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CO_2 Exchange and Acidity Levels in Detached Pineapple, *Ananas comosus* (L.), Merr., Leaves during the Day at Various Temperatures, O_2 and CO_2 Concentrations[1]

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ABSTRACT

The effects of temperature, O_2, and CO_2 on titratable acid content and on CO_2 exchange were measured in detached pineapple (*Ananas comosus*) leaves during the daily 15-hour light period. Comparative measurements were made in air and in CO_2-free air. Increasing the leaf temperature from 20 to 35 C decreased the total CO_2 uptake in air and slightly increased the total CO_2 released into CO_2-free air. Between 25 and 35 C, the activation energy for daily acid loss was near 12 kcal mol^{-1}, but at lower temperatures the activation energy was much greater.

Increasing O_2 or decreasing the CO_2 concentration decreased the total CO_2 fixation in air, whereas the total CO_2 released in CO_2-free air was increased. The total acid content remained constant at 20 C, but it decreased progressively with increasing temperature both in air and in CO_2-free air. The total acid content at 30 C remained constant in 2% O_2 irrespective of CO_2 concentration. The total acid content decreased in 21 and 50% O_2 as the CO_2 increased from 0 to 300, and 540 μl/l of CO_2. The data indicate that photorespiration is present in pineapple. The lack of acid loss in 2% O_2 suggests that light deacidification is dependent upon respiration and that higher O_2 concentrations are required to saturate deacidification.

Much of the evidence today on carbon metabolism in plants with a capacity for Crassulacean acid metabolism suggests that CAM[2] is a very flexible metabolic system whose operation is quite sensitive to such environmental parameters as photoperiod, thermoperiod, atmospheric concentration of CO_2 and O_2, soil moisture pressure, and salinity. C_3 and C_4 plants primarily assimilate CO_2 during the day. In contrast, CAM plants assimilate major quantities of CO_2 at night in green tissues which results in a massive increase in malic acid.

CAM plants not only exhibit an unusual diurnal pattern of CO_2 and acid metabolism, they also exhibit an unusual O_2 metabolism. High O_2 concentrations $\geq$ 10% are required to approach saturation of respiration, and the response to respiration seems to change at O_2 concentrations above 10% in some CAM plants (7, 10, 12). Extensive studies on the respiration quotient (CO_2 release/O_2 uptake) in CAM plants (15-18) also revealed that the RQ is low or zero during dark acidification in CO_2-free air. In air, however, a negative RQ is observed since CO_2 is taken up by the leaves. After maximum dark acidification is reached, the RQ increases and often rises well above 1.00 (15).

The effect of O_2 on photosynthetic CO_2 uptake has been studied by several investigators (4, 9, 11). It is well established that O_2 concentrations above roughly 2% inhibit CO_2 fixation in C_3 plants, whereas C_4 photosynthesis is relatively insensitive to changes in O_2 concentration (4, 9, 11). This inhibitory effect of O_2 on C_3 photosynthesis is postulated to be due to photorespiration.

The presence of photorespiration in CAM plants was suggested by Crews *et al.* (5) when they observed a biphasic postillumination CO_2 burst (PIB) in pineapple and other CAM plant leaves. Decreasing O_2 or increasing CO_2 eliminated the primary PIB peak without affecting the secondary peak. An increase in light-dependent CO_2 fixation with increasing CO_2 concentration also was reported both in *Kalanchoe daigremontiana* (1) and in pineapple leaves (5). Osmond and Björkman (14) showed in *K. daigremontiana* that O_2 at concentrations from 4 to 36% substantially inhibited CO_2 fixation in the light. But with CAM plants, changing the gas phase around a leaf will give a variety of responses throughout a day (5); so that data are complex to interpret or explain since the strong diurnal CAM cycles are involved.

On the basis of these observations, a series of experiments in controlled environments was initiated to study photorespiration, photosynthesis, and other aspects of carbon metabolism in detached pineapple leaves. This paper describes the effects of varying O_2 and CO_2 concentrations and temperature on the rates and patterns of titratable acidity and of CO_2 exchange with pineapple leaves only during the light period.

