of weeks (5).

[2] Abbreviations: CAM: Crassulacean acid metabolism; $c^o_{CO_2}$: external CO_2 concentration; $c^i_{CO_2}$: CO_2 concentration in intercellular air spaces just interior to stomates; g_{wv}: water vapor conductance; J_{CO_2}: net CO_2 uptake; PAR: photosynthetically active radiation; Δc_{wv}: water vapor concentration drop from leaf to air.

To avoid possible prior environmental effects, plants were maintained under a particular condition for 1 week before each experiment.

Gas exchange was measured as described previously (15) on mature leaves averaging 280 g in fresh weight and 29 cm in length. Except where indicated otherwise, the gas phase in the assimilation chamber was air containing 340 μl l^{-1} CO_2 and 6.9 ± 0.1 g m^{-3} water vapor, surface temperature of the leaf was 15.0 ± 0.1 C, and measurements were made 1 to 5 hr after the beginning of the 10-hr dark phase or 4 to 8 hr after the beginning of the light phase (times when stomatal opening was normally fairly constant). A 15-min measurement period was routinely used followed by a 15-min period where the leaves acclimated to the next condition. For gas exchange measurements during the light phase the PAR averaged 1.0 mE m^{-2} sec^{-1} in the planes of the leaf surfaces. Data are expressed on the basis of total area (both sides) for these succulent leaves.

The water vapor conductance (g_{wv}) equaled the net rate of water loss per unit area (J_{wv}) divided by the water vapor concentration drop from leaf to air (Δc_{wv}):

$$g_{wv} = J_{wv}/\Delta c_{wv} \quad (1)$$

The CO_2 concentration in the intercellular air space just interior to the stomates ($c^i_{CO_2}$) was estimated from

$$c^i_{CO_2} \cong c^o_{CO_2} - 1.56 \, J_{CO_2}/g_{wv} \quad (2)$$

where $c^o_{CO_2}$ is the external CO_2 concentration, J_{CO_2} is the net rate of CO_2 uptake, and 1.56 is the ratio of the diffusion coefficient of water vapor to that of CO_2. Approximations involved in using equations 1 and 2 for *A. deserti* have been discussed previously (15).

RESULTS

Illumination. When *A. deserti* in the CAM mode was illuminated at various times during the dark period, the water vapor conductance increased (Fig. 1A). When it was in a C_3 mode, darkening during the daytime caused g_{wv} to decrease over 50% in an hour (Fig. 1B). Figure 1 also indicates that both types of responses were reversible.

CO_2 Concentration. When the external CO_2 concentration for *A. deserti* in the CAM mode at night was raised above 340 μl l^{-1} or $c^i_{CO_2}$ (equation 2) was raised above 200 μl l^{-1}, some stomatal closure occurred (Fig. 2). A reduction in g_{wv} of only 14% from the maximum value took place for a $c^o_{CO_2}$ of 800 μl l^{-1}. This experiment was repeated on four different plants with comparable results; when $c^o_{CO_2}$ was progressively decreased, the response of J_{CO_2} and g_{wv} was the same within experimental error as the results obtained upon increasing $c^o_{CO_2}$. The low water vapor conductance of *A. deserti* causes the guard cells to be exposed to a considerable CO_2 gradient, indicated by the large difference between $c^o_{CO_2}$ and
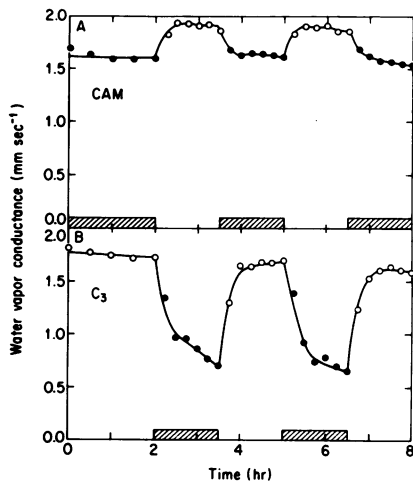
FIG. 1. Effects of light-dark alternation on stomatal opening. A: plants during nighttime in CAM mode (preincubated 1 hr in dark); B: plants during daytime in C_3 mode (preincubated 3 hr in light). (▨):darkness.
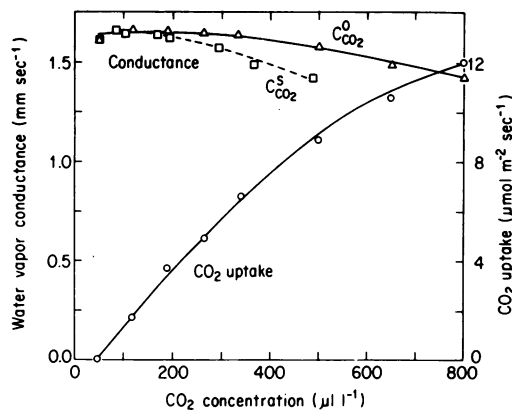

