

THE EFFECT OF NIGHT TEMPERATURE ON CO₂ ASSIMILATION,
TRANSPIRATION, AND WATER USE EFFICIENCY IN
AGAVE AMERICANA L.

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Abstract

The CO₂ and water vapour fluxes arising from the tops of a plant of *A. americana*, growing in nutrient solution, were continuously measured at night temperatures of 15, 25, and 36°C, the day temperature being held constant at 25°C.

Night temperatures of 36°C inhibited both the large nocturnal uptake of CO₂ and the accumulation of titratable acidity. The highest rate of CO₂ assimilation by day was about half that of the maximum rate observed by night. From the observed rhythms in transpiration rate, it was inferred that high night temperatures also reversed the usual "inverted" stomatal rhythm found in *A. americana* and other crassulacean plants. Relative to measurements made at a low night temperature (15°C), high night temperatures induced greater stomatal closure at night and opening during the day. The changes in the gas-exchange pattern induced by high night temperature caused the water use efficiency of this plant to fall to values normally associated with non-crassulacean plants. It is concluded that the normal high water use efficiency of *A. americana*, and its consequent adaptation to arid climates, is causally associated with a requirement for low night temperatures.

I. INTRODUCTION

The adaptive significance of crassulacean acid metabolism (CAM) is usually described as conferring on plants the ability to grow in arid climates where cool night temperatures are found (Evans 1971; Pearcy *et al.* 1971; Ting 1971). The physiological adaptation conferring high water use efficiency comes from the ability to assimilate CO₂ at night and to reduce stomatal aperture during the day (Neales *et al.* 1968). The postulation of adaptation to low night temperatures comes mainly from the demonstration that the amplitude of titratable acidity of plant sap was greatest when night temperatures were low (Bennet-Clark 1933; Vickery 1954; Queiroz 1965), and also from the circumstantial evidence of the climates within which CAM plants grow naturally. The effects of night temperature on the patterns of day and night gas exchange in intact CAM plants have apparently not been studied.

In this paper is described the effect of night temperature on the fluxes of CO₂ and water vapour emanating from the tops of a plant of *Agave americana* L.—a species which is known to possess both a powerful CAM metabolism (Neales *et al.* 1968) and to be adapted to arid regions. The aim of these experiments was to find

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out if the effect of night temperature was to alter the gas-exchange pattern in a manner which supported the hypothesis of the adaptive significance of CAM, described above.

II. METHODS

(a) *Plant Material*

A single plant of *A. americana* (c. 8 months old, of six leaves—the largest of which was 15 cm long—and of total leaf area 189 cm²) was carefully transferred from soil to an aerated nutrient solution [half-strength Hoagland's No. 2 (Hoagland and Arnon 1938)] held in a 7-litre container. The plant was set in a square of black Perspex in a hole through which the roots were passed. The tops were then sealed off from the roots with Silastic rubber (Dow Corning, type A RTV), and were enclosed in a transparent chamber made from 25 cm diam. Perspex tube.

(b) *Gas-exchange Measurements*

Air, which was drawn from the laboratory roof and passed through two 40-gal drums, was reduced in humidity to a dew-point of 3.8–4.0°C. This was then passed through the plant chamber at a measured flow rate (c. 4.0 litres/min). The gradient of water vapour and CO₂ concentration across the chamber, induced by the plant, was measured by i.r. analysis, using two Grubb Parson SB2 analysers. From the resultant continuous records and appropriate calibration factors, the hourly fluxes of CO₂ and water vapour attributable to the plant were calculated.

(c) *Environmental Conditions*

The plant, enclosed in the assimilation chamber, was placed in a growth cabinet 10 days before the beginning of the experiment. The approximate illuminance at the top of the plant was 3500 f.c. and the light source was 30 120-W Sylvania "cool white" tubes plus 22 40-W Mazda incandescent globes. The photoperiod used was 12 hr and the day and night temperatures were independently controlled. The experimental design was such that gas-exchange measurements were made continuously over 8 days during which time the ambient day temperature was set at 25°C throughout. The night temperature (T_N) was altered in the following sequence (Table 1): 15, 15, 25, 25, 36, 36, 36, 15°C.