MATERIALS AND METHODS

Growth of Plant Material. Pineapple, *Ananas comosus* (L.) Merr., plants were rooted from crowns purchased in a local grocery and grown in a greenhouse. Greenhouse plants were watered daily and fertilized with a 20-20-20 fertilizer every 15 days. To insure CAM, plants were maintained in a growth chamber at least 2 weeks prior to use in any experiment. Experimental plants were placed in a growth chamber with day and night temperatures of 30 and 15 C, respectively. Plants were exposed to a cycle of 15-hr light and 9-hr dark periods. Light intensity of 2000 ft-c and a relative humidity of 65 to 70% were maintained in the growth chamber. In these environments, a diurnal acid fluctuation occurs which reflects CAM (5).

Experimental Conditions in the Leaf Chamber. Throughout this study, the daily 15-hr light period was the experimental period. Fifteen different environmental conditions were em-

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[2] Abbreviations: CAM; Crassulacean acid metabolism; C_3: reductive pentose phosphate; C_4: C_4-dicarboxylic acid; OAA: oxaloacetic acid; PEP: phosphoenolpyruvate; PEPCK: phosphoenolpyruvate carboxykinase; PIB: postillumination CO_2 burst; RuDP: ribulose 1,5-diphosphate; RQ: respiratory quotient; E_a: activation energy.

ployed with respect to leaf temperatures and to the concentrations of O_2, CO_2, and N_2 in the gas mixtures passing over the leaves. The environmental combinations were varied in individual experiments as shown in each figure. The patterns of CO_2 exchange were recorded continuously over the 15-hr light period for each environment.

Gas Exchange Measurement Apparatus. An open system measured CO_2 uptake and release. Treatment gases were bubbled through distilled H_2O at a flow rate of 1 liter/min. One-half of the gas stream was passed progressively through a flow meter, the leaf chamber, a condenser, a second flow meter, and finally the sample cell of a Beckman model 215B infrared CO_2 analyzer. At the same time, the reference gas sample was passed through a flow meter, a condenser, and through the reference cell of the analyzer. The readings from the infrared CO_2 analyzer were recorded on a Sargent model SRG recorder. The infrared CO_2 analyzer was calibrated with standard CO_2 gas mixtures, and N_2 was used to zero the instrument. An air seal type leaf chamber described by Wolf *et al.* (21) was used for gas exchange measurements. A water jacket was attached to the bottom of the leaf chamber to maintain a constant leaf temperature. The volume of the leaf chamber was 110 ml.

Four 150-w incandescent floodlamps provided a light intensity of 2000 ft-c at the level of the leaf chamber as measured by a Weston model 756 light meter. In order to control temperature, the light was filtered through a temperature-controlled water tank to absorb the IR portion of the spectrum. The O_2 concentrations were measured with an O_2 analyzer model Servomex, type OA 150. Leaf surface area was measured with a Hayaski Denko automatic leaf area meter.

For each experiment, sets of leaves of approximately the same age were detached at 6:00 AM, immediately recut under water, and placed in a leaf chamber with the cut end dipped into water. Respective treatment gas mixtures were passed over the leaves and the leaf temperatures were maintained for the 15-hr experimental light period. One leaf was used for gas exchange and others for titratable acidity measurements.

Measurement of Leaf Titratable Acidity. At timed intervals throughout the light period, a 1-g leaf sample was removed from the leaf chamber and ground vigorously in a mortar containing 4.5 ml of distilled H_2O. The ground mixture was transferred to a test tube and boiled for 10 min. The homogenate was cooled to room temperature, brought to a known volume, centrifuged at 10,000*g* for 10 min, then an aliquot was removed and titrated with 4 mM NaOH to pH 8.3. The data are expressed as meq of acid/100 g fresh wt.