FIG. 2. Relation between external (△) or substomatal (□) CO_2 concentration and leaf water vapor conductance at night in CAM mode. Also shown is the net CO_2 uptake rates at various $c^o_{CO_2}$ (○), which were increased stepwise.

$c^a_{CO_2}$ (Fig. 2). Also plotted in Figure 2 is the net CO_2 uptake, indicating a CO_2 compensation point of 48 μl l^{-1} in the CAM mode, although the compensation point of *A. deserti* can vary depending on leaf temperature and elapsed time in the dark (15).

As another test of the effect of CO_2 on nocturnal stomatal opening, three external CO_2 levels were imposed for the entire dark period (Fig. 3). When $c^o_{CO_2}$ was raised from 340 to 700 μl l^{-1}, the CO_2 uptake rate for the first 2 hr nearly doubled, but then it dropped rapidly and became zero after about 6 hr in the dark. The total CO_2 uptake found by integrating under the curves in Figure 3A was 153 mmol m^{-2} for 700 μl CO_2 l^{-1} and 150 mmol m^{-2} for an external CO_2 concentration of 340 μl l^{-1}. When the external CO_2 level was reduced to 140 μl l^{-1}, the maximum CO_2 influx was lower than previously, but it continued at a substantial rate throughout most of the dark period, leading to an uptake of 93 mmol m^{-2} and a nonzero influx at 10 hr (Fig. 3A). Qualitatively similar effects of $c^o_{CO_2}$ on nocturnal CO_2 uptake have been reported for *Bryophyllum daigremontianum* (6).

In the latter half of the dark period, the water vapor conductance of *A. deserti* varied greatly with $c^o_{CO_2}$ (and $c^a_{CO_2}$). For instance, g_{wv} decreased 50% from its maximum in less than 5 hr at an external CO_2 concentration of 700 μl l^{-1}, in nearly 8 hr at 340 μl l^{-1}, while it never decreased this much for the night at 140 μl l^{-1} (Fig. 3B). Thus g_{wv} responded substantially to CO_2 (or something correlated with it) only after the plants had been in the dark for several hr,

again suggesting that the stomates did not respond directly to $c^o_{CO_2}$ or $c^a_{CO_2}$.

For *A. deserti* in the C_3 mode, the CO_2 compensation concentration during the daytime was 98 μl l^{-1} (Fig. 4). The higher CO_2 compensation point in the C_3 mode is consistent with results for *Kalanchoë daigremontiana* (= *B. daigremontianum*), where the compensation point is near zero in the CAM mode and is 46 to 60 μl l^{-1} for daytime stomatal opening (1, 16). Stomatal opening for *A. deserti* decreased slightly as the CO_2 concentration was raised stepwise, very similar to observations for the CAM mode at night (Fig. 2). Data for Figures 2 and 4 were obtained over a 4-hr period; when $c^o_{CO_2}$ was raised in one step from 340 to 800 μl l^{-1}, the decrease in g_{wv} was less than 4% (experiment repeated three times in each mode).

