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Respiration and Gas Exchange in Stem Tissue of *Opuntia basilaris*[1]

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ABSTRACT

Respiration and gas exchange in the light were studied manometrically with tissue slices from stem material of *Opuntia basilaris* Engelm. and Bigel. Dark respiration rates were greater in young stems than in mature stems. The timing of the experiment in the day/night cycle influences the magnitude and pattern of respiration and gas exchange in the light. Net dark respiration has a temperature optimum between 35 and 40 C, and is maintained at 60% of the control rate in tissue equilibrated with experimental osmotic potentials of −25 bars. Net gas exchange in the light is regulated by the titratable acidity of the tissue and by the tissue temperature. Increased rates of net CO_2 evolution and net O_2 consumption occur in the light with high levels of titratable acidity and high temperatures. An efflux of CO_2 and influx of O_2 occur following light/dark transitions. These patterns are reversed following dark/light transitions. Similar results were demonstrated at 15, 25, and 35 C, and are interpreted as a mechanism of adaptation to desert environments.

Cacti possess a variety of physiological adaptations for growth and survival in desert environments. These plants have CAM,[3] which is characterized by the occurrence of nocturnal transpiration (11) and CO_2 assimilation (14), and the continuous day/night reassimilation of endogenous gases (21). The adaptive significance of CAM has a 2-fold interpretation. First, strict stomatal regulation enhances water use efficiency, while reducing the loss of endogenous gases. Second, the continuous day/night reassimilation of endogenous gases maintains a moderate level of metabolic activity during periods without atmospheric gas exchange.

The following study was initiated to investigate the mechanism by which respiration and gas exchange in the light contribute to the maintenance of a moderate level of metabolic activity. *Opuntia basilaris* was selected for this study since it is a typical CAM plant (20), which was studied in earlier *in situ* investigations of acid metabolism and gas exchange (21).

MATERIALS AND METHODS

Plant Material. Stems of *Opuntia basilaris* Engelm. and Bigel. were removed from natural stands of plants at the University of California's Philip L. Boyd Deep Canyon Desert Research Center, near Palm Desert, Calif. (116° 07′ W, 33° 30′ N). The detached stems were immediately sealed with a viscous grease, returned to the laboratory, and stored in growth chambers. Temperature and light/dark conditions in the growth chambers were set according to the thermoperiod and photoperiod of the desert environment. Storage of all stems did not exceed 72 hr postharvest, and in all experiments stems of uniform storage periods were utilized. Most experimental stems were young, *i.e.*, without fruiting scars and less than 1 year old, and free from tissue deterioration.

The *Opuntia* stem material was sectioned by hand, using a sharp razor blade on a cylinder of tissue (8 mm diameter). The longitudinal slices were approximately 1 to 2 mm thick, and consisted of parenchymatous tissue. There was a gradient of chlorophyllous cortical tissue to nonchlorophyllous tissue in the center of the cylinder of stem material. Six to eight slices could be sectioned from each cylinder of stem material. The total dry weight was determined with a duplicate cylinder, removed from adjacent stem material, after 48 hr of oven drying at 80 C. The slices were immediately rinsed with 200 ml of glass-distilled H_2O or buffer solution to remove cellular contents liberated by the sectioning. Additional stem material was collected for the determination of the titratable acidity. In all experiments sufficient stem material could be obtained from a single stem.

Respiration and Gas Exchange Measurements. Measurements of respiration and gas exchange were performed in a Gilson respirometer (Photosynthesis Model, Gilson Medical Electronics). Three manometer flasks without tissue slices were used for thermobarometers during each experiment. All manometer flasks contained 3 ml of glass-distilled H_2O or buffer solution, in which the slices were placed after rinsing. The tissue slices were equilibrated in the manometers for 2 hr prior to the beginning of the respiration measurements. The manometer flasks used for the measurement of dark respiration were coated with several layers of silver paint.