RESULTS

Effects of Temperature on CO_2 Exchange. The patterns of CO_2 exchange in air and in CO_2-free air throughout a day at 20, 25, 30, and 35 C are shown with the solid curves in Figures 1 and 2, respectively. Several general features stand out in Figure 1. First, in air, the rate of apparent photosynthesis was higher at 20 C compared to other temperatures throughout the light period. Indeed at 35 C, little photosynthesis was observed (Fig. 1). In CO_2-free air, increasing the temperature from 20 to 35 C had little influence on CO_2 release.

The effects of temperature on CO_2 exchange were summed and are presented in Figure 3. The total CO_2 uptake in air decreased from 47 mg of CO_2 dm^{-2} of leaf area 15 hr^{-1} at 20 C to about 1.5 mg of CO_2 dm^{-2} of leaf area 15 hr^{-1} at 35 C. Total CO_2 release in CO_2-free air only increased slightly as the temperature increased (Figs. 2 and 3).

Effects of Temperature on Titratable Acid. The daily patterns of titratable acidity at 20, 25, 30, and 35 C in air and in CO_2-free air are shown in Figures 1 and 2, respectively. Again, several general features are evident. The titratable acid content of the

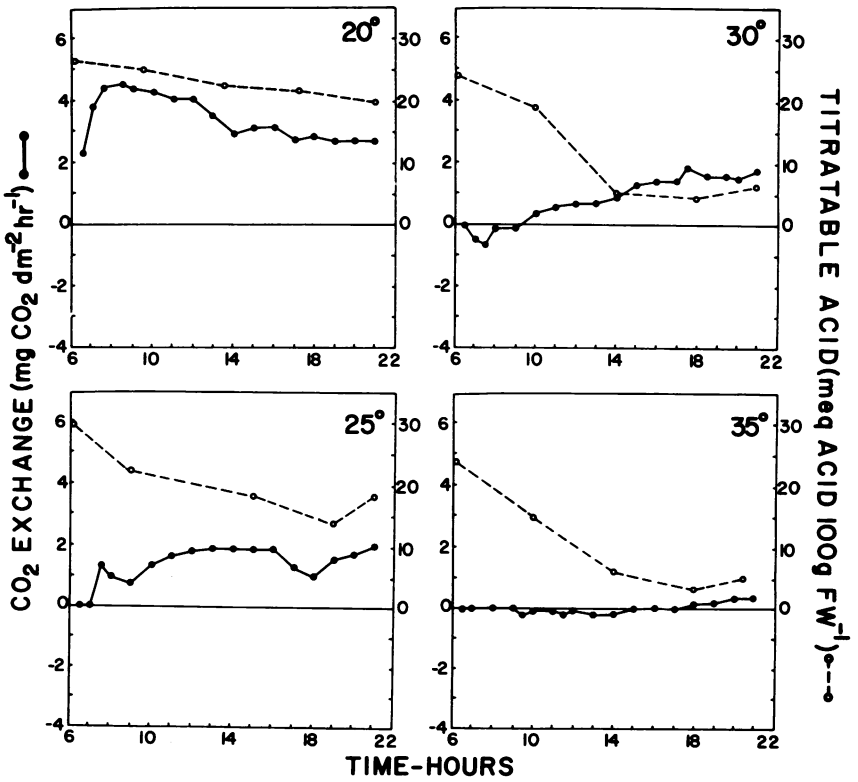

FIG. 1. Effects of temperature on titratable acid and CO_2 exchange in detached pineapple leaves in air, 21% O_2, and 300 μl/l CO_2 during a 15-hr day.