(d) *Measurement of Sap Acidity*

Leaves, from similar plants to that used for the gas-exchange measurements, were taken at the beginning and end of the dark period from plants presented with the same night temperatures as already described. Twenty grams of material were blended with 100 ml of water, after which the mixture was boiled for 5 min, filtered, and made up to 250 ml. Five separate 10-ml aliquots were each titrated against standardized 0.018M NaOH using phenolphthalein as an indicator. The results were expressed as moles of (monobasic) acid per gram fresh weight of tissue.

III. RESULTS

(a) *Effect of Variation in Night Temperature on the Total CO₂ and Water Vapour Exchange by Day and Night*

Table 1 gives the total water vapour and CO₂ exchange of the plant over the 8 days of the experiment in which night temperatures were changed in the sequence 15 → 25 → 36 → 15°C. Day temperatures were set at 25°C throughout this period. Over a complete 24-hr cycle, a night temperature (T_N) of 15°C induced the highest total net uptake of CO₂, which was associated with the lowest total loss of water vapour.

Night temperatures of 25 and 36°C successively decreased the total net assimilation of CO₂ and increased the total transpiratory loss of water vapour (Table 1).

Changes in gas exchange in the light and dark are shown in Figure 1 in which is plotted the mean values (from Table 1) for each of the three night temperatures used.

TABLE 1
EFFECT OF NIGHT TEMPERATURE ON TOTAL WATER VAPOUR AND CO₂ EXCHANGE OF *A. AMERICANA*
BY DAY AND NIGHT, EACH OF 12 HR DURATION

Day No.	Temp. (°C)		Total net CO ₂ exchange (mg/plant)*			Total water exchange (mg/plant)			10 ³ × WUE (24 hr)† (mg CO ₂ /mg water)	Proportion of CO ₂ fixed at night (%)
	Night	Day	By night	By day	Total	By night	By day	Total		
1	15	25	-146	-3	-149	3314	1822	5136	29.0	98
2	15	25	-160	0	-160	3467	1333	4800	33.3	100
3	25	25	-73	-5	-78	3525	2069	5594	13.9	94
4	25	25	-97	-3	-100	4768	1736	6504	15.4	97
5	36	25	+20	-39	-19	1279	5446	6725	2.8	0
6	36	25	+14	-51	-37	2504	6858	9362	4.0	0
7	36	25	+16	-60	-44	1610	8065	9675	4.5	0
8	15	25	-189	—	—	4288	—	—	—	—

* Negative values indicate CO₂ uptake; positive values indicate CO₂ output.

† Water use efficiency (mass of CO₂ assimilated per mass of water transpired) for 24-hr period.

The main effect of increasing T_N from 15 to 25°C was to decrease the night assimilation of CO₂ by 44%, with small effects only on the total day and night transpiration and on the daytime assimilation of CO₂ (Fig. 1). However, increasing T_N from 25 to 36°C

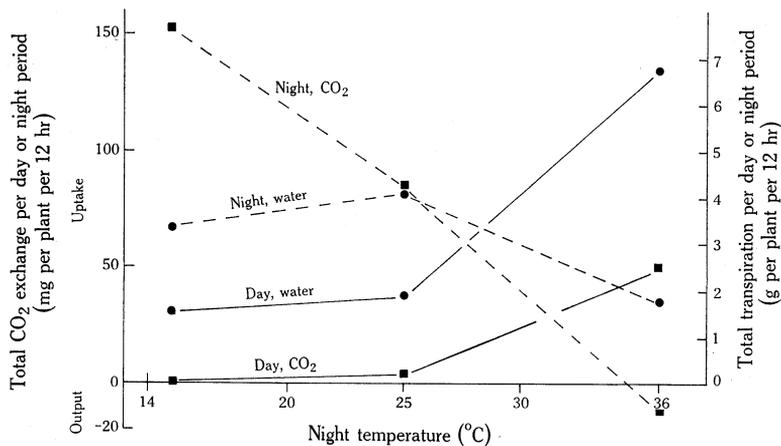


Fig. 1.—Effect of night temperature on the total day and night exchange of CO₂ and water vapour by *A. americana*. (Day and night were each of 12 hr duration and the day temperature was constant at 25°C.)

not only induced a change to a condition of a small net efflux of CO₂ by night, but induced large increases in the daytime assimilation of CO₂ and also in the daytime total transpiration; night-time total transpiration was decreased by 57%. It is of

interest that a high night temperature (36°C) evoked a daytime net assimilation of CO₂ of 50 mg per plant per 12 hr, which is approximately one-third of that assimilated at night (153 mg per plant per 12 hr) in a cool (15°C) night. The net assimilation of CO₂ from ambient air in the dark appears to have a higher potentiality than that in the light.