Respiration and gas exchange in the light were measured by the direct method of Warburg manometry. Each manometer flask used for measurement of O_2 exchange contained 0.2 ml of 20% (w/v) KOH, and a 1.5 × 1.5 cm pleat-folded piece of filter paper in the center well. Each manometer flask used for the measurement of the simultaneous exchange of O_2 and CO_2

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[3] Abbreviations: CAM: Crassulacean acid metabolism; PEG: polyethylene glycol; ψ: tissue water potential.

did not contain KOH in the center well. CO_2 retention was determined after Umbreit, Burris, and Stauffer (24). Initial CO_2 retention was determined after the 2-hr equilibration interval by tipping in 0.5 ml of 60% (w/w) perchloric acid from the manometer sidearms. Final CO_2 retention was determined by the same procedure in a duplicate flask at the end of the experiment. All CO_2 bound as bicarbonate ions was considered liberated from the experimental solution after addition of perchloric acid, since the resulting pH was 2.5 or lower. All the experiments in this study using buffer solutions report corrected total CO_2 evolution or consumption.

Experimental Solutions. Tissue slices were placed in either glass-distilled H_2O or buffer solutions in the various experimental treatments. Sodium phosphate buffer, pH 6.8, solutions of 0.05 M concentrations were used in all experiments. The osmotic potential experiments were conducted with standard grade PEG-6000 (Carbowax 6000, Union Carbide Corporation). The specific osmoticum concentrations for the different experimental treatments were determined from the PEG-6000 calibration table of Michel and Kaufmann (13). Plus-osmoticum treatments and minus-osmoticum control treatments were all conducted with 0.05 M buffer solutions. The osmotic potentials were determined by thermocouple psychrometry (Wescor, Inc.).

Light. Light was provided by twenty 30-w tungsten lamps and six 15-w fluorescent lamps. The total irradiance at the level of the tissue slices was 13.5 mw/cm^2, as measured with a YSI-Kettering radiometer (Model 65 radiometer, Yellow Springs Instrument Company, Inc.). However, as much as 43% of this radiant energy was provided by far-red and infrared wavelengths of light. The total illumination (400–700 nm) at the level of the tissue slices was 2200 ft-c, as measured with a Weston illumination meter (Model 756 illumination meter, Weston Instruments, Inc.). The water bath of the Gilson respirometer was changed every other week to reduce light scattering during the course of the experimental period.

Titratable Acidity. The determination of stem tissue acidity was conducted with a technique modified from Sideris *et al.* (18). Five grams fresh weight of tissue were homogenized in 100 ml of glass-distilled H_2O, and titrated with 0.01 N NaOH to a pH 6.4 endpoint. Experimental results from our laboratory indicate that 95% of the organic acids contributing to the circadian acid fluctuation are neutralized at a pH 6.4 endpoint.

Plant Water Potential. Plants grown in natural stands were sampled to determine the *in situ* ψ. Water potentials were determined by thermocouple psychrometry. A ceramic-capped psychrometer probe (Wescor, Inc.) was placed in the center of an intact stem, through an incision in the epidermis. This opening was sealed with a viscous grease and allowed to equilibrate for at least 12 hr. The ψ was determined shortly after sunrise to minimize temperature distortions within the psychrometer probe.

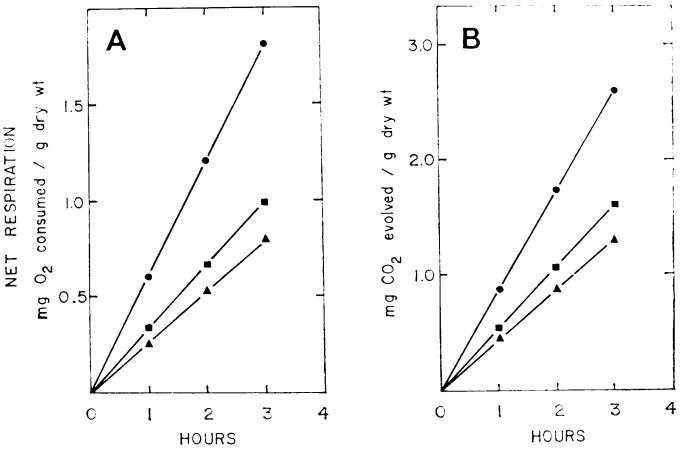

FIG. 1. A: Effect of stem developmental stage on net dark O_2 consumption; B: effect of stem developmental stage on net dark CO_2 evolution. Developmental stages: young, newly formed stem (●); mature, nonflowering stem (■); mature, flowering stem (▲). Tissue slices were rinsed at 15 C in glass-distilled H_2O. Initial acidity was 96 ± 6 μeq/g fresh weight.