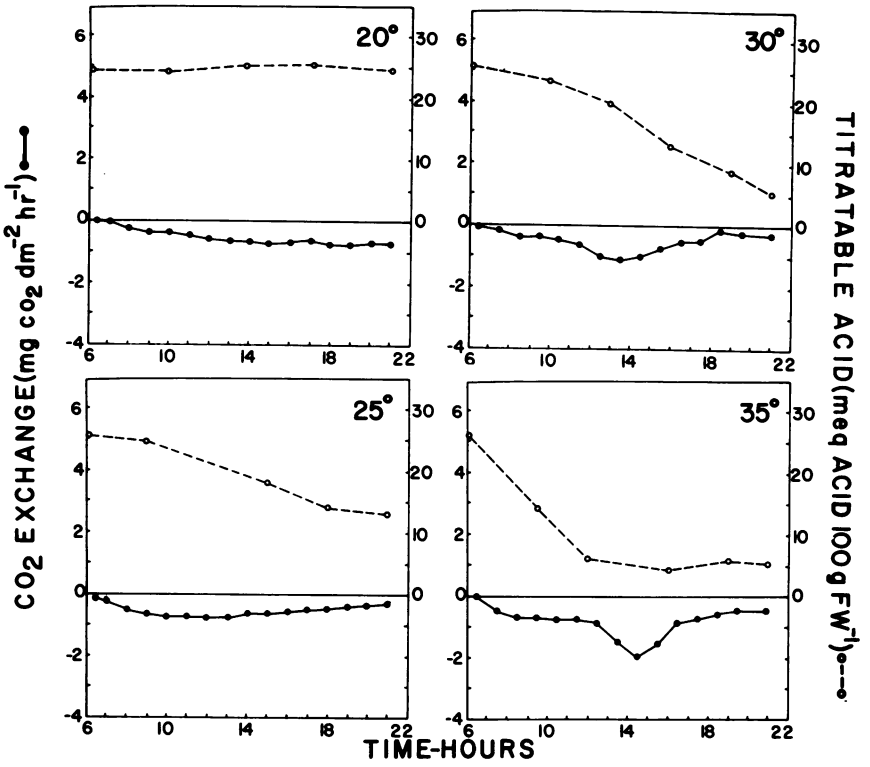

FIG. 2. Effects of temperature on titratable acid and CO_2 exchange in detached pineapple leaves in CO_2-free air, 21% O_2, and zero CO_2 during a 15-hr day.

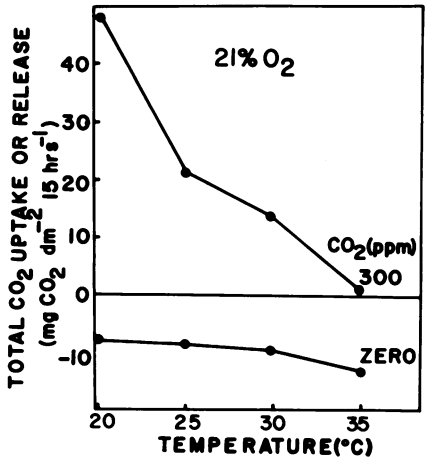

FIG. 3. Effects of temperature and CO_2 concentration on total CO_2 uptake or release during a 15-hr day in detached pineapple leaves at 21% O_2.

leaves remained high at 20 C both in air and in CO_2-free air, but it decreased progressively with increasing temperature. The total acid loss during the day at 30 and 35 C were equal both in air and in CO_2-free air (Fig. 4, upper frames). The maximum rate of acid loss in air and in CO_2-free air increased with increasing temperature (Fig. 4, lower frames). Arrhenius plots of the temperature dependence of the maximum rate of acid loss in air and in CO_2-free air are shown in Figure 5. The activation energy for acid loss in air and in CO_2-free air was about 12 kcal mol^{-1} above about 25 C. Near 20 C, the E_a was much higher. There would be a discontinuity in the Arrhenius plot at low temperatures, but we do not have sufficient data to determine accurately the temperature of the discontinuity.

