

Water Relations and Photosynthesis of a Desert CAM Plant, *Agave deserti*¹

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ABSTRACT

The water relations and photosynthesis of *Agave deserti* Engelm., a plant exhibiting Crassulacean acid metabolism, were measured in the Colorado desert. Although no natural stomatal opening of *A. deserti* occurred in the summer of 1975, it could be induced by watering. The resistance for water vapor diffusion from a leaf (R_{wv}) became less than 20 sec cm^{-1} when the soil water potential at 10 cm became greater than -3 bars, as would occur after a 7-mm rainfall. As a consequence of its shallow root system (mean depth of 8 cm), *A. deserti* responded rapidly to the infrequent rains, and the succulent nature of its leaves allowed stomatal opening to continue for up to 8 days after the soil became drier than the plant. When the leaf temperature at night was increased from 5 to 20 C, R_{wv} increased 5-fold, emphasizing the importance of cool nighttime temperatures for gas exchange by this plant. Although most CO_2 uptake occurred at night, a secondary light-dependent rise in CO_2 influx generally occurred after dawn. The transpiration ratio (mass of water transpired/mass of CO_2 fixed) had extremely low values of 18 for a winter day, and approximately 25 for an entire year.

Desert perennials must cope with extreme heat and desiccation, and yet sustain a net CO_2 uptake over a year. One of the most successful groups of plants for desert environments are the CAM² plants, characterized by diurnal fluctuations in tissue acidity and nighttime stomatal opening (6, 20, 22, 23). Although 18 families have CAM species (22), their physiology has been relatively little studied under natural conditions. CAM plants are usually found in regions having cool night temperatures (5, 13), which were shown by Nishida (16) and Ting *et al.* (24) to favor stomatal opening. Stomatal opening on cool nights minimizes transpirational loss of water, since the tissue temperatures are then lower, and hence, the water vapor concentration gradients from the leaves to the air are considerably less than daytime values. Nisbet and Patten (15) noted that the rate of CO_2 influx decreased as the pad temperature of *Opuntia phaeacantha* increased, and Lange *et al.* (11) observed a decrease in net CO_2 fixation upon heating *Caralluma negevensis* at night. In fact, the temperature optimum for dark CO_2 fixation by CAM plants is low, *e.g.* near 15 C (10, 13), although seasonal variations in temperature response can occur (15). The present study is with *Agave deserti* Engelm. (Agavaceae), a common plant in

the Colorado, Mojave, and Sonoran deserts, which can withstand frosts as well as rainless years (5, 12).

The first reports that agave stomates open at night, as is characteristic of CAM plants, were made independently by Neales *et al.* (14) and Ehrler (4). Ehrler (4) found that the transpirational water loss by *Agave americana* over a 70-day period was 71 times the gain in dry weight, representing a very good water-use efficiency (20, 22). Neales *et al.* (14) measured photosynthesis and transpiration by *A. americana* maintained in a nutrient solution and clearly demonstrated the CAM nature of this plant. Here, a number of both specific and general questions were posed for *A. deserti* in its native habitat. For instance, what is the response of the soil water potential (Ψ_{soil}) to specific amounts of rainfall? What Ψ_{soil} causes stomatal opening of *A. deserti* and how long do stomatal responses (nighttime opening) continue once the soil starts to dry? Do agave stomates open at all during the dry, hot summer? To answer such questions, biweekly field measurements of Ψ_{soil} and gas fluxes for agave leaves were made throughout 1975. Photosynthetic productivity and transpiration were then estimated over a year so that the water-use efficiency could be evaluated under natural conditions. To help provide insight into some of the adaptive strategies of this plant as well as information on water conservation by CAM plants in their native habitats, the relation between stomatal resistance of *A. deserti* and leaf temperature was also investigated.

MATERIALS AND METHODS

Plant Material. *A. deserti* Engelm. is a succulent perennial found in sandy and rocky areas below 1700 m in the western Colorado, southern Mojave, and Sonoran deserts (5, 12). Based on 12 50-m line transects set out in November 1975, *A. deserti* had a ground cover of 6.12% at the research site. In terms of above ground biomass and ground cover, it was the dominant plant (29% of the total ground cover) and had a frequency of 1 plant/1.01 m². The study site, known as "Agave Hill," has an elevation of 850 m, lies at 116° 24' west longitude and 33° 38' north latitude, and is in the University of California Philip L. Boyd Deep Canyon Desert Research Center near Palm Desert, California.

Ψ_{soil} . Based on the observed root depth for *A. deserti*, the probes for monitoring Ψ_{soil} were routinely placed 10 cm below the soil surface. Except where indicated, Ψ_{soil} was the average water potential determined at 0600 for five probes (probes agreed $\pm 8\%$) using a Wescor HR 33 dew point microvoltmeter and PT 51-05 soil thermocouple psychrometers.

Leaf Temperature, R_{wv} , and Ψ_{leaf} . Leaf temperatures were determined with a Barnes Engineering PRT-10 IR field thermometer and also using 36 British-Standard-gauge copper-constantan thermocouples along with a Biddle 72-3110 mv potentiometer. The resistance to water vapor loss from the leaves was directly measured using a Lambda Instruments LI-60 diffusive

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² Abbreviation: CAM: Crassulacean acid metabolism.

resistance porometer with an LI-20S sensor and using the leaf chamber described below. Ψ_{leaf} was determined with a PMS Instrument Scholander-type bomb using excised tips of leaves and with a soil probe inserted halfway through a leaf in a hole made with a cork borer. Tissue sap was mechanically squeezed out of leaves using a vise, and its osmotic pressure was measured with an Advanced Instruments 31LAS osmometer.