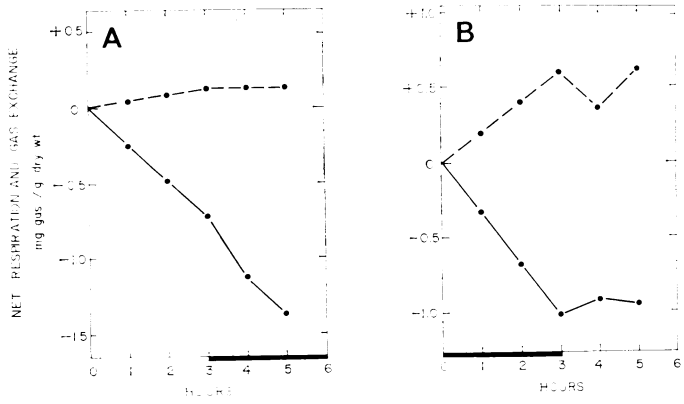

FIG. 2. A: Net gas exchange in the light and respiration using stems harvested at the end of daytime; B: net respiration and gas exchange in the light using stems harvested at the end of nighttime. CO_2 (– – –); O_2 (——). Gas evolution (+ values); gas consumption (— values). Tissue slices from young stems at 10 C in 0.05 M sodium phosphate buffer, pH 6.8.

RESULTS

Physiological and Experimental Conditions. Physiological variations in the stem material influenced the magnitude of the net dark respiration rate (Fig. 1). Mature stems, either flowering or nonflowering, demonstrated comparable dark respiration rates. The respiration rate of a young stem was significantly greater, despite a titratable acidity level comparable to the mature stems. Experimental variables influenced both the magnitude and the pattern of respiration and gas exchange in the light. The most dramatic variation in the magnitude and pattern of the respiration rate was related to the timing of the experiment in the day/night cycle (Fig. 2). Stems harvested at the end of the daytime demonstrated net O_2 consumption in the light and dark (Fig. 2A). A low rate of net CO_2 evolution occurred in the light, although no significant net exchange of CO_2 occurred after darkening. The pattern of respiration and gas exchange in the light shown in Figure 2B is significantly different. Net O_2 consumption and CO_2 evolution occurred in the dark. In the light net O_2 evolution occurred at a low rate. Net CO_2 evolution continued in the light despite an initial short period of net CO_2 consumption.

In order to standardize such variables, the experiments reported below were conducted with young stems, which were harvested at the end of the nighttime, placed in dark conditions, and subsequently exposed to light. Tissue slices were treated with either glass-distilled H_2O or 0.05 M buffer, pH 6.8.

Dark Respiration. The temperature optimum for net dark respiration occurred between 35 and 40 C (Fig. 3). The dark respiration decreased dramatically above 40 C, although respiration rates were unchanged after 7 hr at temperatures as high as 50 C. Dark CO_2 evolution rates generally decreased below O_2 consumption rates at temperatures less than 15 C, resulting in RQ values less than unity. The RQ values, calculated on a μmole basis, were consistently less than unity in those experi-