Water Vapor Concentration Drop. The leaf water vapor conductance of *A. deserti* in the CAM mode at night remained constant ± 5% when Δc_{wv} was varied from 3.8 to 14.1 g m^{-3}, an experiment repeated four times with similar results. This insensitivity also persisted throughout the night when the water vapor concentration drop from leaf to air was oscillated hourly between 5 and 14 g m^{-3}. (To increase the experimental range for Δc_{wv}, leaf temperatures of 20 C were employed in these experiments.) To check whether differences in the sensitivity of the stomates to Δc_{wv}
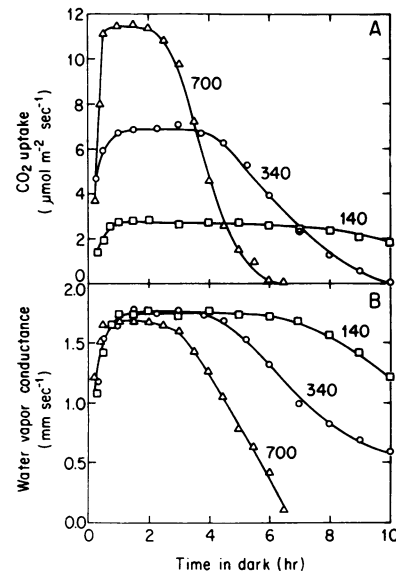

FIG. 3. Effect of various CO_2 levels during the entire night on CO_2 uptake (A) and water vapor conductance (B) in CAM mode. External CO_2 level in μl l^{-1} is indicated next to the curves.
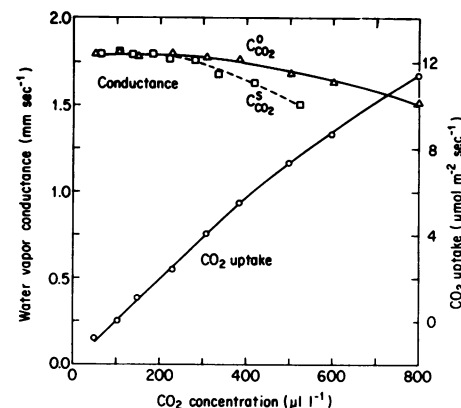

FIG. 4. Relation between external (△) or substomatal (□) CO_2 concentration and leaf water vapor conductance for daytime stomatal opening (C_3 mode). Net CO_2 uptake at various $c^o_{CO_2}$ (○) is also indicated.

might develop during the night, leaves were maintained with a Δc_{wv} of either 5.1 or 13.8 g m^{-3} throughout the dark period (Fig. 5). The water vapor conductance was initially similar, but became slightly higher for the lower Δc_{wv} after 5 hr of dark.

When the weekly watering of plants in the CAM mode was stopped for 2 weeks so that the soil water potential near the roots decreased from the usual -0.2 ± 0.2 MPa to -1.5 ± 0.3 MPa, the water vapor conductance for the leaves decreased. Little effect of Δc_{wv} on g_{wv} was evident at the beginning of the night, but the higher water vapor concentration drop led to somewhat more stomatal closure in the latter half of the dark period (Fig. 5). When the watering was ceased for 3 weeks, the water potential near the roots dropped to -8.2 ± 1.5 MPa and the maximum g_{wv} was only 0.17 mm sec^{-1} (Fig. 5).

The leaf to air water vapor concentration drop also had very little effect on the stomatal conductance in the C_3 mode. For instance, when Δc_{wv} was varied in five approximately equal increments from 4.7 to 13.8 g m^{-3} in each of two experiments at a leaf surface temperature of 19.4 $\pm$ 0.2 C, g_{wv} remained at 1.7 $\pm$ 0.1 mm sec^{-1}.

Temperature. The water vapor conductance of *A. deserti* in the CAM mode steadily decreased as the temperature was raised above 10 C, the response being somewhat greater for an external CO_2 level of 340 μl l^{-1} compared with 15 μl l^{-1} (Fig. 6). The 2-fold lower conductance at 25 C compared with 10 C for 340 μl CO_2 l^{-1} was found even when the temperature was oscillated between these values throughout the night (Fig. 7). The tempera-
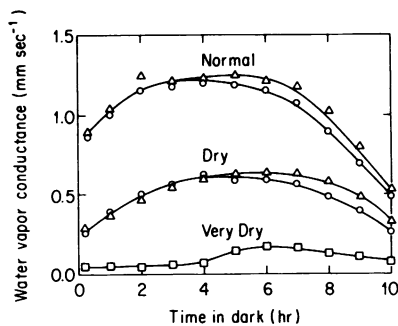