Gas Exchange. Gas fluxes were measured using a Lucite chamber 1 cm high with an opening 2×5 cm sealed onto a leaf with Terostat VII (Teroson GmbH., Heidelberg). Ambient air was pumped through the chamber at $3.33 \text{ cm}^3 \text{ sec}^{-1}$ and the flow rate was monitored at various locations to insure against leaks. The water vapor contents of the incoming and the outgoing air were determined using Cambridge Systems EG & G 880 dew point hygrometers. The water vapor concentration of the outgoing air minus that of the incoming times the flow rate divided by the tissue area exposed within the chamber (10 cm^2) gives the flux of water vapor out of a leaf (J_{wv}). The concentration of water vapor within a leaf (assumed to be the saturation value at the leaf surface temperature) minus that in the ambient air (Δc_{wv}) divided by J_{wv} equals R_{wv} . Using wet filter paper, the boundary layer resistance for the leaf chamber was found to be 0.5 sec cm^{-1} . The porometer measures the resistance of the plant tissue only, since the boundary layer resistance is corrected for in the calibration procedure. Hence, a water vapor boundary layer resistance of 0.5 sec cm^{-1} was added to the porometer readings to give an R_{wv} consistent with the leaf chamber and appropriate for field conditions. The leaf chamber was generally mounted on the inner or adaxial leaf surface and the porometer sensor on the abaxial side. At a given location, similar R_{wv} s were obtained with either technique for both open and closed stomata conditions, but values averaged 10% higher for the abaxial surface (which had a lower stomatal frequency).

The CO_2 concentrations of the gases entering and leaving the leaf chamber were measured with a Beckman 215A IR gas analyzer calibrated with CO_2 standards prepared by Liquid Carbonic Corp. J_{CO_2} was calculated analogously to J_{wv} . Illumination was determined with a Spectra FC 200-TV-B illumination meter, and total hemispherical solar irradiance with a Moll-Gorczynski pyranometer, the sensors being placed in a horizontal plane near the plant. The term "dawn" used below was arbitrarily defined as the time on a cloudless day when the illumination had risen to 40 lux and the solar irradiance to 0.8 w m^{-2} .

RESULTS

Plant and Soil Characteristics. Morphological parameters for the 11 *A. deserti* plants on which gas exchange or stomatal measurements were made are presented in Table I. Leaf wet weights were determined for plants after Ψ_{soil} had been about -0.1 bar (-10^4 Pascals) for 10 days. Counts of stomatal frequencies include some stomata in crypts, and are similar to those for *A. americana* (4). Under the rather thick cuticle ($20\text{--}45 \mu\text{m}$) was a single epidermal layer followed by about 15 Chl-containing mesophyll cell layers on the adaxial surface and 13 on the abaxial one. Vascular, storage, and support tissue occurred between the Chl-containing cells and led to an over-all thickness of 0.65 cm at midleaf. The mesophyll cells were 64 ± 4 (SD) μm in length, $28 \pm 2 \mu\text{m}$ in width, and touched over approximately 16% of their surface area. The area of mesophyll cell walls exposed to the intercellular air spaces per unit leaf surface area (17) was 96 for the adaxial side and 80 for the abaxial one.

The soil was formed from disintegrating granite and had a relatively high gravel and sand content with a low content of organic matter and clay. Based on eight soil samples taken at a depth of 10 cm, the gravel content (particles $>2\text{mm}$) was $24.1 \pm 3.1\%$ (SD) of the soil by weight (3). The particle size distribution determined by the pipette method (3) for the remaining soil was

$76.7 \pm 1.8\%$ sand ($0.05\text{--}2 \text{ mm}$), $16.9 \pm 1.9\%$ silt ($0.002\text{--}0.05 \text{ mm}$), and $6.4 \pm 1.1\%$ clay ($<0.002 \text{ mm}$), which placed the soil on the borderline between the textural categories known as "sandy loam" and "loamy sand" (7). The organic matter determined by the Walkley-Black method (1) was $0.4 \pm 0.2\%$ of the nongravel fraction. Soil samples taken at 2 cm and 20 cm had a composition and particle size distribution within the standard deviations determined for 10 cm. The region of the soil infiltrated by roots could be represented by a circle extending 49 cm radially from the base of the mature plants (Table I) used in this study. Other root properties, including their depth below the soil surface, are summarized in Table I.

Watering. Biweekly field measurements of R_{wv} over 24-hr periods gave no indication of natural stomatal opening for *A. deserti* in the summer of 1975. However, stomatal movements at such a hot and dry time of year could be induced by watering (Fig. 1). Ψ_{soil} before watering was less than -90 bars, the limit of sensitivity of the transducers used. A total of 115 kg of water, equivalent to a water depth of 20 mm, was uniformly sprinkled from 0800 to 1200 (Fig. 1) on the ground in a circle 2.7 m in diameter centered on a plant, which had 29 leaves, a mean above ground diameter of 0.57 m, and essentially all of its roots within a circle 1 m in diameter. This watering raised Ψ_{soil} to -0.1 bar by 1800 and led to partial stomatal opening that night, when R_{wv} decreased about 10-fold to 90 sec cm^{-1} (Fig. 1). The soil remained wet through the following day, and an even greater stomatal opening occurred the next night in that R_{wv} had a minimum value of 11 sec cm^{-1} (Fig. 1). The initial R_{wv} for this plant was between 800 and 900 sec cm^{-1} , indicating that the

Table I. Summary of Plant Characteristics
Data are presented as average \pm SD (no. of samples).