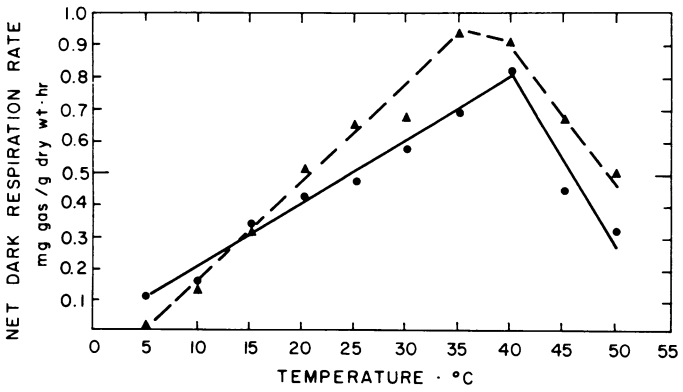

FIG. 3. Effect of temperature on the net dark respiration rate of stem tissue. Net CO_2 evolution (▲, – – –); net O_2 consumption (●, ——). Tissue slices from young stems, in glass distilled H_2O. Experiments were initiated following the end of nighttime.

ments conducted at 5 and 10 C, despite variations in the titratable acidity of the stem tissue. Net CO_2 evolution was virtually terminated at 5 C, although net O_2 consumption persisted.

The water status of tissue slices equilibrated with PEG-6000 solutions of different osmotic potentials influenced the dark respiration rate (Fig. 4). The rate of respiration, expressed as the per cent of the minus-osmoticum control rate was highest at −7 bars, decreased from −7 to −16 bars, and did not decline as rapidly as osmotic potentials decreased beyond −16 bars. Respiration was maintained at 60% of the control rate in tissue equilibrated with experimental osmotic potentials of −25 bars. Few of the highly vacuolated parenchymatous cells were plasmolyzed in the PEG-6000 solutions. Only marginal cells of the tissue slice were plasmolyzed, and the degree of plasmolysis was less than 25% of the marginal cells in osmotic potential solutions of −25 bars. Furthermore, the respiration rate was linear for 7 hr after the tissue slices were placed in the plus-osmoticum solutions, indicating a rapid respiration equilibration to varying levels of tissue water status.

The respiration rate was additionally influenced by the initial *in situ* ψ of the stem material (Table I). In the experiments reported in Table I the osmotic potential of the minus-osmoticum buffer solution was −3 bars. The respiration rate was lower in the −11 to −12 bar tissue than in the −5 to −6 bar tissue collected during a period of rainfall. The respiration rate decreased as the tissue dehydrated after the rainfall, and ψ levels of −10 to −11 bars generated respiration rates similar to those measured prior to the rainfall. The response of respiration to variations in the initial *in situ* ψ were similar to the respiration response using tissue slices equilibrated in various osmotic potential solutions (see Fig. 4).

Net Light Gas Exchange. The patterns of gas exchange in the light were influenced by the initial level of tissue acidity (Fig. 5). Net CO_2 evolution occurred in the light and the highest rate was associated with the highest acidity level (Fig. 5A). The rate of CO_2 evolution decreased as the acidity level decreased in the tissue slices. Figure 5B presents a partially different pattern of net O_2 exchange. In tissue slices with the highest acidity level net O_2 consumption predominated. The pattern shifted to a low rate of net O_2 consumption with the intermediate acidity level and ultimately to the highest rate of O_2 evolution in the light with the lowest acidity level. Similar net CO_2 evolution patterns were demonstrated at 25 and 40 C, and net O_2 consumption predominated in all cases at 25 and 40 C.

The level of stem tissue acidity varied in response to desert environmental conditions. The level of acidity was high following rainfall and nocturnal atmospheric CO_2 assimilation. The level of acidity was low following periods of drought, without nocturnal atmospheric CO_2 assimilation. In order to standardize variations in ψ, all experiments were conducted with tissue slices in glass-distilled H_2O.

The patterns of gas exchange in the light were also influenced by the experimental temperature, using stem tissue at similar levels of titratable acidity (Fig. 6). Figure 6A demonstrates that net CO_2 evolution occurred in the light, and the