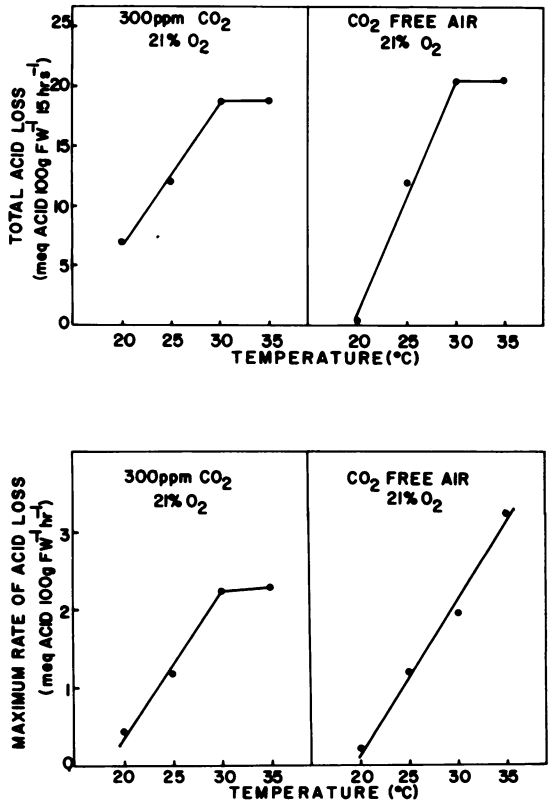

FIG. 4. Effects of temperature and CO_2 concentration on total acid loss (upper) and maximum rate of acid loss (lower) during a 15-hr day in detached pineapple leaves at 21% O_2.

Since the experimental plants were grown at 30 C and a characteristic CAM pattern was observed at 30 C (Fig. 1), a series of experiments varying O_2 and CO_2 in the leaf chamber were conducted at 30 C.

Influence of O_2 and CO_2 Concentration on CO_2 Exchange. As shown in Figure 6, A, D, and G, CO_2 release into CO_2-free air was promoted by increasing O_2 concentrations from 2 to 50%. Net photosynthesis, at both 300 and 540 μl/l CO_2, decreased as the O_2 concentration increased (Fig. 6), whereas net photosynthesis in 2, 21, and 50% O_2 increased with increasing CO_2 concentration from 300 to 540 μl/l CO_2 (Fig. 6 compares frames B and C, E and F, H and I). The maximum CO_2 uptake occurred at 2% O_2 and 540 μl/l CO_2 (Fig. 6C).

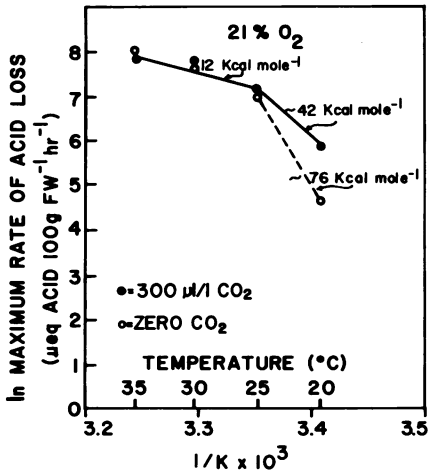

FIG. 5. Arrhenius plots of the temperature dependence of the maximum rate of acid loss at zero and 300 μl/l CO_2 and 21% O_2 during a 15 hr day in detached pineapple leaves.

Influence of O_2 and CO_2 Concentration on Titratable Acid. The concentrations of O_2 and CO_2 in the leaf chamber also influenced the daily loss of acid in pineapple leaves. At 2% O_2, only a slight decrease in acid content of the leaves occurred at all CO_2 concentrations during the entire light period (Fig. 6, A–C). At 21 and 50% O_2, acid loss occurred at all C_2 concentrations (Fig. 6, D–I), but at different rates and amounts of acid loss.

DISCUSSION

It is well established that the magnitude of the diurnal fluctuation of organic acids (malic) and starch, which play key roles in carbon metabolism in CAM plants, is dependent upon the night and day growth temperatures as well as the surrounding O_2 and CO_2 concentrations (15–19). This work confirms many of these early observations and offers some additional explanations about the light portion of CAM.