The changes in gas exchange (induced by changes in T_N) (Table 1, Fig. 1) have large effects on the water use efficiency (WUE) of the plant, where WUE is defined as the ratio of the mass of CO₂ assimilated to the mass of water transpired (column 10, Table 1) and is the inverse, therefore, of the transpiration ratio. For nights of 15°C the mean value of WUE, over a complete 24-hr cycle, is 0·0311 mg CO₂/mg water, whilst when $T_N = 36^\circ\text{C}$, WUE = 0·0039 or 12·5% of that for the cool night temperature. The high value of WUE for $T_N = 15^\circ\text{C}$ (0·0311) corresponds to a transpiration ratio of 32·2, which agrees with values for CAM plants previously published (Joshi *et al.* 1965; Neales *et al.* 1968). At $T_N = 36^\circ\text{C}$, the value of 0·0039 for WUE corresponds to a transpiration ratio of 256 which is within the range reported for non-CAM plants (Slatyer 1964). The results reported above indicate that, in *A. americana*, low night temperatures are causally associated with high water use efficiencies.

(b) Effect of Night Temperature on Titratable Acidity in Sap

The sap from the leaves of plants, held under the same temperature regime as those used for gas-exchange measurements, was extracted and the acidity measured by titration. This was done at the end of the 12-hr dark period and at the end of the subsequent 12-hr day period. The results (Table 2) indicate that, for $T_N = 15^\circ\text{C}$, the

TABLE 2
EFFECT OF NIGHT TEMPERATURE ON THE TITRATABLE ACIDITY IN SAP OF
A. AMERICANA LEAVES, MEASURED AT THE END OF THE NIGHT AND OF THE DAY

Temperature (°C)		Titratable acidity ($\mu\text{moles acid/g fresh wt.}$):	
Night	Day	At end of night*	At end of day*
15	25	222 (100)	20 (100)
25	25	176 (79)	33 (165)
36	25	41 (18)	41 (205)

* The light and dark periods were each of 12 hr duration. Values in parentheses are relative values.

titratable acidity at the end of the night is approximately 10 times that at the end of the day, whereas for $T_N = 36^\circ\text{C}$, the end-of-night value is approximately 0·2 times that for $T_N = 15^\circ\text{C}$, and also that this is the same value as that found at the end of the 25°C day. This indicates that high night temperatures, as expected (Bennet-Clark 1933), eliminate the night-time formation of titratable acidity. The significance of the increase in the end-of-day value of titratable acidity as night temperature is raised (Table 2) may be that in the absence of the night-time accumulation of acidity ($T_N = 36^\circ\text{C}$) the daytime formation of acid is not inhibited (Avadhani *et al.* 1971; Kluge 1971). These authors suggested that in "high malate" CAM plants the daytime formation of malate was inhibited, and vice versa.

(c) Pattern of Gas Exchange by Day and Night

The gross effects of night temperature (T_N) on CO₂ and water vapour exchange given in Table 1 and Figure 1 can be amplified and, to some extent, "explained" by an examination of the hourly rates of CO₂ exchange and transpiration over the 12-hr light and dark period. Figure 2 shows the trends in CO₂ and water vapour exchange