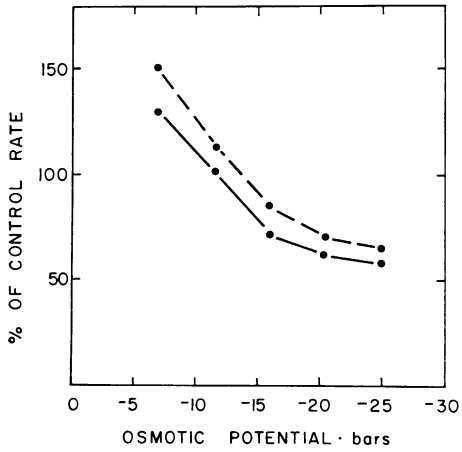

FIG. 4. Effect of osmotic potential on net dark respiration, expressed as percentage of minus osmoticum control rate. Net CO_2 evolution (– – –); net O_2 consumption (——). Tissue slices from young stems at 25 C in 0.05 M sodium phosphate buffer, pH 6.8. Minus osmoticum control with buffer solution only, and plus osmoticum treatments with buffer and PEG-6000. Experiments were initiated following the end of nighttime. Stem tissue water potentials measured prior to experimentation were −12 ± 2 bars.

TABLE I. *Effect of in Situ Tissue Water Status on Dark Respiration Rate*

Tissue slices from young stems of *O. basilaris* at 25 C in 0.05 M buffer, pH 6.8. *In situ* tissue water potentials (ψ) were determined by thermocouple psychrometry. Stem samples were collected 1 week prior (n = 6), during (n = 3), and following (n = 7) rainfalls occurring on August 3 and 5, 1973.

In Situ ψ		Oxygen Consumption Rate
bars	*n*	*mg O_2/g dry wt·hr*
−11 to −12	6	0.40 ± 0.05
−5 to −6	3	0.59 ± 0.06
−10 to −11	7	0.45 ± 0.06

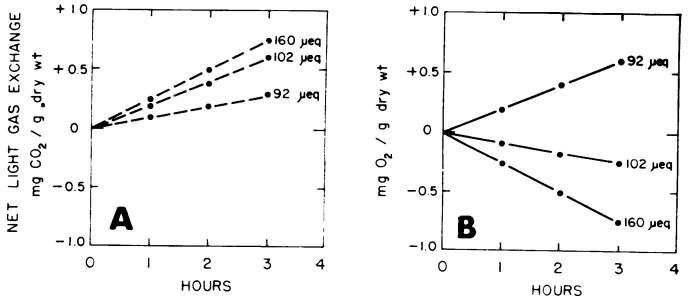

FIG. 5. A: Effect of tissue acidity on net CO_2 evolution in the light; B: effect of tissue acidity on net O_2 evolution and consumption in the light. Gas evolution (+ values); gas consumption (− values). Tissue slices from young stems at 15 C on glass-distilled H_2O. Experiments were initiated following the end of nighttime.

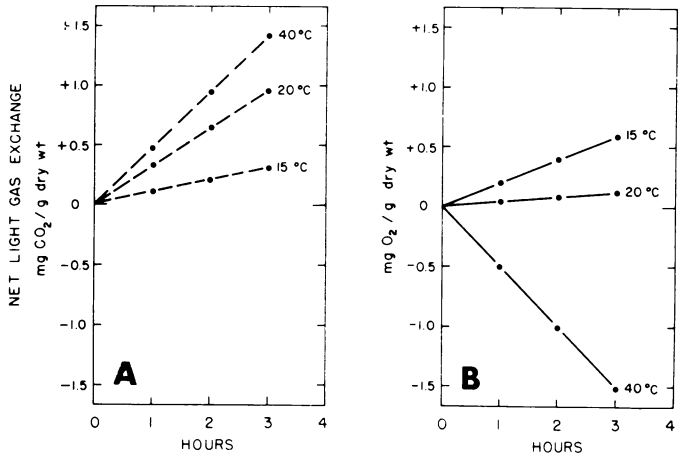

FIG. 6. A: Effect of temperature on net CO_2 evolution in the light; B: effect of temperature on net O_2 evolution and consumption in the light. Gas evolution (+ values); gas consumption (− values). Tissue slices from young stems on glass-distilled H_2O. Experiments were initiated following the end of nighttime.

highest rate was associated with the highest temperature. The rate of CO_2 evolution decreased as the temperature decreased. Tissue slices at the highest temperature demonstrated net O_2 consumption, while net O_2 evolution occurred at 20 C, and the highest rate of net O_2 evolution occurred at the lowest temperature (Fig. 6B). Stem tissue acidity was comparable, *i.e.*, 91 ± 3 μeq/g fresh weight, in the three separate experiments presented in Figure 6. In order to standardize ψ, the three experiments were conducted with tissue slices in glass-distilled H_2O. The *in situ* ψ for the three experiments was −7 ± 3 bars.