Leaves	
Number/plant	29 ± 4 (11)
Surface area (both sides)	380 ± 44 (31) cm^2
Length	30.0 ± 2.6 (50) cm
Wet weight	348 ± 33 (25) g
Dry weight	47 ± 4 (15) g
Stomatal pore length	27 ± 4 (9) μm
Stomatal frequency	
adaxial surface	32 ± 2 (25) mm^{-2}
abaxial surface	30 ± 3 (25) mm^{-2}
Chlorophyll	
adaxial side	93 ± 11 (10) $\mu\text{g cm}^{-2}$
abaxial side	87 ± 10 (10) $\mu\text{g cm}^{-2}$
Roots	
Number/plant	88 ± 16 (7)
Length	46 ± 11 (77) cm
Diameter at mid-length	2.9 ± 0.8 (47) mm
Depth below soil surface	
mean	7.8 ± 2.9 (41) cm
maximum	15.4 ± 5.9 (41) cm

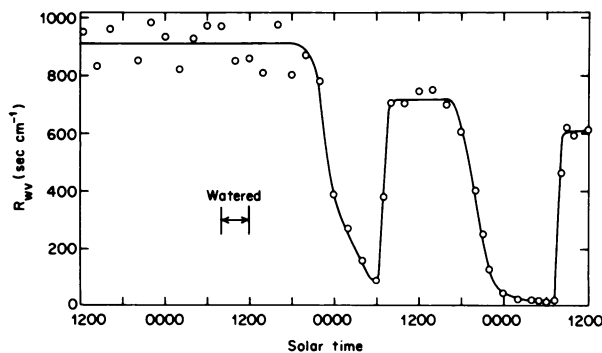


Fig. 1. Stomatal response of *A. deserti* to summertime watering. Twenty mm of water were uniformly sprinkled on the ground about an agave plant on August 10, 1975, a representative clear day when the noontime solar irradiance was 1040 w m^{-2} , the maximum air temperature of 40.8 C occurred at 1430, the minimum temperature of 28.6 C at 0430 (dawn), and the water vapor content of the ambient air ranged from 5.1 to $8.2 \mu\text{g cm}^{-3}$.

cuticle was rather impervious to water. The maximum resistance decreased to about 700 sec cm^{-1} the day after watering, and 600 sec cm^{-1} 2 days later (Fig. 1), probably reflecting a decline in cuticular resistance following water uptake by the plant. The latter R_{wv} was fairly representative of average daytime values for the part of the year when stomatal opening occurred at night for *A. deserti*.

To investigate the dependence of stomatal opening on Ψ_{soil} , potted agave plants in initially dry soil were given carefully controlled amounts of water daily so that Ψ_{soil} was maintained within a narrow range. After 5 days, R_{wv} was measured hourly beginning at 0500 and continuing until stomatal activity ceased (generally 1000), and the minimum R_{wv} is plotted for each Ψ_{soil} in Figure 2. Diurnal changes in R_{wv} were not apparent until Ψ_{soil} became greater than -3 bars, and maximal stomatal opening (minimum $R_{wv} < 10 \text{ sec cm}^{-1}$ for some leaf) did not occur until Ψ_{soil} was greater than -1.2 bars.

Two days after diurnal stomatal movements had ceased due to drying of the soil, Ψ_{leaf} at 25°C was -6.6 ± 0.9 (7) bars determined with a soil probe inserted into a leaf and -5.6 ± 0.7 (8) bars measured at the leaf tip with a Scholander bomb (data are presented as average \pm SD [no. of measurements]). After being maintained for 5 days with $\Psi_{\text{soil}} > -1$ bar, Ψ_{leaf} at 25°C increased to -3.9 ± 0.6 (7) bars determined with soil probe and -3.7 ± 0.5 (8) bars measured with a Scholander bomb. The osmotic pressure of leaf sap (π_{leaf}) was 6.5 ± 0.3 (7) bars for the dry soil condition and 6.0 ± 0.3 (7) bars for a wet soil. The 2- to 3-bar increase in Ψ_{leaf} upon watering with little change in π_{leaf} presumably reflects the generation of a hydrostatic pressure in the leaf cells. When measurements were made on a plant that had experienced a Ψ_{soil} below -90 bars for 5 months, π_{leaf} was 13.4 ± 1.4 (6) bars and Ψ_{leaf} was -13.8 ± 1.5 (6) bars measured with a Scholander bomb. For such a plant, Ψ_{leaf} at $24 \pm 2^\circ\text{C}$ determined at 0600 using an embedded soil probe was initially -13.6 bars, became -11.9 bars 12 hr after watering (at 1800 the previous day), -6.5 bars 36 hr after watering, and -4.3 bars 60 hr after watering (Ψ_{soil} remained about -0.1 bar throughout); R_{wv} was initially 760 sec cm^{-1} , became 550 sec cm^{-1} 12 hr after watering, 38 sec cm^{-1} 36 hr after watering, and 11 sec cm^{-1} 60 hr after watering, rather consistent with the results presented in Figure 1. Water uptake into the leaves, which was evident 12 hr after watering commenced, thus continued for a few days.

Rainfall. Since stomatal opening of *A. deserti* depended on Ψ_{soil} (Fig. 2), the soil water potential resulting from a given amount of rainfall was determined for an initially dry soil (Ψ_{soil}

≤ -90 bars). Areas of approximately 0.6 m^2 were uniformly sprinkled with water at 1800 on various days in September 1975 and Ψ_{soil} at 10 cm was measured at 12-hr intervals. The maximum Ψ_{soil} was achieved at 12 hr for an application of 19 mm water and at 36 hr for an 0.65-mm watering. Such maximum water potentials are plotted versus the depth of water applied in Figure 3. Four natural rainfalls (0.2, 1.1, 3.5, and 19.3 mm) occurring on dry soil during these studies gave values consistent with those created artificially by watering (Fig. 3). To raise Ψ_{soil} above -3 bars for an initially dry soil and thus induce stomatal opening of *A. deserti* (Fig. 2) requires a rainfall exceeding 7 mm (Fig. 3).