FIG. 5. Influence of water vapor concentration drop from leaf to air on nocturnal water vapor conductance in CAM mode. Leaves were maintained at 20 C with a Δc_{wv} of 5.1 $\pm$ 0.2 g m^{-3} ($\triangle$) or 13.8 $\pm$ 0.1 g m^{-3} (O, $\square$). Soil water potential at 10 cm averaged -0.2 MPa ("Normal"), -1.5 MPa ("Dry"), or -8.2 MPa ("Very dry").
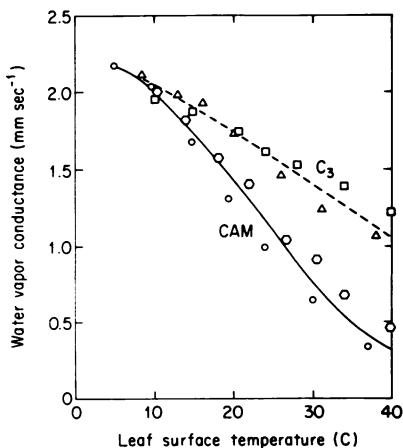

FIG. 6. Temperature dependence of stomatal opening at night in CAM mode (O) and during the daytime in C_3 mode ($\triangle$). Temperature was also increased stepwise when the external CO_2 concentration was reduced to 15 μl l^{-1} for the CAM (O) and C_3 ($\square$) modes. Water vapor content of the air was 5 $\pm$ 1 g m^{-3} below 15 C and 8 $\pm$ 1 g m^{-3} above 15 C.
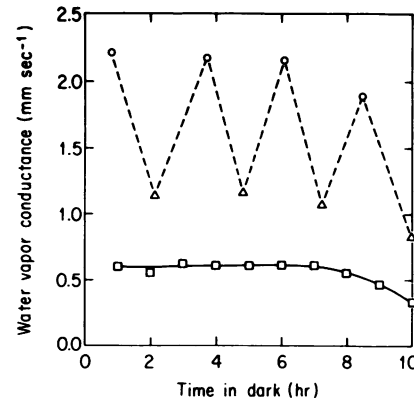

FIG. 7. Effect of leaf surface temperature on nocturnal water vapor conductance in CAM mode. Temperature was oscillated (----) between 9.7 $\pm$ 0.3 C (O) and 25.0 $\pm$ 0.1 C ($\triangle$). Also, the nocturnal temperature was maintained at 29.9 $\pm$ 0.1 C (———, $\square$). Δc_{wv} was 7 $\pm$ 1 g m^{-3}.

ture effect was the same whether the water vapor content of the air was constant (Fig. 6) or Δc_{wv} was constant (Fig. 7).

The lower conductances at higher temperatures occurred throughout the night under steady conditions, although secondary effects were evident near the end of the dark period. For instance, the nocturnal conductance decreased 30% from its maximum value after 6.4 hr at 15 C (Fig. 3), 7.7 hr at 20 C, while it took 9.3 hr at 30 C (Fig. 7). The daytime stomatal conductance in the C_3 mode progressively decreased as the temperature was raised, slightly more so at 340 than 15 μl l^{-1} external CO_2 (Fig. 6), although neither response was as great as in the CAM mode.

DISCUSSION

The illumination response of the stomates of *A. deserti* in both the CAM and C_3 modes was qualitatively similar to that generally found for C_3 and C_4 plants, while the response to CO_2 was less, slower, and may have been indirect (4). In both modes the stomates of *A. deserti* did not close when exposed to low air water vapor levels and increases in temperature led to stomatal closure, contrary to many findings for C_3 and C_4 plants (4). In fact, the response to temperature for stomates open in either the CAM or C_3 mode was the main short term change of *A. deserti* induced by any of the environmental parameters considered.

When *A. deserti* is in a predominantly CAM mode, appreciable CO_2 uptake can occur at the beginning of the light phase, presumably as the C_3 pathway is initiated (5, 13). In the field this enhanced CO_2 uptake at dawn was accompanied by an increase in the water vapor conductance (13), similar to the increase in g_{wv} observed here upon illumination (Fig. 1A). Such stomatal movements have also been observed for other CAM plants (12). The decrease in g_{wv} when leaves in a C_3 mode were periodically darkened during the daytime (Fig. 1B) was similar to that occurring at dusk for *A. deserti* that had previously been converted to daytime CO_2 uptake by watering (5). The integrated daytime illumination also has an effect on the degree of nocturnal stomatal opening in the CAM mode (12), *e.g.* the maximum nighttime g_{wv} for *A. deserti* was about half as great when the total PAR during the previous day was reduced from 37 to 8 E m^{-2} (15).