Momentary shifts in the rates of net respiration and gas exchange in the light occurred following dark/light and light/dark transitions (Fig. 7). The initial equilibrium patterns of dark respiration were net CO_2 evolution and net O_2 consumption in all cases. The dark/light transition induced a CO_2 influx and O_2 efflux. This pattern was consistent at the different experimental temperatures. The maximum effect of the transition occurred after 15 min at 15 and 25 C (Fig. 7, A and B). The pattern was partially different at 35 C, since the maximum O_2 transition occurred at the 15-min interval and the maximum CO_2 transition occurred at the 30-min interval (Fig. 7C). After the subsequent re-establishment of equilibrium gas exchange in the light no net exchange occurred at 15 and 25 C, while net O_2 consumption and net CO_2 evolution occurred at 35 C in the light. The light/dark transition induced a CO_2 efflux and O_2 influx, which was also consistent at the different experimental temperatures. The maximum transition shift occurred at the 30-min interval at 15 C (Fig. 7A), and at the 15-min interval at 25 C (Fig. 7B). The transition at 35 C was more complex, since the effects were not sharply defined (Fig. 7C). The maximum peak of CO_2 efflux occurred at the 45-min interval and the maximum peak of O_2 influx occurred at the 30-min interval. After the subsequent re-establishment of equilibrium respiration rates, the patterns at the different temperatures were similar to the respective CO_2 and O_2 respiration patterns of the initial dark period.

DISCUSSION

In this study, patterns of respiration and gas exchange in the light using tissue slices of stem material from *O. basilaris* have been presented. These patterns are interpreted as representative of the endogenous gas exchange patterns which occur in intact stems of this desert cactus. The results supplement our previous investigations of circadian and annual acid metabolism and gas exchange characteristics of plants of *O. basilaris* growing in a desert environment (20, 21). In these earlier investigations we reported the maintenance of metabolic activity throughout the course of the year.

Physiological differences in the *Opuntia* stem material influenced the magnitude of the net dark respiration (see Fig. 1). Stem developmental stage produced similar effects on respiration at higher temperatures, *i.e.*, 40 C. These stem developmental stage differences are similar to the findings of Richards (16) with intact, whole stems of *Opuntia versicolor*. His earlier experimental findings demonstrated a 90% increase in the rate of CO_2 evolution in the dark by young stems, as compared