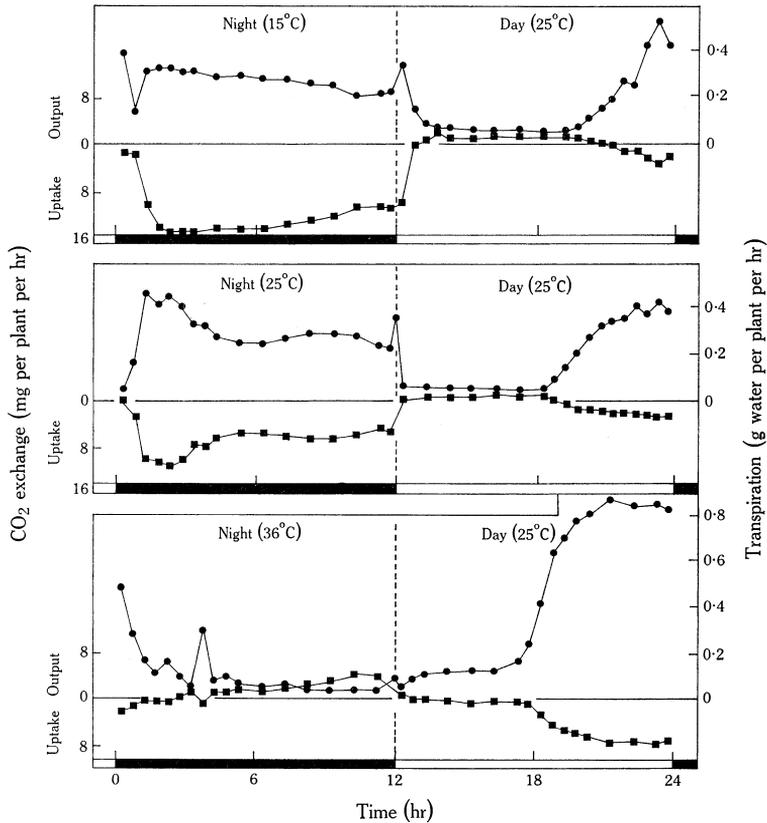


Fig. 2.—Effect of three night temperatures on the patterns of CO₂ (■) and water vapour exchange (●) of *A. americana*, by day and night.

for days 1, 3, and 5 (Table 1), with $T_N = 15, 25,$ and 36°C respectively. The correlation of the changes in CO₂ influx and water efflux is apparent at all three night temperatures, indicating that stomatal movement and CO₂ exchange are interrelated, as has been noted before (Neales *et al.* 1968). For night temperatures of 15 and 25°C the patterns are similar: CO₂ influx at night corresponds with high rates of night-time transpiration and therefore also with open stomata. Stomatal closure takes place at the beginning of the 25°C day, persisting for the first 7¼ hr after a 15°C night and for the first 6¼ hr after a 25°C night, during which periods a small daytime CO₂ efflux accompanies the low (approximately one-quarter of the night-time rate) daytime transpiration rates. After 7¼ and 6¼ hr respectively the transpiration rate increases and CO₂ influx begins; stomatal opening is accompanied by the initiation of net photosynthesis.

The pattern induced by a T_N of 36°C is qualitatively different at night and quantitatively different during the day. In 36°C nights a persistent low net efflux of CO_2 is accompanied by a low transpiration rate having a minimum value of *c.* 0.25 times that at the two higher night temperatures (Fig. 1). It may be inferred therefore that this treatment induces a degree of night-time stomatal closure. The daytime gas exchange following a 36°C night is characterized by an initially (0–5¼ hr) very small rate of CO_2 influx and a higher rate of transpiration than that which occurred in similar periods after nights of 15 and 25°C. Apparently the stomatal closure at the beginning of the day following a 36°C night was less complete than that following the two cooler nights. After 5¼ hr of light, the rates of transpiration and CO_2 influx subsequently induced were obviously much greater than in the days following the cooler nights of 15 and 25°C. It may be inferred, therefore, that the induction of relatively high rates of CO_2 influx was accompanied by a larger stomatal aperture.

(d) *Effect of Night Temperature on the Pattern of Water Vapour Efflux and Stomatal Aperture*

Direct observation of stomatal aperture in *A. americana* is difficult as the stomatal pore is sunk below the leaf surface (Renner 1910). However, the rhythm of the transpiration rate of plants held under standard conditions allows some semi-quantitative observations to be made. These are based on the assumption that the main

TABLE 3
MEAN NIGHT-TIME TRANSPIRATION RATES AND THE CALCULATED APPROXIMATE
LEAF-TO-AIR GRADIENT (Δe) OF ABSOLUTE HUMIDITY

Leaf temp. (°C)	Absolute humidity (e) (g water/m ³):		Δe (g water/m ³)†	Mean night-time transpiration per plant (mg/12 hr)†
	Leaf*	Ambient		
15	12.83	6.12	6.71 (1.00)	3390 (1.00)
25	23.05	5.91	17.14 (2.55)	4146 (1.22)
36	41.75	5.70	36.05 (5.37)	1798 (0.53)

* Calculated from estimated leaf temperature (assumed to be equal to ambient cabinet temperature) and Smithsonian Tables.