Daily measurements of Ψ_{soil} and minimum R_{wv} were made on plants having 25 to 30 leaves, which were placed in a greenhouse during September and October 1975, to determine whether stomatal opening ceased once Ψ_{soil} went below a certain value. Upon increasing Ψ_{soil} from less than -90 bars to -0.1 bar, stomatal opening was induced, consistent with field results. After 4 days, Ψ_{soil} was allowed to drop below -3 bars (not shown). Stomatal opening continued to occur for 2 more days, as evidenced by R_{wv} going below 20 sec cm^{-1} at some time of the day (for at least one of the three leaves examined). Next, Ψ_{soil} was maintained greater than -3 bars for a longer period, 10 days (Fig. 4). Stomatal opening for one of the three leaves monitored continued for 5 days after Ψ_{soil} went below -3 bars. In this case,

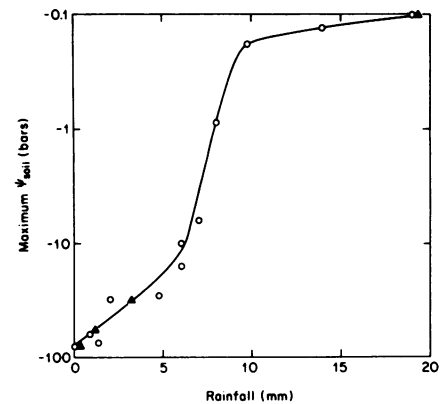


FIG. 3. Relationship between the depth of water applied to dry flat ground at Agave Hill and the resulting soil water potential at 10 cm below the surface. \blacktriangle : values for natural rainfalls on dry ground ($\Psi_{\text{soil}} \leq -90$ bars).

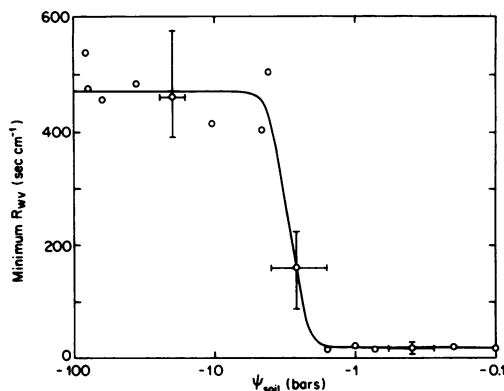


FIG. 2. Relationship between the soil water potential maintained for 5 days and the maximum degree of stomatal opening observed on the last day of that period. Horizontal bars represent the range of daily Ψ_{soil} about the average; vertical bars give the range around the mean in minimum R_{wv} for five leaves on the same potted plant.

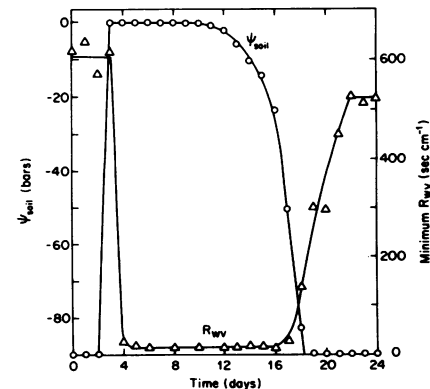


FIG. 4. Time course for change in minimum daily stomatal resistance as a wet soil dries out. A potted plant in an initially dry soil was watered at 1400 on the 2nd day and the following 6 days (Ψ_{soil} determined at 0600), after which it was allowed to dry out. R_{wv} was determined hourly for three leaves from 0500 to 0800 (when Δc_{wv} between the leaf and the air averaged $3.9 \mu\text{g cm}^{-3}$) and the minimum value is plotted.

R_{wv} ceased going below 20 sec cm^{-1} when Ψ_{soil} was about -22 bars (Fig. 4). When *A. deserti* was maintained for 21 days in a wet soil, stomatal opening continued for 7 days after Ψ_{soil} fell below -3 bars, the last day having a Ψ_{soil} less than -90 bars. Thus, cessation of diurnal stomatal opening caused by drying of the soil was influenced by how many days the soil had been maintained in a wet condition.

Next, the measured rainfalls at Agave Hill will be correlated with the predicted and observed changes of Ψ_{soil} and R_{wv} in the field. Stomatal opening of *A. deserti* would be expected in response to the winter rains from mid-November 1972 through February 1973. Based on field observations indicated below, a rainfall total of 36.6 mm throughout March 1973 would ensure stomatal opening to approximately mid-May. The next rainfall (27 mm) did not occur until August 3, 1973, which, together with subsequent rainfalls that month totaling 12.4 mm, would lead to stomatal opening through mid-September. The next major rainfall (27.4 mm) causing R_{wv} to go below 20 sec cm^{-1} was on January 5, 1974, followed by an additional 44.2 mm would have led to stomatal opening to about mid-April. Based on the response of Ψ_{soil} to rainfall (Fig. 3), the next precipitation reducing R_{wv} below 20 sec cm^{-1} (Fig. 2) was 12.9 mm on October 23, 1974. A subsequent total of 44.1 mm would have led to stomatal opening through mid-March 1975, after which the stomatal movements would not be significant until a rainfall of 28.8 mm on April 9, 1975. On May 23, Ψ_{soil} was -21 bars and a minimum R_{wv} of 19 sec cm^{-1} was measured for *A. deserti*, i.e. appreciable stomatal opening occurred for 44 days after the 28.8-mm rainfall. By June 10, stomatal opening had ceased ($R_{wv} > 600$ sec cm^{-1} over a 24-hr period) and Ψ_{soil} had dropped below -90 bars. Although Ψ_{soil} increased to a maximum of -32 bars following a September 19 rain of 3.5 mm (Fig. 3), the next stomatal opening of *A. deserti* did not occur until a 19.3-mm rain on November 28, 1975 (minimum R_{wv} of 12 sec cm^{-1} on November 29). The soil stayed wet ($\Psi_{soil} > -3$ bars) until about January 16, 1976, aided by December rains totaling 8.0 mm.