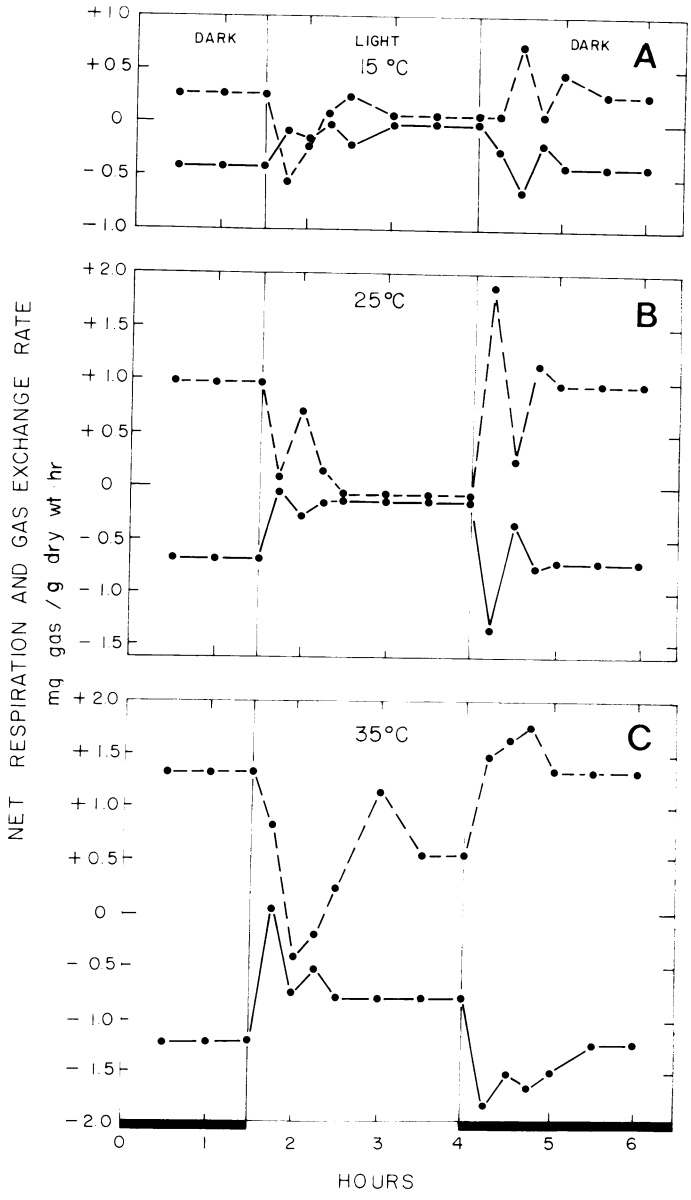

FIG. 7. A: Effect of light/dark transitions on the rate of net respiration and gas exchange in the light of tissue slices at 15 C; B: effect of light/dark transitions on the rate of net respiration and gas exchange in the light of tissue slices at 25 C; C: effect of light/dark transitions on the rate of net respiration and gas exchange in the light of tissue slices at 35 C. CO_2 (– – –); O_2 (——). Gas evolution (+ values); gas consumption (− values). Tissue slices from young stems 0.05 M sodium phosphate buffer, pH 6.8. Experiments were initiated following the end of nighttime.

with mature stems. Such effects of stem developmental stage in *O. basilaris* and *O. versicolor* may be due to differences in the relative amounts of chlorophyllous and nonchlorophyllous parenchymatous tissue in the different aged stems. In young stems chlorophyllous tissue is the most common type, while in mature stems nonchlorophyllous tissue is the most common type. Experimental variables, *i.e.*, the timing of the experiment in the day/night cycle, influenced both the magnitude and the pattern of respiration. No net dark exchange of CO_2 occurred and low rates of dark CO_2 consumption could only be demonstrated with stems harvested at the end of the daytime (see Fig. 2A). Photosynthetic O_2 evolution occurred only with stems harvested at the end of the nighttime (see Fig. 2B). Such results may be due to the photoperiod-controlled circadian rhythmicity of CAM enzyme systems (15). Experimental pH conditions influenced the apparent CO_2 exchange. The pH 6.8 experimental conditions were used to correspond with previous respiration studies with CAM plants (5, 22).

The respiration temperature optimum of 35 to 40 C is consistent with the optimum temperature values demonstrated in respiration and CO_2 fixation in other CAM plants. Richards (16) reported that maximum CO_2 evolution occurred at 45 C with plants of *O. versicolor*, while Bruinsma (4) reported a 30 to 35 C temperature optimum for respiration in the CAM plant *Bryophyllum tubiflorum*. Isolated mesophyll protoplasts from the CAM plant *Sedum telephium* demonstrate optimum $^{14}CO_2$ incorporation activity from 35 to 40 C (17). Furthermore, these results are consistent with the temperature responses of CAM plant enzymes reported by Brandon (3).