† Values in parentheses are relative values.

variable affecting transpiration rate in these experiments is stomatal aperture. The salient relevant observations (Table 3) are as follows:

- (1) The increase in night-time transpiration rate, as T_N is increased from 15 to 25°C, is considerably less than would be expected from the increase induced in water vapour pressure gradient from leaf to air, due to increase in leaf temperature.
- (2) A night temperature of 36°C evokes a mean night-time transpiration rate of 0.53 times that at $T_N = 15^\circ\text{C}$, although the vapour pressure gradient has increased by a factor of 5.37.

It may be concluded that a slight nocturnal stomatal closure is induced by the change of T_N from 15 to 25°C, whereas the change of T_N from 25 to 36°C induces almost complete stomatal closure at night.

The pattern of daytime transpiration (at 25°C) is of two phases (Fig. 2): (1) an initial period in which the rates may be lower than ($T_N = 15\text{--}25^\circ\text{C}$) or similar to ($T_N = 36^\circ\text{C}$) the night-time rates, and (2) a subsequent period in which transpiration rate rapidly increases, achieving a maximum which is greatest for plants which have previously experienced a high (36°C) night temperature. It may be concluded that phase (1) corresponds to a period of stomatal closure, which is only partial in the case of $T_N = 36^\circ\text{C}$ plants, followed by daytime stomatal opening which attains its fullest extent in $T_N 36^\circ\text{C}$ plants. A corollary to this is that the stomata of $T_N 15$ and 25°C plants do not fully open in the day under the 12-hr photoperiod regime used in these experiments.

(e) *Effect of Night Temperature on the Water Use Efficiency during a Dark-Light Cycle*

It is apparent (Table 1, Fig. 1) that a night temperature of 36°C eliminates the night-time gas-exchange pattern that characterizes CAM plants. The daytime gas exchange is also altered by high night temperatures, causing a reduction in the degree and duration of stomatal closure at the beginning of the light period, and also by an enhanced net daytime CO₂ uptake. In view of the adaptive role that has been ascribed to the CAM pattern of carbon metabolism, it is of interest to see if this has also affected the water use efficiency of the plant. Comment has already been made (Table 1) on the effect of T_N on the WUE over the complete 24-hr day-night cycle. The hourly values of WUE for plants held at the three night temperatures of 15, 25, and 36°C are plotted in Figure 3. It is apparent that WUE by night is approximately

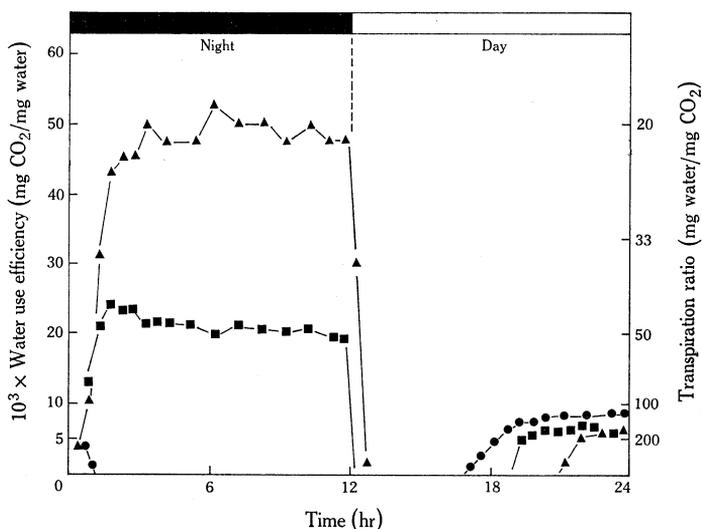


Fig. 3.—Effect of night temperature (T_N) on the water use efficiency of *A. americana*. \blacktriangle $T_N = 15^\circ\text{C}$; \blacksquare $T_N = 25^\circ\text{C}$; \bullet $T_N = 36^\circ\text{C}$; day temperature was constant at 25°C .

halved by the increase of T_N from 15 to 25°C , and that this is mainly due to a decrease in the dark assimilation of CO₂ (Fig. 1). When $T_N = 36^\circ\text{C}$ there was a nocturnal efflux of CO₂ and WUE was negative. The effect of night temperature on the values

of WUE for the following day was such that the higher the night temperature, the longer was the duration of positive WUE values, the largest of which was one-fifth of the maximum nocturnal value.