The high activation energy for acid loss at temperatures below 25 C (Fig. 5) explains the slight decrease in acid content observed in leaves at 20 C both in air and CO_2-free air (Figs. 1, 2, 4) and explains the well known requirement for a high day temperature for maximum diurnal fluctuation of malic acid and starch (15). The increase in activation energy for acid breakdown at low temperatures can be related to enzymes involved in CAM. Using *Aloe vera* leaf extracts, Crews *et al.* (6) showed that at 30 C the activity of PEPCK was twice greater than activities of

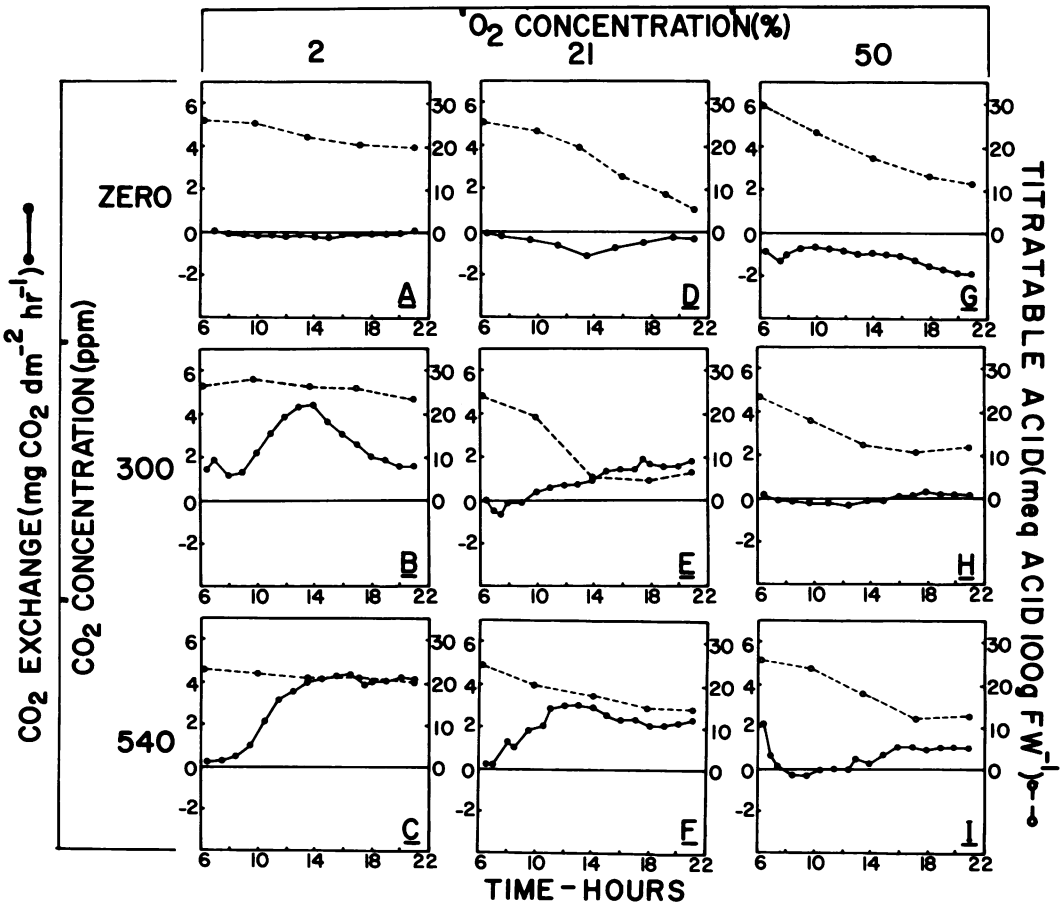

FIG. 6. Effects of various O_2 and CO_2 concentrations on titratable acid and CO_2 exchange in detached pineapple leaves at 30 C during a 15-hr day.

PEP carboxylase and RuDP carboxylase, whereas at temperatures near 15 C, PEP carboxylase was the most active enzyme. In this laboratory, L. Daley has found a discontinuity in the Arrhenius plot for PEPCK from pineapple leaves near 15 C which shows a very high E_a, near 80 kcal mol^{-1} (data unpublished). Thus, the high E_a for acid loss at low temperatures can be interpreted as a decrease in the PEPCK-catalyzed OAA decarboxylation reaction which is essential for CAM. The present results are consistent with the requirements for a high day temperature and a low night temperature for maximum diurnal fluctuation of malic acid in CAM plants.