Table II presents data on the final part of the January 1976 drying period for agave plants at two different locations. Ψ_{soil} fell more rapidly for a well drained south facing slope compared with flat ground, becoming less than -3 bars on about January 14 in the former case, and 4 days later for the flat ground. The minimum R_{wv} became greater than 20 sec cm^{-1} on about January 22 for the plant on the south facing slope, and on January 25 for the one in flat ground (Table II), consistent with the time course observed for potted plants after Ψ_{soil} fell below -3 bars. Stomatal opening where R_{wv} decreased below 20 sec cm^{-1} here occurred for 55 ± 2 days in response to a total rainfall of 27.3 mm.

Transpiration and Photosynthesis. Environmental parameters and gas fluxes were monitored over a 24-hr period on a winter day when the soil was wet following the substantial rainfall on November 28, 1975. The maximum solar irradiance at noon of 590 $w m^{-2}$ (Fig. 5) corresponded to an illumination of 62 klux. The leaf surface temperature reached a maximum of 25 C near 1400 and fell to a low of 2.6 C at 0600 (near dawn, Fig. 5). During most of the daylight period, changes in J_{wv} corresponded

to those in Δc_{wv} , since R_{wv} ($= \Delta c_{wv}/J_{wv}$) was constant at about 500 sec cm^{-1} . At night, stomatal opening led to an increased water vapor efflux, R_{wv} decreasing to 41 sec cm^{-1} when the solar irradiance became negligible at 1830 and then remained near a minimum of 6.8 sec cm^{-1} throughout most of the night (Fig. 5). Stomatal opening was accompanied by a net influx of CO_2 , which reached a maximum of 0.55 $nmol cm^{-2} sec^{-1}$ at about 2200. J_{CO_2} steadily decreased until 0600, then rose about 30% to a secondary peak at 0730, after which it declined to zero at 1000 (Fig. 5).

The secondary rise in J_{CO_2} , which had a peak about 1 hr after dawn, was observed on five of six occasions monitored in December 1975 and January 1976. It did not occur on the morning of January 16, 1976, an especially warm period during which the leaf tissue temperature did not drop below 10.3 C. Also, the secondary increase in J_{CO_2} was prevented on December 18, 1975, by directing heated air at the leaf used for Figure 5 so that the tissue temperature was always above 12 C.

Figure 6 presents data on another experiment where heating the leaf by 8.4 C (above that of a leaf which averaged 0.9 C cooler than the one in Fig. 5) eliminated the secondary rise, as J_{CO_2} decreased to zero by dawn and then remained negligible thereafter. This decrease in J_{CO_2} was accompanied by a steady rise in R_{wv} from 6.4 sec cm^{-1} at 0400 to 430 sec cm^{-1} at 0624, with similarly high resistances continuing thereafter. Eliminating the sunlight incident on a leaf by means of an opaque cover prevented air movements and caused a change in leaf surface temperature, so porous screening was used. Reducing the light by 95% without materially changing the leaf temperature essentially eliminated the secondary increase in J_{CO_2} occurring after dawn, although considerable net photosynthesis still took place after 0600 (Fig. 6). R_{wv} for the darkened leaf increased from 8 sec cm^{-1} at 0410 to 17 sec cm^{-1} at 0540, remained fairly constant until 0810, and then increased to 300 sec cm^{-1} by 0910. The "normal" leaf exhibited a secondary rise in J_{CO_2} leading to a peak near 0730, which coincided in time with a local minimum in R_{wv} of 7.4 sec cm^{-1} . Thus, increased illumination accompanied by lowered stomatal resistance appeared to be responsible for the rise in net photosynthesis after dawn.

The closure of the stomates caused by heating (Fig. 6) was investigated at a series of leaf temperatures (Fig. 7). To avoid

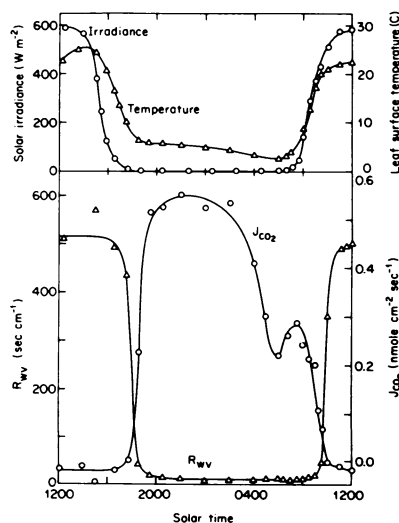


FIG. 5. Solar irradiance, leaf temperature, and gas fluxes for an agave leaf in the wintertime. Data were obtained on clear days, December 16 and 17, 1975. Leaf surface temperature was measured at midleaf adjacent to a leaf chamber mounted on the adaxial side. The water vapor content of the ambient air was 2.0 to 3.9 $\mu g cm^{-3}$, the maximum air temperature of 18.6 C occurred at 1300, and the minimum of 6.9 C at 0550.