IV. DISCUSSION AND CONCLUSIONS

(a) *Effect of Night Temperature on CO₂ Assimilation*

An increase in the night temperature inhibited both the nocturnal assimilation of CO₂ (Fig. 1; Table 1) and also the accumulation of titratable acidity (Table 2) in *A. americana*. The association of nocturnal CO₂ assimilation and acidification is well known (Thomas and Ransome 1954), and McWilliams (1970) has extended these observations to families other than the Crassulaceae and Cactaceae. High night temperatures, however, do not completely convert *A. americana* to the "square wave" pattern of gas exchange (Neales *et al.* 1968) associated with non-crassulacean plants: even when nocturnal CO₂ assimilation is prevented, a period of partial stomatal closure persists at the beginning of the light period (Fig. 2).

The highest hourly rates of CO₂ assimilation at night ($T_N = 15^\circ\text{C}$) was *c.* 16 mg CO₂ per plant per 12 hr (Fig. 2) whilst the highest daytime rate recorded ($T_N = 36^\circ\text{C}$, Fig. 2) was *c.* 8 mg CO₂ per plant per 12 hr. This maximum daytime rate was also associated with the highest transpiration rate (Fig. 2). Assuming that the total diffusive resistance to CO₂ transfer was similar in each of the above instances, this indicates that the affinity of the "dark" carboxylation process was greater than that of the "light" process.

These observations, therefore, support the idea that the dark carboxylation in *A. americana* proceeds via a carboxylating enzyme [which by analogy with other CAM plants (see Ting 1971 for review) is probably phosphopyruvate carboxylase] which has a higher affinity for CO₂ than that responsible for daytime CO₂ assimilation. In *Bryophyllum tubiflorum*, Kluge (1971) and Avadhani *et al.* (1971) suggest that ribulosediphosphate carboxylase may be partially responsible for CO₂ assimilation in the light.

(b) *Night Temperature and the Water Use Efficiency of CAM Plants*

The salient observations in this regard are that both the nocturnal assimilation of CO₂ (and acidity) in *A. americana* and the pattern of stomatal rhythm are strongly influenced by night temperature (Tables 1 and 2, Fig. 2). It is possible, therefore, that the normal pattern of nocturnal stomatal opening in CAM plants is caused by the existence of "dark" CO₂ assimilation; when this is inhibited by high temperature, nocturnal stomatal closure results. The mechanism of this control may be via the internal CO₂ concentration in the substomatal air spaces, as has been suggested before from other evidence (Neales 1970).

The consequence of the effects of high night temperature on the CO₂ assimilation pattern and on the rhythm of stomatal aperture are summarized in Figure 4. This figure shows that when CO₂ assimilation in *A. americana* is predominantly nocturnal then this condition is associated with low daytime transpiration. The converse is also true (Fig. 4). As has already been shown [Sections III(a) and III(e)], these changes

greatly affect the WUE of *A. americana*, whether this be assessed over a complete 24-hr cycle (Table 1) or from the hourly rates of gas exchange (Fig. 3). High values of WUE are associated with high rates of nocturnal CO₂ assimilation.

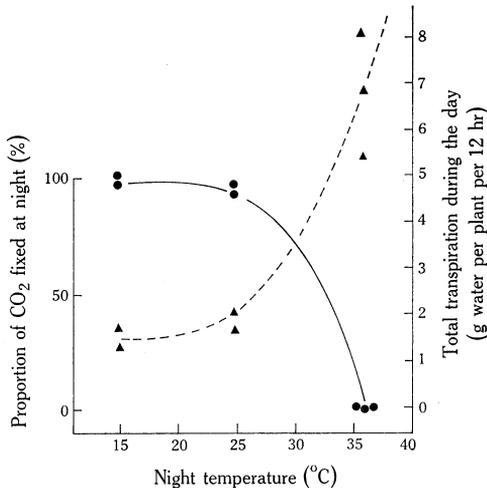


Fig. 4.—Effect of night temperature on the relationship between the nocturnal assimilation of CO₂ (●) and total transpiration during the following day (▲). Day temperature was constant at 25°C; the two curves were drawn by eye.