In addition to temperature, O_2 concentration influences malic acid breakdown during the day (Fig. 6) and malic acid formation at night (unpublished data). It is clear that O_2 concentrations over 2% are required for complete daily acid loss (Fig. 6) which raises the question of the site of action of O_2 during acid decarboxylation. Others (13) propose that the dependency of the acid loss on O_2 may be a consequence of the need for regeneration of NADP in the reaction catalyzed by malic enzyme, but in pineapple leaves, acid decarboxylation is catalyzed by PEPCK. Using *Aloe arborescens* leaf slices, Denius and Homann (8) report that the decrease in malic acid content was completely inhibited by amytal and rotenone, inhibitors of mitochondrial electron transport. They concluded that light-dependent deacidification depends upon the operation of the tricarboxylic acid cycle and the mitochondrial electron transport chain. If light deacidification in pineapple leaves is dependent upon mitochondrial respiration, it can be concluded that mitochondrial respiration is not saturated at 2% O_2. Bulky or fleshy tissues do require more O_2 for maximum respiratory rates (10). Denius and Homann (6) reported that in *Aloe* leaf slices, respiration saturation was obtained near 10% O_2. The respiratory rate was 19.6 μmol O_2/mg Chl/hr at 21% O_2 compared to 7.6 at 3% O_2. With *Bryophyllum* leaves (12), the respiratory rate was proportional to the logarithm of the O_2 tension. Plotting respiration rates *versus* the logarithm of the O_2 tension gave two straight lines with a break in the slope at about 10% O_2. Ducet and Rosenberg (10) suggested that the mechanism of respiration changes at O_2 concentrations higher than 10%. Moyse (12) also measured dark acidification in leaves of *B. daigremontianum* in CO_2-free atmospheres with O_2 concentrations ranging from 0.1 to 100%. As the O_2 concentration was increased from 0.1 to 5%, the acid content of leaves increased. On the basis of available information, we agree that "dark" respiration in pineapple leaves is not saturated at 2% O_2, and that the diurnal acid turnover is somehow related to respiration.

In addition to temperature and O_2, CO_2 concentration influences the daily acid loss (Fig. 6). Increasing the CO_2 concentration decreases total acid loss (compare Fig. 6, E and F). This is expected since the PEPCK-catalyzed OAA decarboxylation reaction can proceed readily in either direction depending upon substrate and product concentrations. It also has been shown that PEP carboxylase is inhibited by high CO_2 concentrations (20).

The observed effects of temperature, O_2, and CO_2 concentrations on CO_2 release and photosynthetic CO_2 uptake in detached pineapple leaves are similar to those observed in C_3 plants. In C_3 plants, increasing temperature favors photorespiration apparently at the expense of photosynthesis. The presence of photorespiration in pineapple leaves is indicated by an equal loss of total acid at the higher temperatures and by a decrease in CO_2 uptake with increased temperature (Fig. 1). The O_2 inhibition of light CO_2 uptake in pineapple leaves (Fig. 6) is in agreement with that obtained in *K. daigremontiana*, *A. vera*, and in C_3 plants (3-5, 9, 11). The PIB in *Kalanchoe* was shown to be O_2-insensitive (3), whereas the PIB in pineapple was sensitive to changes in O_2 and CO_2 concentrations (5). It has also been shown in *Kalanchoe*, like pineapple (5), that the steady rate of light CO_2 fixation increased with increasing CO_2 concentration (1). Badger *et al.* (2) reported that in leaf extracts of *K. daigremontiana*, O_2 inhibited RuDP carboxylase in a competitive manner with respect to CO_2 and the RuDP oxygenase activity was inhibited by CO_2 in a manner similar to that observed in C_3 plants. These data are consistent with postulating that photorespiration is present in pineapple leaves and that CAM photorespiration also is favored by high temperature, low CO_2, and high O_2 concentrations.

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