Table II. Changes in Minimum Stomatal Resistance and Soil Water Potential during a Rainless Drying Period

Date	Plant on 8° slope facing south-southeast		Plant in flat ground	
	Minimum R_{wv} sec cm^{-1}	Ψ_{soil} bars	Minimum R_{wv} sec cm^{-1}	Ψ_{soil} bars
January-1976				
8	8.6	-1.0	7.8	-0.7
12	8.0	-2.4	7.9	-1.4
16	8.4	-3.9	8.3	-2.0
20	12.7	-5.8	9.1	-3.8
24	25.6	-8.6	13.0	-5.0
27	48.3	-17.6	34.1	-7.9

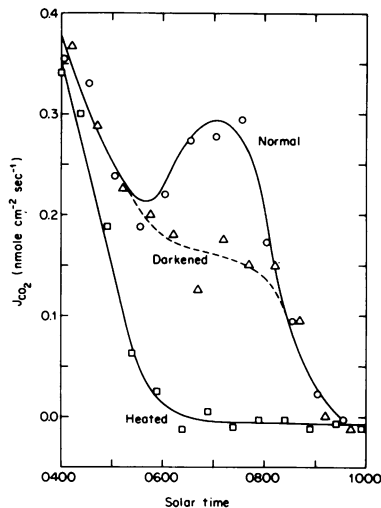


FIG. 6. Factors affecting the secondary rise in net photosynthesis occurring after dawn. "Darkened" refers to a leaf covered by porous neutral density screens such that the solar irradiance was reduced 95%, but the surface temperature was within ± 0.8 C of the leaf under natural conditions ("Normal"). "Heated" refers to a leaf whose temperature was raised 8.4 ± 1.1 C by directing heated air at it beginning at 0430. Data were for three leaves on one plant on December 30, 1975, under environmental conditions similar to those for Figure 5.

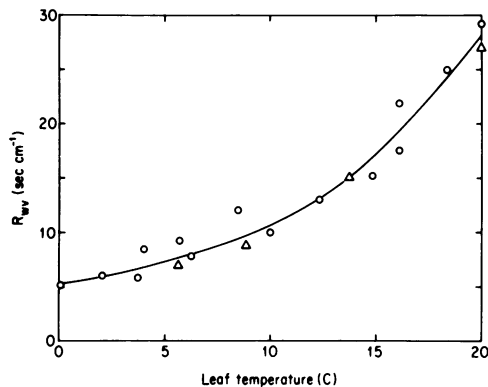


FIG. 7. Effect of leaf temperature on stomatal resistance. Four leaf chambers were used on the nights of January 5 to 7, 1976, and heated air was directed at the leaves to vary their temperatures. The water vapor content of the air entering the leaf chamber was 2.6 to $3.4 \mu\text{g cm}^{-3}$ (\circ) or adjusted by passing a fraction of the incoming air over moistened filter paper so that Δc_{wv} from leaf to air was $5.2 \pm 0.2 \mu\text{g cm}^{-3}$ (Δ).

complications due to illumination, experiments were performed between 2300 and 0400. Leaves were maintained at a given temperature ± 0.5 C for 30 min before determining J_{wv} . As the leaf temperature was raised from 0.1 to 20.0 C, R_{wv} increased from 5.2 sec cm^{-1} to 29 sec cm^{-1} . Simply heating the air directed at the leaves also causes Δc_{wv} to vary, and hence the leaf-to-air water vapor concentration drop was maintained at $5.2 \mu\text{g cm}^{-3}$ while the leaves were heated (triangles in Fig. 7). This led essentially to the same changes in R_{wv} , indicating that the stomata were responding primarily to temperature. The net rate of photosynthesis was $0.52 \text{ nmol cm}^{-2} \text{ sec}^{-1}$ at 5.8 C, 0.52 at 10 C, 0.40 at 14.8 C, and $0.28 \text{ nmol cm}^{-2} \text{ sec}^{-1}$ at 20 C.

DISCUSSION

Plant-Soil Water Relations. Four aspects of the water relations of *A. deserti* deserve special emphasis: (a) the root system is shallow, having a mean depth of about 8 cm, which means it can respond quickly to rainfall; (b) the sandy soil in which it grows can remain wet ($\Psi_{\text{soil}} > -3$ bars at 10 cm) for 43 ± 7 days

after 28 ± 1 mm of winter or spring rainfall; (c) the succulent nature of the leaves allows water storage so that stomatal opening can occur after the soil becomes drier than the leaves; and (d) the plant does not readily lose water to a dry soil. Another CAM plant growing in the same area, *Opuntia basilaris*, showed water uptake in response to a 6-mm rainfall (20), similar to the requirement (7 mm) for *A. deserti* (Fig. 2). *A. deserti* responded in about 12 hr to rainfall, with a nearly full stomatal opening occurring in 48 hr (Fig. 1), whereas *O. puberula* formed new roots within hours after watering (9) and water uptake by *O. basilaris* took place 24 to 36 hr after a rainfall (20, 21).