It may be concluded, therefore, that adaptation of this plant to arid climates is, in part, conferred by mechanisms that depend upon cool night temperatures. This substantiates previous speculation on this point that was largely dependent upon circumstantial evidence arising from the climatic distribution of CAM plants.

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VI. REFERENCES

- AVADHANI, P. M., OSMOND, C. B., and TAN, K. K. (1971).—Crassulacean acid metabolism and the C₄ pathway of photosynthesis. In "Photosynthesis and Photorespiration". (Eds. M. D. Hatch, C. B. Osmond, and R. O. Slatyer.) pp. 288–93. (Wiley-Interscience: New York.)
- BENNET-CLARK, T. A. (1933).—The role of organic acids in plant metabolism. I. *New Phytol.* **32**, 37–71.
- EVANS, L. T. (1971).—Evolutionary, adaptive and environmental aspects of the photosynthetic pathway: assessment. In "Photosynthesis and Photorespiration". (Eds. M. D. Hatch, C. B. Osmond, and R. O. Slatyer.) pp. 130–6. (Wiley-Interscience: New York.)
- HOAGLAND, D. R., and ARNON, D. I. (1938).—The water culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Stn No.* 347.
- JOSHI, M. C., BOYER, J. S., and KRAMER, P. J. (1965).—Growth, carbon dioxide exchange, transpiration and transpiration ratio of pineapple. *Bot. Gaz.* **126**, 174–9.
- KLUGE, M. (1971).—Studies in CO₂ fixation by succulent plants in the light. In "Photosynthesis and Photorespiration". (Eds. M. D. Hatch, C. B. Osmond, and R. O. Slatyer.) pp. 283–7. (Wiley-Interscience: New York.)

- McWILLIAMS, E. L. (1970).—Comparative rates of dark CO₂ uptake and acidification in the Bromeliaceae, Orchidaceae and Euphorbiaceae. *Bot. Gaz.* **131**, 285–90.
- NEALES, T. F. (1970).—Effect of ambient CO₂ concentration on the rate of transpiration of *Agave americana* in the dark. *Nature, Lond.* **228**, 880–2.
- NEALES, T. F., PATTERSON, A. A. and HARTNEY, V. J. (1968).—Physiological adaptation to drought in the carbon assimilation and water loss of xerophytes. *Nature, Lond.* **219**, 469–72.
- PEARCY, R. W., BJÖRKMAN, O., HARRISON, A. T., and MOONEY, H. A. (1971).—Photosynthetic performance of two desert species with C₄ photosynthesis in Death Valley, California. *Yb. Carnegie Instn Wash.* No. 70. pp. 540–50.
- QUEIROZ, O. (1965).—Sur le metabolisme acide des Crassulacees. *Physiol. Vég.* **3**, 203–13.
- RENNER, O. (1910).—Beitrage zur physik der transpiration. *Flora, Jena* **100**, 451–547.
- SLATYER, R. O. (1964).—Efficiency of water utilization by acid zone vegetation. *Ann. Arid Zone* **3**, 1–11.
- THOMAS, M., and RANSOME, S. L. (1954).—Physiological studies on acid metabolism in green plants. III. *New Phytol.* **53**, 1–27.
- TING, I. P. (1971).—Non autotrophic CO₂ fixation and crassulacean acid metabolism. In “Photosynthesis and Photorespiration”. (Eds. M. D. Hatch, C. B. Osmond, and R. O. Slatyer.) pp. 169–85. (Wiley-Interscience: New York.)
- VICKERY, H. B. (1954).—The effect of temperature on the behaviour of malic acid and starch in leaves of *Bryophyllum calycinum* cultured in darkness. *Pl. Physiol., Lancaster* **29**, 385–92.