High CO_2 levels induced only about a 15% decrease in stomatal opening over the course of hours for the CAM and C_3 modes (Figs. 2 and 4). Major decreases in g_{wv} occurring during the course of a night were correlated with higher $c^0_{CO_2}$ (Fig. 3B), but the effect was apparently indirect, since organic acid levels and other cellular factors would change drastically on such a time scale where the cumulative CO_2 uptake becomes important. The lack of response of open stomates of *A. deserti* to CO_2 concentrations is surprising, since it has long been proposed that CO_2 concentrations in the

intercellular air spaces of the chlorenchyma controlled the stomatal aperture of CAM plants (7, 8, 11). The water vapor conductance of young leaves of *Agave americana* maintained in a nutrient solution apparently decreased 6-fold as the ambient CO_2 concentration at night was raised from 0 to 1,340 $\mu l \, l^{-1}$ (9), and the conductance decreased 2-fold for *K. daigremontiana* seedlings in both the CAM and C_3 modes when the external CO_2 concentration was raised from 100 to 1,000 $\mu l \, l^{-1}$ (2). The lack of a major short term response of open stomates of *A. deserti* to CO_2 may be a characteristic of mature CAM plants, since the responses observed for *A. americana* and *K. daigremontiana* were for young plants. Alternatively, open stomates of *A. deserti* may be relatively insensitive to CO_2 levels at all stages of leaf development, just as certain non-CAM species also do not exhibit major stomatal responses to CO_2 (4).

Increasing Δc_{wv} at constant temperature did not influence stomatal opening for *A. deserti* in the CAM or C_3 mode, in agreement with previous studies on *F. acanthodes* in the CAM mode (14). On the other hand, for *Opuntia compressa* a lower daytime g_{wv} was correlated with a higher Δc_{wv}; however, there was considerable inconsistency in the data and also the mean water vapor conductance was often about the same in the light and the dark (3). The effect of Δc_{wv} on stomatal opening of *A. deserti* was not materially changed upon allowing the soil to dry substantially. Such a treatment did lower the leaf water vapor conductance appreciably over a period of weeks (Fig. 5), an example of a long term influence of water status on g_{wv} (13). Similar responses of g_{wv} to gradual soil drying and hence decreasing tissue water potentials have also been observed in the field for *Opuntia basilaris* (17) and *F. acanthodes* (14). The succulent nature of the leaves of *A. deserti* (the ones used here averaged nearly 2 cm in thickness) may provide such a large water reservoir that changes in transpiration induced by changes in Δc_{wv} are an ineffectual short term stomatal control. For instance, the maximal nocturnal water loss for a leaf at 20 C exposed to a rather high Δc_{wv} of 13.8 g m^{-3} amounted to only 7% of its water content. Measurements of the components of the water potential in guard cells, adjacent epidermal cells, and the mesophyll region as well as determination of water conductances along the pathway from the soil to the leaves is necessary before the water relations of stomatal responses of this CAM plant can be fully understood.

The mechanism for the marked decrease of g_{wv} at increasing temperature for *A. deserti* in the CAM and C_3 modes is also unclear, although such stomatal closure has been observed for other succulent plants in the CAM (10, 12, 13–15) and C_3 modes (18). Moreover, it is consistent with the well known requirement of low nighttime temperatures for optimal gas exchange by CAM plants (12, 14, 18). Neales (10) proposed that the influence of temperature on stomatal opening for *A. americana* was mediated by the substomatal CO_2 concentration. This seems unlikely as the main cause in the present results for *A. deserti*, inasmuch as the temperature effects on g_{wv} are more rapid and larger than $c^s_{CO_2}$ influences (Figs. 2, 4, 6). The temperature effect on g_{wv} was changed only moderately upon reducing the external CO_2 concentration from 340 to 15 $\mu l \, l^{-1}$ (Fig. 6). Thus temperature seems to exert a direct influence on stomatal opening for *A. deserti* independent of water vapor concentration drops and CO_2 levels, the effect being greater when the plant is in the CAM mode. Understanding this temperature response, which may involve membrane properties such as changes in the fluxes of solutes across the plasmalemmas of guard cells, should provide insight into the control of stomates in CAM plants.

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