In general, for water to move from the soil to the leaves, Ψ_{leaf} should be lower than Ψ_{soil} . Agave plants exhibiting diurnal stomatal opening had a Ψ_{leaf} of -3.8 bars, consistent with the observation that water was not effectively taken up from the soil until Ψ_{soil} was above -3 bars (Fig. 2). When a wet sandy soil begins to dry, Ψ_{soil} will initially remain close to 0 bars because of the small surface area per unit volume and large pores, whereas in a clay of the same water content, Ψ_{soil} can be quite negative (7) due to the many more interfaces present in clays. The water retention at a high Ψ_{soil} by sandy soils is important for water uptake by *A. deserti*, since its high Ψ_{leaf} (-3.8 bars) means that it is unable to take up water from a soil once Ψ_{soil} drops below this rather high value. Potted agave plants in wet soil for 4, 10, or 21 days had stomatal opening (minimum R_{wv} below 20 sec cm^{-1}) for 2, 5, or 7 days, respectively, after Ψ_{soil} went below -3 bars (see Fig. 4). In field studies where Ψ_{soil} was above -3 bars for about 50 days, stomatal opening continued for 8 days after the soil had become drier than the plant (Table II). In summary, both the soil water retention at high Ψ_{soil} and the water available in the succulent leaves can allow stomatal opening and hence gas exchange to proceed for a considerable period after a rain. As the soil dries and Ψ_{soil} becomes less than Ψ_{leaf} , the plant will lose water to the soil. The fact that this occurs slowly compared with the reverse process suggests that the regions of the root where appreciable water is absorbed die back when Ψ_{soil} decreases, the water permeability of the entire root decreases, and/or air gaps develop between the root and the soil particles thereby limiting water loss, aspects that need further investigation.

Stomatal Movements and Fluxes. When agave leaves were heated above 15 C, R_{wv} increased to such an extent (Fig. 7) that transpiration at the accompanying higher water vapor gradients actually decreased. Using the data of Neales (13) on *A. americana* growing in nutrient solution with a constant daytime temperature of 25 C, but variable night temperatures of 15, 25, or 36 C, the average R_{wv} for the nighttime can be calculated. As the leaf temperature increased, R_{wv} went from 16 to 34 to 164 sec cm^{-1} , similar to the present results. The temperature effect on the water vapor concentration within a leaf, which affects the leaf-to-air water vapor concentration drop (Δc_{wv}), should be distinguished from other influences of temperature on stomatal movements. Increases in Δc_{wv} at constant temperature cause stomatal closure in other plants (18, 19), but apparently, there was little influence of Δc_{wv} on R_{wv} for *A. deserti*, since essentially all of the increase in R_{wv} caused by heating occurred when Δc_{wv} was kept constant (Fig. 7). Hence, there is another and more crucial influence of temperature on stomatal opening for *A. deserti*, possibly related to changes in the internal CO_2 concentrations (13). Thus, the common observation that CAM plants fare better at cool night temperatures (5, 10, 11, 13, 15, 16, 24) may reflect not only the acute water conservation requirements for these arid region plants, but also metabolic factors such as enzyme rates, internal CO_2 levels, and possibly other effects of temperature on guard cell physiology.

The maximum CO_2 uptake for *A. deserti* (Fig. 5) occurred nearly 4 hr after sunset, similar to the J_{CO_2} pattern observed for another CAM plant, *Caralluma negevensis*, under natural conditions (11). After the maximum, J_{CO_2} progressively decreased through the night for both plants. However, a secondary rise in

J_{CO_2} occurred after dawn (Figs. 5 and 6). In the morning before appreciable solar irradiation, the leaf temperature did not rise substantially from its nighttime minimum, and so the slight additional stomatal opening accompanying the secondary rise in J_{CO_2} occurred before Δc_{wv} from the leaf to the air became large, and hence transpiration excessive. The observations that the light of sunrise can lead to increased stomatal opening and increased J_{CO_2} for *A. deserti* (Fig. 6) have also been made with other CAM plants, especially when water is not limiting (2, 10, 11, 16, 22). In fact, heavily watering *A. deserti* for 12 weeks under laboratory conditions can cause it to shift its stomatal movements so that 97% of the CO_2 uptake occurs during the daytime (6).

Minimum stomatal resistances are generally 2 to 10 sec cm^{-1} for CAM plants (20), consistent with the minimum R_{wv} of 5 sec cm^{-1} for *A. deserti*. In the daylight throughout the dry part of the year, R_{wv} was about 900 sec cm^{-1} (Fig. 1), similar to the cuticular resistances reported for three *Opuntia* species: 620 sec cm^{-1} for *O. basilaris* and *O. acanthocarpa*, 1020 sec cm^{-1} for *O. bigelovii* (22). Neales *et al.* (14) reported a maximum dark CO_2 uptake rate of 0.74 $nmol\ cm^{-2}\ sec^{-1}$ for *A. americana*, similar to that observed for *A. deserti* (0.55 $nmol\ cm^{-2}\ sec^{-1}$, Fig. 5). An upper limit for the mesophyll resistance for CO_2 fixation ($R_{CO_2}^{mes}$) can be estimated using data in Figure 5, where the maximum CO_2 uptake actually occurred when R_{wv} was 8.3 sec cm^{-1} . By equating the gas phase resistance for CO_2 to 1.56 R_{wv} (17) and assuming that the internal CO_2 concentration is negligible (which overestimates $R_{CO_2}^{mes}$), $R_{CO_2}^{mes}$ is 11.9 sec cm^{-1} for *A. deserti*. Multiplying the estimated $R_{CO_2}^{mes}$ by the ratio of exposed mesophyll surface area per unit leaf area (an A^{mes}/A of 96 for the adaxial surface considered here), the CO_2 resistance expressed per unit cell wall area ($R_{CO_2}^{cell}$, Ref. 17) would be 1140 sec cm^{-1} , a rather large value. Apparently, the very high A^{mes}/A for *A. deserti* (A^{mes}/A is generally 15–30 for C_3 species, Ref. 17) is necessary to reduce its mesophyll resistance so that an adequate CO_2 influx occurs. It remains for future research to understand fully the relations between $R_{CO_2}^{cell}$, actual CO_2 concentrations, daytime illumination, and nighttime temperature for nocturnal CO_2 uptake by CAM plants.