The rate of respiration was additionally influenced by the tissue water status. The first evidence of water stress effects on CAM plant respiration was reported by Richards (16) with plants of *O. versicolor*, as was shown by increased dark CO_2 evolution rates in mature, turgid stems compared with mature, flaccid stems. The rate of respiration in *O. basilaris* tissue slices was also increased in low plant water stress conditions (see Fig. 4 and Table I). Respiration rates decreased with decreasing osmotic potentials below -7 bars. This respiration response was similar to the pattern previously demonstrated with beet tissue slices in different osmotic potential solutions (19). However, the response of *O. basilaris* contrasts markedly with the response of other plants, which demonstrate significant decreases in dark respiration with ψ values ranging from -20 to -35 bars (1, 2, 7). The inhibition of respiration is probably due to turgor loss and dehydration, although severe cellular disorganization from plasmolysis occurred only in some of the marginal cells of the tissue slices. Apparently PEG-6000 does penetrate the cell wall or plasmalemma to cause plasmolysis (12).

The relationship of net light CO_2 exchange and titratable acidity represented in Figure 5A was probably the result of the evolution of CO_2 which was not endogenously reassimilated by photosynthesis. Malate is the source of CO_2 evolved in the light (10), and the endogenous reassimilation of CO_2 generated from malate was demonstrated earlier (23). Assuming the amount of endogenous CO_2 which can potentially be reassimilated by photosynthesis is less than the amount of CO_2 generated from photodeacidification, CO_2 evolution would be expected to occur when the level of titratable acidity is high and in excess of the amount which can be endogenously reassimilated. The relationship of net light O_2 consumption and level of titratable acidity represented in Figure 5B was opposite the pattern reported in the CAM plant *Aloe arborescens* (5). In this earlier study, increased rates of O_2 evolution in the light were directly related to higher levels of titratable acidity. Experimental differences in the two separate studies, *i.e.*, differences in O_2 concentrations and use of nonchlorophyllous tissue, may account for the occurrence of the opposite pattern.

In *Opuntia* stem material with high acidity net CO_2 evolution occurred in the light at low and high temperatures (see Fig. 6A). The results of Figure 6B indicate that net O_2 consumption occurred at temperatures of 40 C, while net O_2 evolution occurred at lower temperatures. This pattern in *O. basilaris* could possibly occur if photorespiration was present and the temperature optimum for net O_2 consumption by photorespiration was higher than the temperature optimum for net O_2 evolution by photosynthesis.

Momentary shifts in respiration and gas exchange in the light following dark/light and light/dark transitions occur in plants with photorespiration (9). The first evidence of the light/dark transitional efflux of CO_2 by a CAM plant was demonstrated with *Kalanchoe blossfeldiana* (8), and subsequently other CAM plants were reported to possess photorespiration (6, 10, 25). In *O. basilaris* the maximum transitional shift occurred after 15 min or longer. A study with the saguaro cactus *Carnegia gigantea* demonstrated that the maximum shift also occurred after 15 or 20 min (6). However, nonsucculent plants and other CAM plants demonstrate more rapid CO_2 transitional shifts (25). The effect of photorespiration on the net carbon balance of *O. basilaris* would be increased rates of net CO_2 evolution in the light.

The results of this study elucidate the mechanism by which endogenous gas exchange in *O. basilaris* maintains a moderate level of metabolic activity during periods without atmospheric gas exchange. Since the cuticular gas diffusion resistance is very high in whole stems of this cactus (20), the net carbon balance of the plant is stabilized when the assimilation of atmospheric CO_2 is terminated by stomatal closure. Dark respiration occurs over a wide range of temperatures, and is maintained at tissue temperatures as great as 50 C. Additionally, dark respiration continues at a moderate rate in tissue water stress conditions greater than the seasonal levels of *in situ* water stress (21). In the light, endogenous gas recycling continues by photosynthesis and photorespiration. We have previously reported that the circadian organic acid fluctuation continues without atmospheric gas exchange (21). The continuous day/night recycling of endogenous CO_2 provides a constant supply of NADPH, by the transhydrogenation from NADH produced during acid synthesis (22). The production of NADPH from a nonphotosynthetic pathway provides an additional energy source for reductive biosynthetic reactions, which supplements photosynthetic NADPH synthesis. Thus, *O. basilaris* appears capable of continuously providing biosynthetic energy to sustain a moderate level of metabolic activity, solely by the recycling of a stabilized supply of endogenously available gases.

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