Annual Transpiration. By combining the data on water-retention properties of the soil and water storage by the leaves with the recorded rainfall at Agave Hill, the number of days with substantial stomatal opening can be estimated for 1973 through 1975 (see "Results"). Stomates opened significantly as evidenced by R_{wv} decreasing to 20 sec cm^{-1} or less for approximately 175 days in 1973, 172 in 1974, and 154 in 1975, or 45% of the days. More importantly, 83% of the days when stomatal opening occurred during this 3-year period were in November through April, the cooler half of the year (mean daily temperatures at Agave Hill of 13.8 C versus 26.4 C for the other 6 months) when J_{wv} would be less for a given R_{wv} . Also, the longer nights at this time would mean that a greater fraction of the diurnal cycle was available for net CO_2 uptake by a CAM plant.

Transpiration was estimated for a year period by using the stomatal resistances determined biweekly, measured leaf temperatures, and the recorded air water vapor concentrations. For the 6-month period from May 28 to November 28, 1975, during which R_{wv} was always greater than 50 sec cm^{-1} (usually about 900 sec cm^{-1}), the total water loss was 0.52 $g\ cm^{-2}$ or 198 g for a leaf of 380 cm^2 . Five months in a dry soil at this time of year would lead to a loss of 165 g $H_2O/leaf$ (just over half the leaf maximum water content of 301 g, Table I), which should cause the tissue osmotic pressure to more than double. Indeed, π_{leaf} increased from 6.1 bars for a plant in wet soil to 13.4 bars after 5 months in a dry soil. For the other 6-month period (January 1–May 28 and November 28–December 31, 1975), the water loss was 0.21 $g\ cm^{-2}$ when R_{wv} exceeded 20 sec cm^{-1} and 1.11 $g\ cm^{-2}$ for that part of the 154 days when the stomatal resistance was less than 20 sec cm^{-1} . Thus, 60% of the annual water loss

occurred during the open stomate condition favoring net CO_2 uptake, which was 19% of the year. The water loss would have been much greater had stomatal opening occurred during the summer and/or the warmer part of the day (daylight). The total water loss per year was 1.84 $g\ cm^{-2}$ or 699 g for a mature leaf of 380 cm^2 surface area. The loss/plant of 29 leaves would be 20.3 kg in 1975, which was taken up from the soil in a circle 49 cm in radius centered on the plant. Using a water density of 1 $g\ cm^{-3}$, the annual transpiration of 20.3 kg for this plant corresponds to a water depth of 26.9 mm for uniform extraction over a 49-cm radius circle. This represents 35% of the 77.7-mm total rainfall at Agave Hill in 1975, 62% of which occurred in two major storms that had considerable surface runoff. Water would also be lost due to evaporation from the soil and percolation down to lower depths. The shallow root system of *A. deserti* allows the plant to take advantage of light rainfalls and to obtain over one-third of the water annually incident within 0.5 m of its base.

Transpiration Ratio. For a representative winter day when *A. deserti* exhibited a substantial stomatal opening (Fig. 5), the total water loss from 1000 to 1800 was 57 $\mu mol\ cm^{-2}$, concomitant with a net CO_2 loss of 0.5 $\mu mol\ cm^{-2}$. Such a low net CO_2 efflux during the day had been noted by Neales *et al.* (14) for *A. americana*, and may be attributable to an internal recycling of CO_2 , which has been suggested for other CAM plants (20, 21). From 1800 to 1000 the next day, the water loss was 910 $\mu mol\ cm^{-2}$ and the net gain of CO_2 was 22.6 $\mu mol\ cm^{-2}$. The conventional transpiration ratio (mass of H_2O lost/mass of CO_2 gained) is then 18 for the 24-hr period, apparently the lowest value that has been reported (8, 20, 22, 23). Such a high water-use efficiency is a well known characteristic of CAM plants, *e.g.* the transpiration ratio is 34 (8) to 53 (14) for *Ananas comosus*, 47 to 48 for *A. americana* (4, 14), and about 50 for *O. basilaris* (20), but generally 200 to 700 for C_3 and C_4 plants (8, 20, 22), depending on the prevailing Δc_{wv} (Refs. 4 and 8 reported water loss/dry weight gain, which was here converted to a transpiration ratio consistent with the others by assuming that the dry weight was all carbohydrate).

Using the net photosynthesis of 22.6 $\mu mol\ cm^{-2}$ for the 16-hr period appropriate to December 16 to 17 (Fig. 5), allowing for shorter nights further from the winter solstice, adjusting for higher R_{wv} s as the soil dries, using 154 days for stomatal opening in 1975, and correcting for the small net CO_2 efflux (0.5 $\mu mol\ cm^{-2}$ in an 8-hr period), the net photosynthesis would be about 1700 $\mu mol\ cm^{-2}$ for the year. This corresponds to an annual production of 0.051 g carbohydrate per cm^2 . Hence, a mature agave leaf of 380 cm^2 total area would annually take up 28 g CO_2 to produce 19 g carbohydrate, which is 40% of its dry weight (47 g). Since some photosynthate would be required for root growth as well as for additional metabolic requirements during leaf development, an *A. deserti* plant at Agave Hill might increase its leaf number by about one-quarter to one-third annually, which is consistent with preliminary studies on its growth under natural conditions. Also, projection of this growth rate is consistent with observations found for other agaves, which generally live 10 to 25 years and have 60 to 80 leaves at maturity (5). Using the predicted annual net photosynthesis and water loss, the transpiration ratio for 1975 would be 699 g $H_2O/28\ g\ CO_2$ or 25. In spite of having a net photosynthesis on only 42% of the days in 1975, but losing water throughout the year, *A. deserti* apparently has the lowest annual transpiration ratio so far reported under field conditions (20, 22).

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